

COMMISSION OF THE EUROPEAN COMMUNITIES  
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# RAW MATERIALS

RESEARCH AND DEVELOPMENT

STUDIES ON SECONDARY RAW MATERIALS

## IV. FERMENTATION-HYDROLYSIS PROCESSES FOR THE UTILISATION OF ORGANIC WASTE MATERIALS

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COMMISSION OF THE EUROPEAN COMMUNITIES  
(Directorate General XII - Research, Science, Education)

FERMENTATION - HYDROLYSIS PROCESSES  
FOR THE  
UTILISATION OF ORGANIC WASTE MATERIALS

Report by

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## O. GENERAL REMARKS

In agreement with our colleagues from the University of Gent (R.U.G.) and the "Services de la Programmation de la Politique Scientifique" (S.P.P.S., Brussels), we have elaborated this final report according to a framework defined below.

Following the meeting with our colleagues of Ireland, Germany and Italy in Dublin (31.03.1977), Berlin (31.05.1977) and Brussels (27.06.1977), the final report was prepared according to a draft table of contents and to the adopted resolutions.

The literature survey will be kept to the essential papers and to unpublished reports edited by national and international organisations, all in possession of the authors, and hence accessible to other members of the working group upon request.

### Framework of the report

#### 1 Introduction :

To be written up and forwarded by the S.P.P.S. The introduction was prepared jointly by the R.U.G., the U.C.L. and the S.P.P.S. during a meeting in Brussels on 21.06.1977.

#### 2. Organic waste utilization processes

##### 2.1. Anaerobic Digestion

###### 21.1. Raw materials

To be done for the flemish-speaking part of the Benelux by Mr. Pipyn

(R.U.G.) and for the french-speaking part of the Benelux by Dr. Naveau (U.C.L.).

#### 21.2. Commercial processes

- a. The technology of possible processes will be reviewed by Mr. Pipyn (R.U.G.).
- b. An essay on the basic technology and science of methanogenesis will be done by Dr. Nyns (U.C.L.).
- c. The existing plants will be listed for the flemish-speaking part of the Benelux by Mr. Pipyn (R.U.G.) and for the french-speaking part of the Benelux by Dr. Nyns (U.C.L.).

#### 21.3. Status of R & D

A review of existing R & D for the flemish-speaking part of the Benelux will be done by Mr. Pipyn (R.U.G.) and for the french-speaking part of the Benelux by Dr. Nyns (U.C.L.).

#### 21.4. Economic evaluation

An essay on the basic economics of methanogenesis will be done by Dr. Nyns (U.C.L.).

#### 2.2. Composting

The study will be carried out by Mr. Vanacker (R.U.G.)

#### 2.3. Carbohydrate hydrolysis

- 23.1. a. The review of existing raw materials, such as molasses, cellulosic material (including paper and wood, starch and whey) will be done for

the flemish-speaking part of the Benelux by Dr. Vandamme (R.U.G.) and for the french-speaking part of the Benelux by Dr. Naveau (U.C.L.).

- b. The review of end products will be done for the flemish-speaking part of the Benelux by Dr. Vandamme (R.U.G.) and for the french-speaking part of the Benelux by Dr. Naveau (U.C.L.).

### 23.2. Commercial processes

The review of the existing plants will be done for the flemish-speaking part of the Benelux by Dr. Vandamme (R.U.G.) and for the french-speaking part of the Benelux by Dr. Naveau (U.C.L.).

### 23.3. Status of R & D

The review of the status of R & D for the fermentation and enzymatic processing will be done by Dr. Vandamme (R.U.G.) and for chemical or physical pretreatment and chemical processing by Dr. Naveau (U.C.L.).

### 23.4. Economic evaluation

The economic evaluation of the enzymatic and fermentation processes will be done by Dr. Vandamme (R.U.G.) and of the pretreatment and chemical processes by Dr. Naveau (U.C.L.).

## 2.4. Protein recovery

- 24.1. The survey of raw materials will be done for the flemish-speaking part of the Benelux by Dr. Verstraete (R.U.G.) and for the french-speaking part of the Benelux by Dr. Naveau (U.C.L.).

- 24.2. a. A study of the possible processes using residues from slaughter houses and the existing plants within the Benelux will be made by Dr. Verstraete (R.U.G.)

b. Studies of the possible using animal residues and residues from other sources will be carried for the flemish-speaking part of the Benelux by Dr. Verstraete (R.U.G.) and for the french-speaking part of the Benelux by Dr. Nyns (U.C.L.).

#### 24.3. Status of R & D

The survey of the status of R & D will be made for the flemish-speaking part of the Benelux by Dr. Verstraete (R.U.G.) and for the french-speaking part of the Benelux by Dr. Nyns (U.C.L.).

#### 24.4. Economic evaluation

The economic evaluation of the processes covered by Dr. Verstraete (R.U.G.) will be done by Dr. Verstraete (R.U.G.) and for the processes covered by Dr. Nyns (U.C.L.) by Dr. Nyns (U.C.L.).

### 3. Competitive processes

Processes like hydrolysis processes not involving fermentation, and processes not using residues as raw material but both producing end-products similar to those ascertained in § 2 will be listed here.

3.1. A study concerning protein and/or metabolite production from hydrocarbons and simply related compounds will be carried by Dr. Nyns (U.C.L.)

### 4. Preliminary research proposals

4.1. Preliminary research proposals concerning anaerobic digestion will be done by Mr. Pipyn (R.U.G.) and Dr. Nyns (U.C.L.).

- 4.2. Preliminary research proposals concerning compositing will be done by Mr. Vanacker (R.U.G.).
- 4.3. Preliminary research proposals concerning carbohydrate hydrolysis will be done by Dr. Vandamme (R.U.G.) and Dr. Naveau (U.C.L.).
- 4.4. Preliminary research proposals concerning protein recovery will be done by Dr. Verstraete (R.U.G.) and Dr. Nyns (U.C.L.).

## 2. ORGANIC WASTE UTILIZATION

### 2.1. Anaerobic digestion

#### 21.1. Raw materials and end products

The contractor feels that the amount of biogas produced in an anaerobic digester for excess sludge of waste water treatment plant, gives a poor idea of the potentialities of the methane production by bioconversion of organic residues in general.

The present study is therefore an assay of a quantitative evaluation of methane biogas potentialities from available knowledge of existing sources of organic residues.

#### 211.1. Methane production by bioconversion of animal residues

The total amounts of animal residues in Belgium are given in the following Table :

| Year    | 1970 | 1980 | % increase in<br>ten years |
|---------|------|------|----------------------------|
| Cattle  | 40   | 60   | 50                         |
| Swine   | 6.3  | 12.6 | 100                        |
| Poultry | 1.0  | 1.3  | 30                         |
| Total   | 47.3 | 73.9 | 56.2                       |

The units are tons x  $10^6$  x year<sup>-1</sup>. The figures refer to weight of manure which is a mixture of 10 to 15% solids with 85 to 90% liquids, eventually diluted with variable amounts of water.

The amount of dry matter in the manure may vary considerably depending

upon its origin and composition. For swine manure, the dry matter content lies between 60 and 80 kg x tons<sup>-1</sup>. This dry matter usually contains 65% organic matter.

The present evaluation is based on the sole swine manure. Indeed, cattle manure is presently not a problem in Belgium because the cattle farms are of small size and geographically widespread. Furthermore, cattle manure is widely disposed of by spreading on agricultural lands. On the other hand, swine manure becomes an increasingly stressing problem because (a) the swine farms are geographically concentrated (84% in the Flanders) and (b) the 100% increase of the number of swines in 10 years.

Assuming a linear increase in the number of swines between 1970 and 1980, one may expect  $10 \times 10^6$  tons x year<sup>-1</sup> of swine manure in 1977, that is  $700 \times 10^6$  kg dry matter or  $455 \times 10^6$  kg organic matter. Assuming a  $\frac{Y_{m^3 CH_4}}{\text{kg organic matter}} = 1$  (see 214.4b), the potential production of methane

biogas from swine manure amounts to  $455 \times 10^6 \text{ m}^3 \text{ CH}_4$ . This represents 4% of the total gas sold in Belgium or 20% of the gas distributed for domestic use (see 214.5b).

#### 211.2. Methane production by bioconversion of organic industrial residues

Figures are available for the province Antwerp in Belgium (E.R.A., 1974) and are given in the following Table :

| Year               | 1971 | 1974 | 1980 | Yearly % increase |
|--------------------|------|------|------|-------------------|
| Residue            |      |      |      |                   |
| Oils               | 2    | 7    | 9    | 6                 |
| Sludges            | 153  | 338  | 401  | 3.5               |
| Non-toxic organics | 121  | 394  | 540  | 7.5               |
| Bio-organics       | 8    | 21   | 27   | 5                 |
| Total              | 284  | 760  | 977  |                   |

The units are tons  $\times 10^3 \times \text{year}^{-1}$ . Oils are not suitable for anaerobic digestion. Sludges have a high water content : assuming a 1% organic dry matter content,  $3.4 \times 10^3$  tons  $\times \text{year}^{-1}$  organic matter were available in 1974. Non toxic organics contain a high amount of paper, cardboard, wood. One may consider that 70% of the given weights are organic matter. The remaining splits in 12% water and 18% non-organic matter. Assuming that only 50% of this organic matter is anaerobically digestible, due namely to the content in non biodegradable lignin , then, were available in 1974,  $393/2$  or  $197 \times 10^3$  tons of organic matter from non toxic organic industrial residues. Assuming, finally, that 50% of the bio-organics are organic matter, then there were available in 1974,  $21/2$  or  $11 \times 10^3$  tons of organic matter from bio-organic industrial residues.

Summing up the figures from the preceeding paragraph yields a disponibility of  $210 \times 10^3$  tons of organic matter. Assuming that the province Antwerp represents 1/6 of the industries of Belgium, a total amount of industrial organic residues of  $1,260 \times 10^3$  tons  $\times \text{year}^{-1}$  may be expected. Assuming again a  $Y_{m^3\text{CH}_4}$  of 1 (see 214.4b), one may expect

kg organic matter

a potential methane biogas production of  $1,260 \times 10^6 \text{ m}^3 \text{ CH}_4$ . This represents 13% of the total amount of gas sold in Belgium or 70% of the gas distributed for domestic use (see 214.5b).

It should be noted that sludges, if quantitatively negligible, are more important qualitatively because of their high content in nitrogen, usually lacking in other industrial organic residues.

### 211.3. Methane production by bioconversion of domestic residues

Assuming that the amount of domestic residue in Belgium is one half that of the U.S. per capita, one may evaluate the total amount of domestic residues in Belgium in 1977 at  $3.6 \times 10^6 \text{ tons} \times \text{year}^{-1}$ .

Domestic residues contain (a) 50% organic matter, (b) 8% metals, (c) 9% glass, (d) 3% plastics, (e) 5% other inorganics and (f) 25% water.

The total amount of organic matter from domestic residues, can thus be evaluated at  $1.8 \times 10^6 \text{ tons} \times \text{year}^{-1}$ . Assuming the same yield as above, the corresponding potential biogas production can be evaluated at  $1.8 \times 10^9 \text{ m}^3 \text{ CH}_4$  per year, that is 15% of the total gas sold in Belgium or 75% of the gas distributed for domestic use (see 214.5b).

### 211.4. Photosynthetic production of biomass : energy crops vs residues

The production costs of vegetal crops amount in Belgium to 20,000 BF  $\times \text{year}^{-1} \times \text{ha}^{-1}$ . The production potentialities (based on corn) are 12 to 15 tons dry matter  $\times \text{ha}^{-1}$  or 10 tons organic matter  $\times \text{ha}^{-1}$ .

Assuming the same yields figures as above (see also 214.4b), a potential gas production of  $10 \times 10^3 \text{ m}^3 \text{ CH}_4 \times \text{ha}^{-1}$  can be expected. Combining the production costs and yield figures, the price of raw material alone amounts to 2 B.F.  $\times \text{m}^{-3} \text{ CH}_4$ , which is too high. (see 21.4). The only possibility would then be to increase the yield per ha. In the U.S. some vegetal species can produce up to 35 tons dry matter  $\times \text{ha}^{-1}$ .

Assuming that one wants to satisfy 10% of belgian national consumption of gas (see 214.5b), one would have to produce  $1 \times 10^9 \text{ m}^3 \text{ CH}_4 \times \text{year}^{-1}$ . This would requires  $10^6$  tons of organic matter, which could be gained (as corn) from  $10^5$  ha. This area is precisely what is actually farmed in Belgium for the production of corn. This area is  $1/300^{\text{th}}$  of the total national area.

## 21.2. Commercial processes in the French-speaking part of the Benelux

There are two industrial plants known to the contractor in the french-speaking part of the Benelux, both treating excess sludge from waste water treatment :

212.1. Station d'épuration de et à Wasmuel. Association Intercommunale pour le développement économique et l'aménagement des régions du Centre et du Borinage (I.D.E.A.), rue des Paturages, 74, 7300 Quaregnon. MM. Hanon ou Douillez. Tel. 065/66.57.01.

212.2. Station d'épuration de et à Waterloo (not yet fully identified).

## 21.3. Status of R & D in the French-speaking part of the Benelux

213.1. There is only one R & D project known to the contractor in the french-speaking part of Benelux "Correlation between biochemical and microbiological properties of the methane-producing biomass and the rate and yield of methane production".

National program for the economy of residues and secondary metabolites (S.P.P.S., Public Authority), University of Louvain, Prof. H. Naveau and E.J. Nyns, 3 years, 3 man year.

213.2. In order to evaluate priorities in research proposals, it seemed interesting to the contractor to assay the present status of knowledge both on the technology and on the basic mechanisms of methanogenesis.

2132.2. Present status of knowledge of the basic mechanisms of methanogenesis.

The basic process for methane biogas production by bioconversion of organic residues can be subdivided in the following subprocesses :  
(a) pretreatment, (b) liquefaction, (c) acidogenesis, (d) interface between acidogenesis and methanogenesis, (e) methanogenesis, (f) gas purification.

21321.1. Pretreatment of organic residues

The conditioning of an organic residue in order to make it more suitable to methane biogenesis, has hitherto been little examined. Up to now, the organic residues, excess sludge from waste water treatment, domestic residues and animal residues have been introduced in the digester without pretreatments at the exception of some crude grinding and mixing.

The idea that adequate pretreatment by physicochemical means could result in a major progress in gas yield or in retention time reduction, is quite obvious. But curiously, only two R & D projects are known to the contractor dealing with this idea : one in the U.S., the second in Genth, Belgium. A first approach found in both projects deals with a heat pretreatment to feed the digester with killed organisms instead of live organisms or denaturated proteins instead of nat proteins.

#### 21321.2. Liquefaction of the organic residues

The liquefaction of the organic residues is the phenomenon by which essentially anaerobic bacteria, using enzymes which are localized outside the periplasmic membrane, either bound at the outer surface, or secreted in the outer medium, hydrolyzes highly polymerized compounds into smaller soluble units. Proteins yield anions acids, polysaccharides and namely cellulose and hemicellulose, yield sugars, fatty compounds yield fatty acids with a carbon atom number of 16 to 18). Lignin is untill now poorly degraded and, as such, not suitable for further bioconversion into methane. Due to the proper constitution of cellulosic natural compounds, lignin often protects cellulose and hence prevents its hydrolysis. The state of cellulosic natural compounds in the influent will thus directly influence its fate, up to a point that it has been written that the hydrolysis of cellulose may well be the determining step of methanogenesis (Hobson et al., 1974).

#### 21321.3. Acidogenesis of the liquefied organic residues

Acidogenesis is the phenomenon by which fatty acids and sugars are

transformed into organic acids of low number of carbon atoms : from 1 to 5.

Fatty acids are transformed to small organic acids by  $\beta$ -decarboxylation, a well known biochemical pathway in aerobiosis. The rate limiting step is the activation step by which a coenzyme A becomes bound to the fatty acid (Novak and Carlson, 1970). When this reaction occurs in anaerobiosis, a major problem remains unsolved : the mechanism of regeneration of the reduced coenzymes, although the end product : hydrogen has been known for a long time.

Sugars are transformed either through glycolysis or through the hexose monophosphate shunt finally to pyruvate (Toerien et al, 1970) from which a number of small organic acid can be produced such as lactate, succinate, propionate, formate and mainly acetate. Some low molecular weight alcohols are also produced such as ethanol, butanol, isopropanol, 2,3-butanediol . Some hydrogen also is formed (Andrews and Pearson, 1965). These metabolic pathways seem rather well understood.

Bacteria responsible for the acidogenesis of liquified organic residues, have a rather good growth rate, which has never been reported hitherto as rate-limiting step for the overall methanogenesis process. These bacteria obtain their energy from substrate level phosphorylation either during glycolysis or the Krebs cycle. They obtain their nitrogen from the amino acids which are deaminated during acidogenesis to yield  $\text{NH}_4^+$ .

Most of the knowledge on the methanogenesis through anaerobic bioconversion of organic residues, has been gained from numerous studies on the functioning of a rumen. Let it be remembered that one cow produces daily 600 l of methane. In the rumen however, as well as in most anaerobic digesters; bioconversion occurs in one single phase where liquefaction, acidogenesis and methanogenesis occur in a completely mixed homogeneous system. Under these conditions, it appears difficult to study the optimization of each sub process and to define exactly what are the end products of acidogenesis and the starting materials for methanogenesis, not to speak of the individual influence (activation or inhibition) of each of these products.

From a number of papers dealing with this interface problem (Wolfe, 1971; Stafford, 1974; Keefer and Urtes, 1974; McCarty, 1964; Edeline, 1976; Andrews and Graes, 1971), it can only be concluded that much disagreement exist among authors. To take but one example, whereas low molecular fatty acids are found in the interface and thought to be appropriate starting materials for methanogenesis, with pure cultures in laboratory experiments methane is mostly only produced from hydrogen and carbondioxide.

Finally, in a completely mixed system, the pH will be a compromise between the optimum pH for acidogenesis and the optimum pH for methanogenesis. Quite obviously it can be expected that both optimum pH's will not be identical. Acidogenesis will probably best occur in more acidic conditions, whereas methanogenesis occurs better at slightly alkaline pH, not because hydrogen ions are inhibiting per se but because a slightly alkaline pH would better regulate the membrane permeation to organic acids of the methanogenic bacteria.

## 21321.5. Methanogenesis

Methanogenesis remains at the present time a black box. It has been seen in the previous paragraph (21321.4) that confusion exists about the starting materials for methanogenesis. From a survey of the scarce literature (Wolfe, 1971; Toerien et al., 1970; Stafford, 1974) on the subject, the following facts further arise :

- a. There seems to be more than one metabolic pathway which yields  $\text{CH}_4$  although not a single one is clearly, completely and unambiguously understood.
- b. Nothing at all is known about the way methanogenic bacteria gain energy from redox processes. The contractor is since long engaged in energy conservation studies in aerobiosis where the chemiosmotic theory of Mitchell is now unanimously accepted. In the very few cases of anaerobic non substrate level energy conservation, but among which none deals with methanogenesis, elucidated up to now, it does well seem that a mechanism based on the chemiosmotic theory of Mitchell must apply.
- c. Methanogenic bacteria have a very slow growth rate. The maintenance of an appropriate amount of methanogenic biomass in the digester is another rate-limiting step in methane biogas production : high retention times are required to avoid washing out of the methanogenic biomass.

## 2132.2. Present status of knowledge of the technology of methanogenesis

One of the limits in the design of anaerobic methanogenic digesters, is the accumulation at the surface of the fermenting medium of a stable foam-like layer which resists mixing. Therefore, digesters were build

with a little a top surface as possible and were therefore constructed as very high, egg-shaped constructions. Such constructions are very expensive. More recently, in Belgium, Fabricom builded a prototype where these mixing problems were mechanically solved, and which, therefore, could be build as a flat construction. Studies in these directions are also pursued in the U.S. mainly for small size units at the single agricultural exploitation level.

Another limit in the desing of anaerobic methanogenic digesters is the high retention time which, in turn, results in large specific volumes (volume of digester per weight of organic residue treated per day). As one of the rate-limiting steps is the high generation time of the methanogenic bacteria, attempts have been made, first at the bench level (Hobson et al., 1974; Pohland and Gosh, 1975), and later at the pilot plant level (Gosh et al., 1974), to operate the bioconversion of organic residues into methane in two-stages biological units : a first stage devoted to liquefaction and acidogenesis and a second stage devoted to methanogenesis. The sludge decanted after the second stage is however recycled with the influent of the first stage.

#### 21.4. Economic evaluation

- 214.1. The contractor feels that the economic evaluations based on existing processes, namely excess sludge treatment in waste water treatment plants, is erroneous because :
  - a. The primary material for anaerobic digestion is solely the excess sludge gained from the aerobic waste water treatment. This sludge is

selected for its best ability to purify aerobically waste water, not for its best suitability for anaerobic digestion.

b. This excess sludge is for a large part made of live cells, which, by definition of life itself, should not constitute adequate food for other cells.

c. The anaerobic process in waste water treatment plants has, as first target, the obtention of a residual excess sludge stabilized, non putrescible, odorless, with appropriate and improved properties for dewatering through filtration. This anaerobic process has not, as primary target, a good yield or a high rate of methane production, which is nevertheless a useful by-product to be used within the plant.

Therefore, it seemed suitable to the contractor to assay the economic evaluation by a more fundamental approach which will be described below.

#### 214.2. Fundamental economic evaluation of methanogenesis

214.2.1. Let us assume an anaerobic digestion plant of an effective digestion capacity of  $10,000 \text{ m}^3$  and an anaerobic digestion plant of an effective digestion capacity of  $100,000 \text{ m}^3$ . The yearly costs of these plants can be respectively evaluated at 2,067 and 2,670 B.F.  $\times \text{m}^{-3}$  (as demonstrated below under 214.3).

214.2.2. Let us assume that each effective  $\text{m}^3$  of a digester can work at a concentration of 80 kg organic matter  $\times \text{m}^{-3}$  ( $80 \text{ g} \times \text{l}^{-1}$ ) (as demonstrated below under 214.4.a).

214.2.3. Let us assume that each kg of organic matter can produce  $1 \text{ m}^3$  of methane

(as demonstrated below under 214.4.b). Then, each  $\text{m}^3$  of digester will be able to produce  $80 \times 1 = 80 \text{ m}^3$  of  $\text{CH}_4$  in one turnover.

2142.4. Considering a mean residential time of 10 days (as demonstrated below under 214.4.c), then each  $\text{m}^3$  of digester is able to produce, under optimum conditions  $80 \text{ m}^3 \times \left(\frac{365}{10} = 36.5\right) = 2,920 \text{ m}^3$  of  $\text{CH}_4$  of which 35% are needed as energy by the plant itself. Remains  $1,898 \text{ m}^3 \text{ CH}_4$ .

2142.5. Hence, under present optimum conditions, the production cost of  $1 \text{ m}^3$  of methane amounts respectively to 2,067/1,898 or 2,670/1,898, that is from 1.08 to 1.40 F.B. These prices compare well with the present production costs of natural gas, evaluated at B.F.  $1.0 - 2.0 \times \text{m}^{-3}$  methane (as demonstrated below under 214.5).

### 214.3. Yearly costs of methane-producing plants

The construction price per effective digestion  $\text{m}^3$  of an anaerobic digestion plant can be evaluated in Belgium at 20,000 B.F. in 1977. This appears from a turn-key offer made in 1972 by Fabricom, a Belgian constructor, still valid presently. At that time, a  $2000 \text{ m}^3$  plant was offered at  $13 \times 10^6$  B.F.. The plant included the storage tank, the digester itself, the final aerobic treatment of the liquid effluent, the annex buildings. The offer included the civil engineering and the electromechanics including an electrical power generator. From Fabricom itself, it appears that this offer could reasonably well be expressed in B.F. of 1977 at  $20 \times 10^6$  B.F. Let us assume that a somewhat more improved plant would cost twice as much.

A  $10,000 \text{ m}^3$  plant would thus cost  $200 \times 10^6$  B.F. and a  $100,000 \text{ m}^3$  plant,  $2 \times 10^9$  B.F.

Exploitation requires the following personnel :

1 engineer, per year : 1,000,000 B.F.

1 technical assistant, per year 700,000 B.F.

One round the clock shaft of 2  
persons, that is 10 workmen at

400,000 B.F. each 4,000,000 B.F.

Energy needs will be deduced from the methane production.

The maintenance of the buildings and equipment is evaluated at  
1,000,000 B.F.

Hence, a first subtotal of exploitation costs amounts to  $6,7 \times 10^6$  B.F.  
 $\times \text{year}^{-1}$ .

Capital costs, on a twenty year basis, amount either to  $20 \times 10^6$  or to  
 $200 \times 10^6$  B.F., yielding the two respective totals of  $26,7 \times 10^6$  or  
 $206,7 \times 10^6$  B.F., that is 2.67 or 2.067 B.F.  $\times \text{m}^{-3}$ .

A large scale anaerobic digestion plant of organic residues will require  
the following unitary processes :

a. A pretreatment unit, consisting of an organic residue reception  
subunit, a separation subunit (to remove non digestible materials,  
grinding and mixing subunits with eventual pH adjustment.

b. A digestion unit, consisting of the digesters, including mixing and  
heating devices, pumping, parameter (pH,  $\text{O}_2$ , T ...) control devices and  
addition devices for basic or nitrogenous compounds.

c. A biogas-treating unit for the elimination of  $\text{CO}_2$ ,  $\text{H}_2\text{S}$ , for the drying of the gas, and consisting also of a compressor subunit, storage and heat or electricity generator.

d. An effluent-treating unit for the final conditioning of the residual sludge and for the treatment of liquid residues. Although this differs in some aspects from the content of Fabricom's offer above, this will not substantially modify the figure of  $20,000 \text{ B.F.} \times \text{m}^{-3}$  calculated above.

No transportation costs are included in this evaluation. Domestic residues are collected anyway and the anaerobic biogas production plant can be considered as a methane trap, located somewhere on the transport way of organic residues.

Even if the manpower defined above is largely underestimated particularly in the  $100,000 \text{ m}^3$  plant, it can easily be seen that this will not substantially affect the exploitation costs per  $\text{m}^3$ .

#### 214.4. Available technological data for methane production

##### 214.4a. Density of organic charge in the digester.

The upper limit of density of organic charge in the anaerobic digester, compatible with pumping and mixing, lies around 8% solids (Pfeffer, 1976). Assuming that 50% of the solids are converted to methane biogas, the concentration of this feed to the digester may be as high as 16%. For a yield of 75% instead of 50%, this concentration can rise to 32%.

An increase in yield from 50 to 75% will thus not only increase the amount of biogas produced but also the reduction of the dimensions of the digester, and the reduction of the costs of effluent treatment.

#### 14.4b. Thermodynamic yields of the bioconversion of organic matter to methane

Let  $Y$  be the yield in  $\text{m}^3 \text{CH}_4$  per kg of dry organic matter.

Various figures of  $Y$  have appeared in the literature. Gady et al. (1974) report the low value of 0.08 for the anaerobic bioconversion of animal residues. Kispert et al. (1975) report the value of 0.25 for the anaerobic bioconversion of a mixture of domestic residues and excess sludges from waste water treatment. For the same mixture, Hitte (1976) reports the close value of 0.2. Pfeffer and Liebman (1976) also report values of 0.2 to 0.3 for the anaerobic bioconversion of a mixture of domestic residues and excess sludges from waste water treatment, but point out that the yield may rise to 0.45 under thermophilic conditions. Cooney and Wise (1975), in laboratory scale experiments, also points to a beneficial effect of temperature. A 0.47 yield at 37°C increases to 0.69 at 65°C. At 95°F a yield value of 0.5 is reported by Diaz and Trezek (1974). Also in laboratory experiments, Andrews and Peason (1965) report that 1 l of gas is produced per g of organic matter consumed. This means 0.7 l  $\text{CH}_4$  per g of consumed organic matter, thus a  $Y = 0.7$ . Also per kg of organic matter consumed, Eckenfelder (1966) reports a yield value of 1 which varies depending upon the nature of the organic matter : whereas low values of 0.7 are found for brewery residues, high values of 1.4 can be obtained with wine industry residues. Finally in laboratory experiments but in a two stage pilot plant, Ghosh et al. (1975) report that, under

these conditions, (a) the Y value increases to 1, (b) the mean solids retention time may become inferior to 10 days, namely 7.5 days and (e) the bioconversion of organic matter affects 40% of its dry weight.

It may be concluded that a Y value of 1 is within reach on large scale bioconversion, provided the process be studied with a real aim at methane production. Therefore, this value will be taken into consideration for this evaluation.

214.4c. Mean residential time of organic residues in an anaerobic digester.

A large number of values for the mean residential time of organic residues in an anaerobic digester are reported in the literature, which will not be summarized here, ranging from 7 to 30 days. The minimum value encountered was 7.5 days in a two stage system with re-incubation of solids from the effluent of stage II to the influent to stage I. From kinetic studies, a value as low as 3-4 days may be expected.

It appeared therefore reasonable to utilize the value of 10 days for the mean residential time in this evaluation, if one also wants to consider a good  $Y_m^3 \text{CH}_4$  value.  

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kg organic matter

214.5a. Actual market prices for natural gas.

According to Distrigaz (a Belgian Company for the distribution of natural gas), the market prices of natural gas for domestic uses,

amounts to 4.62 B.F. par  $N m^3$  GN where N stands for dry,  $0^\circ C$  and 1013 mbar and GN for natural gas. Also according to Distrigaz, the market price for direct delivery of natural gas to large industries amounts to 2.25 B.F. per  $N m^3$  GN. Production or fossil gas exploitation costs may thus range between 1.0 and 2.0 B.F.

214.5b. Yearly consumption of city plus natural gas in Belgium

The reading of the 1975 yearly report of Figaz (Figaz, 1975) yields the following figures :

Yearly consumption of city plus natural gas :

|                                       |                           |
|---------------------------------------|---------------------------|
| (a) Distributed : domestic            | $15,904 \times 10^3$ Gcal |
| non domestic                          | $6,053 \times 10^3$ Gcal  |
| (b) Delivered directly to<br>industry | $65,686 \times 10^3$ Gcal |
| Total :                               | $87,643 \times 10^3$ Gcal |

Knowing that 1 Gcal can be obtained from  $119 N m^3$  GN at  $8,400 \text{ Kcal}/N m^3$ , hence the total yearly consumption amounts for Belgium to  $8.76 \times 10^7 \times 1.19 \times 10^2 N m^3$  GN =  $10.43 \times 10^9 N m^3$  GN.

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## 2.3. Carbohydrate hydrolysis

### 2.3.1. Raw materials

#### 2.3.1.1. Wood residues

Wood residues are present in large quantities in many parts of the community. These are found mainly in the form of sawdust and shavings from industry and of logging residues, although this last category will not be considered at this time, due to collections problems. Sawdust alone represents 3,5 millions tons of wood residue within the European Community Countries.

#### 2.3.1.2. Urban solid wastes

The amount of urban solid wastes is 1kg/person.day in Brussels and of the same order of magnitude in other big cities of Belgium. It is estimated at around 0,7 kg/person.day in rural areas : precise determinations are under way in the French speaking part of Belgium.

Composition analyses show that in urban-industrial areas, urban solid wastes contain 22 to 41 % ( $\pm 30$  %) paper and 40 % putrescible matter; in semi-rural (semi-industrial) areas, these proportions are 15 to 26 ( $\pm 20$  %) for paper and 50 to 64 ( $\pm 57$  %) for putrescible and in rural areas 16 % paper and 54 % putrescible.

After separate collection of paper, it can be estimated that paper represents at least 20 % and putrescible 50 % of urban solid wastes. This corresponds to approximately 60 kg of waste paper per inhabitant per year which end up in the household refuse.

Current research is directed toward evaluation of wood residues as a source of digestible energy or as a roughage in ruminant rations. In vitro and in vivo tests have shown that digestibility of wood and bark may vary very much for different species of trees from 0 to 50 %. But most untreated woods can contribute little to the energy needs of animals, even ruminants. However, they still can play a useful role as roughage substitute. Oak sawdust, for instance, is an effective roughage substitute when used as 5 to 15 % of the total ration of beef and sheep while aspen sawdust, which is about 35 % digestible, could replace one third of lactating cows rations while maintaining their milk production.

Several physical and chemical pretreatment have been tested for their ability to increase digestibility of wood cellulose : electron irradiation, vibratory ball milling, gaseous and liquid ammonia gaseous sulfur dioxide, dilute sodium hydroxide and whiterot fungi. The coniferous species and most deciduous species are quite resistant to all treatments with the exception of gaseous sulfur dioxide to increase their digestibility. The sulfur dioxide appears interesting as yields are high and the products are accepted by animals.

Sodium hydroxyde treatment is also valuable. It has been patented for straw by Beckman in 1919 : 24 hr in 1.5 % NaOH increased the digestibility from 30-50 to 60-70 %, making it equivalent to high quality hays. The treatment has been adapted to minimize hemicellulose losses. Several commercial plants are now being installed to process the straw using the modified Beckman process. A small quan-

As such this waste paper can be recuperated from the refuse by either wet or dry mechanical sorting systems. Because it is polluted with food residues, faeces, etc, it can only to a limited extent be reutilized for paper production. Nevertheless, extremely large quantities of waste paper are potentially available, e.g. 600.000 tons per year for Belgium and 13-15 millions tons per year for the community.

The amount of urban solid wastes which is now disposed of by various way (incineration, composting, and filling...) has to be determined so as to know the amount available for the processes considered in this study.

### 2.3.2. Commercial processes

#### 2.3.2.1. Cellulose and hemicellulose hydrolysis

No plant in the french-speaking part of Benelux.

### 2.3.3. Status of R-D

#### 2.3.3.1. Wood-based residues in animal feed

Research on use of hydrolyzed wood in animal feed has begun in 1918 in USA. It was concluded that feeding hydrolyzed wood was possible but practical only when natural feed grains were in short supply. Later on, research was conducted to produce concentrated sugar solutions. The tests indicated that wood-sugar molasses is a highly digestible carbohydrate feed and that the protein value of Torula yeast grown on neutralized dilute wood hydrolyzate was equivalent to casein when supplemented with methionine.

tity of 3-5 % W/W sodium hydroxide solution is added to chopped straw which is then pressed so as to generate high pressure and temperature resulting in a breakdown of lignin. The cooled product can be stored at 80 - 85 % dry matter. The net energy is claimed to be twice that of untreated straw.

In such treatments with wood, the results are related to the amount of lignin, 18 % lignin corresponding to 50-60 % digestibility and 26 % to 0 % digestibility. Treatments with anhydrous ammonia yield similar results.

Wood delignified by normal pulping methods has high rumen digestibility; it depends upon the level of lignin removal and not upon the method of lignin removal. Waste paper has the disadvantage that it often contains glue, clay, ink and plastics that could introduce undesirable additive in the feed.

Successful treatment has been obtained with a fungal treatment followed by spraying with sodium hydroxide solution. This increased the organic matter digestibility of beech and of oak sapwood from 14 to 56 %.

It can be concluded that wood-based residues can be effectively used in animal feed. However, their efficiency is much reduced by their resistance to micro-organisms, which render them partially or wholly unavailable as an energy source. This is related to the lignin-carbohydrate complex and the cristallinity of the cellulose.

#### 2.3.3.2. Pretreatment of ligno-cellulosic materials

The pretreatment of lignocellulosic material is very important to attain high yields of conversion in biological processes utilizing them, which can be

envisionned in this syudy, that is anaerobic diges-  
tion, hydrolysis, protein recovery.

Its aim is to improve the accessibility of the  
carbohydrates and particularly cellulose by decrea-  
sing the lignin content of the material, or dis-  
rupting the lignin-carbohydrate complex, and  
reducing the cristallinity of cellulose, so as to  
allow access of the enzymes to the cellulose mole-  
cule.

#### 2.3.3.2.1. Chemical pretreatments

Swelling agents have an intercrystalline or inter-  
crystalline action on cellulose. The later one  
involves a penetration of the crystalline regions  
providing one pathway toward alteration of cellu-  
lose crystalline structure, up to complete solution,  
with the possibility of enhanced hydrolytic reacti-  
vity.

Concentrated mineral acids have been abandonned  
due to high plant and opeating costs and metal  
chelates solvents due to produce separation, sol-  
vent recovery and interference of lignin.

Mercerization with NaOH, regeneration by the vis-  
cose process and cellulose "decrystallisation" by  
liquid ethylamine are effective treatments which  
increase the hydrolysis rate with dilute hot acids  
by 40 - 50 %. The need for high levels of che-  
micals and the problems of chemical recovery make  
it doubtful that these processes can function eco-  
nomically in actual conditions for dilute acid sac-  
charification.

Delignification is naturally very interesting since lignin is one of the roadblock on the way to cellulose saccharification. However, pulp production is a costly procedure. What are the prospects for less costly techniques, or better, what degree of lignin removal is really needed for a reasonable carbohydrate availability. Studies have shown a digestibility of 60 % at 15 % lignin content for hardwoods and 9 % for softwoods and a reasonable growth of the fungus *Aspergillus fumigatus* at 14 % lignin for hardwoods and 2 % for softwoods. Yet the delignification procedures are not selective and considerable carbohydrate material is commonly lost in the waste stream.

An other delignification possibility is that of disrupting the lignin-carbohydrate association without removing any constituent. This has been done by gaseous sulfur applied to moist wood during 2 - 3 hr at 120°C. Accessibility to enzymatic saccharification is dramatically increased up to 60 - 67 % for hardwoods and 45 to 50 % for softwoods. Although no lignin was removed, "Klason" lignin values are less than 9 % for hardwoods and 19 % for ponderosa pine, suggesting an extensive depolymerization of the original lignin. Carbohydrate to sugars conversion is almost 100 % for hardwoods after SO<sub>2</sub> treatment and 70 - 85 % for softwood : this last value could probably be increased.

Residues from pulpmills (groundwood fines, semi-chemical pulping fines, screen rejects, chemical pulping fines) show digestibility or accessibility in direct relation with the parent wood and the treatment they have received, that is from very low to high in the order of listing.

#### 2.3.3.2.2. Physical Pretreatments

High-energy electron irradiation enhances the dilute acid saccharification of both cellulose and lignocellulosic materials. Dosages of  $10^8$  R are necessary for a maximum effect with about 15 % carbohydrate destruction, higher dosages promoting destruction of too much carbohydrate. However, there are once again strong species specificity in favor of hardwoods even after opening up of cellulose crystallinity; the presence of lignin at levels of 30 % may still prevent enzyme access to that carbohydrate. However, price is in the order of \$ 180 / T.

Grinding and ball-milling markedly enhance the susceptibility of cellulose materials to hydrolytic, enzymatic and microbiological attack. However, production of fine wood particles yields expensive substrates (from \$ 2.80 for 40 mesh screen size to \$ 180 for 400 mesh). Vibratory ball milling is appreciably more effective than ordinary ball milling and cellulose crystallinity is eliminated within 30 min under proper conditions, allowing complete saccharification of the cellulose. Elevated temperature also increase the efficacy. Since effective ball milling requires an essentially dry environment, the added drying effect of heat may also account for the enhanced reactivity observed when milling at 220°C. Work, thus far, has been largely on laboratory mills and practical application of vibratory ball milling as an industrial pretreatment for ligno-cellulosic residues will depend upon availability and efficiency of equipment and overall energy costs for drying and milling.

Compression of hydrocellulose and pulp sheets increase their reactivity, but this does not seem to yield better results than ball milling.

#### 2.3.3.2.3. Biological pretreatments

Biological pretreatments are also possible. These are mostly by enzymes solutions or by fungi.

Enzyme solutions contain enzymes able to decrystallize the cellulose and partly to hydrolyse it can be obtained through culture of *Trichoderma viride*. Fungi (white rot fungi) can also directly degrade lignin as well as cellulose. However, these biological methods produce also inhibitors so that caution must be taken in the use of these pretreated materials. However, there is still very much to learn in the application of these biological pretreatments.

#### 2.3.3.3. Chemical hydrolysis of cellulosic materials

Chemical hydrolysis of cellulosic materials has been used following several technical processes, which fall into four basic categories according to the nature of the hydrolysis agent.

- A.1 Concentrated sulfuric acid
- A.2. Concentrated hydrochloric acid
- B. Dilute sulfuric acid
- C. Hydrogen chloride gas

##### 2.3.3.3.1. Concentrated acid processes

In the concentrated acid types of treatment, the acid is used as a swelling agent and as a catalyst for the reaction. Recovery and corrosion problems are critical for the economy of the system. Sulfuric acid processes have been used in USA

and Europe during World War II but later abandoned after the end of the state of emergency. The Hokkaido process has been developed in Japan. Hydrochloric acid processes are the Rheinau-Bergius, the modified Rheinau and the Udic-Rheinau. Hydrogen chloride gas is used in the Noguchi-Chisso and the Chalo processes.

#### 2.3.3.3.2. Dilute sulfuric acid processes

Several processes based on dilute sulfuric acid have been used commercially, the Scholler process being the oldest. It consists of hydrolyzing wood chips under pressure with dilute sulfuric acid and steam, removing the hydrolyzate as rapidly as possible, and neutralizing the excess acid to ferment the resulting sugar solution with yeast to produce ethyl alcohol.

This German process was improved at Madison U.S. Forest Products Laboratory from a batch process yielding 210 l alcohol in 13 to 20 hours to a semi-continuous process yielding 245 l in 3 hours with 0.5 to 0.6 % sulfuric acid at 150 - 180°C, these figures being based on laboratory-scale pilot-plant studies. Yields of alcohols, raw-materials requirement and by-product output would be approximately as follow :

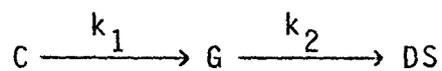
|   |          |
|---|----------|
| - daily production of alcohol (95 %)      | 43.500 l |
| - saw-mill residues (dry-wood, bark free) | 200 T    |
| - lignin residue (dry basis)              | 60 T     |
| - calcium sulfate dihydrate (gypsum)      | 18 T     |
| - still-bottom liquors                    | 1.450 T  |

In addition, furfural, methanol, pentose sugars (10 T/d), carbon dioxide (40 T/d) and small amounts of acetic acid could be recovered. A plant was constructed at Springfield, Oregon and went on stream in 1947. However, operations disclosed engineering problems in operational difficulties as well as the need for minor changes and operating techniques. These could not be predicted in designing a full-scale plant on basis of laboratory scale pilot plant studies. Due to conditions at this time, these changes were not made, but trial percolations demonstrated rather conclusively that the plant can be made to function.

A modification of the Madison process has been introduced to produce sugar solutions of high concentration (molasses) for feed. It is known as the TVA (Tennessee Valley Authority) process and is also no more in use.

#### 2.3.3.3.3. Kinetic of the reaction

Studies on the kinetics of the hydrolysis reaction point out to possibilities for drastic improvement in the economics of the process. It has been shown that, with dilute sulfuric acid, saccharification is proceeding in two steps :



where C = cellulose

G = glucose

DS = decomposed glucose

$k_1$  = reaction rate constant, cellulose to glucose hydrolysis

$k_2$  = reaction rate constant, glucose decomposition.

As the activation energy for the hydrolysis step is greater than the activation energy for the decomposition step, increasing the reaction temperature increase the rate of the first step over that of the second.

Thus, for any given hydrolysis conditions (acid concentration and temperature), there is an optimum reaction time for maximum sugar yield after which the temperature must be sharply reduced to quench the reaction and stabilize the yield. High temperature and low acid concentration are recommended and Porteous has found that 0.4 %  $H_2SO_4$  and  $230^\circ C$  are the appropriate upper limits for controllable reaction. The optimum residence time for maximum yield in a continuous reactor is then 1.2 minutes with a 55 % conversion to fermentable sugars.

Porteous has proposed a scheme to process the cellulosic fraction of urban solid wastes in this way. The plant economics appear extremely interesting and, although the materials are said to have been costed on the excess side, the fermentable sugar cost is estimated, at 40 tp, \$ 55 / ton as compared with a free marked price of sugar solutions over \$ 100 / ton (1974) this being without credit to the plant for disposal of refuse but without cost of transport of urban solid wastes. One very important fact is certainly the small size of the reactor, due to the very short reaction time.

2.3.3.4. Utilisation of residual products from hydrolysis :  
Lignin utilisation

2.3.3.4.1. Availability

In hydrolysis of lignocellulosic materials, lignin remains as a residue as it does in the pulping of wood. In the pulping of wood, lignin is either burnt to recover the chemicals used for the pulping and the heat from the dissolved organics (kraft and soda processes) or discharged (sulfite processes); in this last case, lignin is sometimes recuperated (vanillin, liginosulfonates for various uses but never in high amount). Sulfite lignin (lignosulfonates) is responsible for nearly the whole of lignin disposal. Hydrolysis lignin is found only in Eastern Europe ( $\pm$  500.000 T / year) since wood hydrolysis is now only found in these countries. It is mainly burnt. The same considerations apply to lignin from enzymic hydrolysis, which is likely to become of importance.

2.3.3.4.2. Actual uses

Hydrolysis lignin can be used as a large polar thermoplastic molecule as are kraft and soda lignins or it can be degraded in monomers such as phenols.

Uses of the large molecule are as components of resins as filler or dispersing agent in rubber or as a binder resins by itself. They could be used for the production of active carbon, although there is no proof that the products have any special properties. These markets are in specialty fields and it seems difficult to give them an important extension.

### 2.3.3.4.3. Production of chemicals

Several chemical or thermo-chemical treatments can be applied to hydrolysis lignin in view of producing simple chemicals.

Pyrolysis : it has been practised in the form of wood distillation since antiquity. The components obtained include acetic acid, acetone, catechol, phenols and phenol-ethers, hydrocarbons etc... but the main product is charcoal. The weak point is that there is no market for this charcoal. Pyrolysis in solution gives a conversion of about 50 % in ether-soluble products of which homoprotocatechuic acid represents 5 % yield on total organic matter.

Gaseification : it does not offer much advantage over simple burning and the scale would be too small for considering an ammonia or methanol synthesis plant.

Pyrolytic hydrogenolysis : the technical process developed for high pressure hydrogenolysis of coal was applied to lignin. Due to its higher reactivity and molecular homogeneity, lignin would seem to offer higher throughput and more valuable products (phenols rather than hydrocarbons) although a closer control of the degradation is necessary. The only process to approach commercial viability is that of Noguchi, developed by Crown-Zellerbach. The product contained 21 % of monophenols (of which 60 % phenol and 20 % of cresols), 5 % of light hydrocarbons and only 9 % of alcohol.

Hydrolytic hydrogenolysis : hydrogenolysis in the presence of alkali offers advantages but it has not been developed. Satisfactory yields of catechols

and monophenols are obtainable and the process is economically attractive because a substantial fraction of the hydrogen requirement may be obtained from alcohols of little value formed in the reaction.

Hydrolysis : simple hydrolysis is not effective with hydrolysis lignin.

Alkali fusion : this can be used to give over 20 % yield of catechol or of photocatechuic acid, according to conditions.

Oxidation : vanillin is made commercially by alkaline oxidation of softwood lignosulfonate in 25 % yield. The yield with hydrolysis lignin would not be so high, and the market is narrow as no other uses as flavouring exists.

#### 2.3.4. Economic evaluation

##### 2.3.4.1. Economic evaluation of pretreatment of ligno-cellulosic materials

The economic possibility of using wood-based residues in animal feed is much linked with the price of the treatment necessary to increase digestibility and the price of substituted feed : hay, feed grains, etc... and on the transport cost of the wood-based residues. Indeed, the cost of pretreatment is important in any biological use of ligno-cellulosic residue.

Grinding is costly due to high energy expenses. Irradiation is reported at \$ 150 per ton and the chemical methods, such as pulping methods, cost over \$ 200 per ton. It seems appropriate to say that prices should be halved for wood feed to be competitive but this is very dependent upon the price of the substituted feed. Long-term prospects are considered to be good.

The costs of pretreatment before any other biological transformation are to be considered as a whole with the costs of that transformation. An evaluation is therefore difficult.

#### 3.4.2. Economic evaluation of chemical hydrolysis

Economic performance of the various processes of chemical hydrolysis is dependent upon the final product form and the necessity of more or less acid-resistant materials.

In the processes using concentrated acid, the major portion of the plant must be acid resistant and estimates of investment cost indicate that such plants would require an investment per annual ton of product similar to that of expensive chemical plants. Plants using dilute sulfuric acid require much less corrosion-resistant equipment and its cost are about one-half of that required for the strong acid processes.

Plant heat requirements depend largely on the end-use of the hydrolyzate : for crystalline glucose and molasses, the heat load is high. Also, heat is required to recover strong acids. Methods for increasing the sugar concentration in the hydrolyzates of the dilute acid process have been employed which give an economic advantage to the dilute-acid processes. If the sugar is to be used in solution for the production of yeast, alcohol, or any product easily separable from dilute solution, the heat load is greatly reduced, giving a decided advantage to the dilute-acid over the strong-acid processes.

Chemical cost for the dilute-acid processes is small but it is significant for the strong-acid processes

Considering the high costs of handling the raw material, of investment, of chemicals and of the heating load, it is evident that these should be reduced in all available ways.

Reducing the investment seems possible for dilute-sulfuric acid processes in the way opened up by Porteous : its results show the possibility to use a much smaller reactor (100 to 200 times smaller) due to the short reaction time (1 to 2 minutes) as compared with the reaction-time in the Madison process (3 hours). High temperature and associated pressures could be coped with the actual knowledge in chemical engineering.

Reduction in cost could also be attained through use of the residue from saccharification, that is the pentosan fraction and the lignin.

#### 2.3.4.3. Economic evaluation of lignin utilization

##### Production of chemicals from hydrolysis lignin

Hydrolysis lignin is used mainly as a fuel, which gives it a defined value. Other uses represent a small amount as large thermoplastic molecule, with no signs of expansion. As a chemical resource, lignin should take its place alongside coal and petroleum by being exploited for its characteristic chemical products. Unfortunately, these tend to be somewhat outside existing markets and considerable development would be necessary to launch guajacol, protocatechuic acid or vanillic acid : this does not seem reasonable. Preference should thus be given to reactions giving clear yields of commodity chemicals, such as phenols, cresols, benzene. There is a large market for them.

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## 2.4. Protein recovery

It seemed interesting to the contractor to oppose to more standard processes, the concept of upgrading. By upgrading are meant processes by which an organic residue is essentially submitted to an aerobic conversion (with eventual physico-chemical pre- and post-treatments), in order to transform it partially to single-cell protein, the mixture of residual raw material, not or slightly modified, with the produced biomass being used as animal feed. This concept will be discussed according to the draft Table of content adopted at the meeting in Dublin.

The organisms used for this biological conversion must have a number of properties : (a) capability to grow on a wide range of carbon sources ; (b) have a high growth rate to minimize the size of the fermentation system ; (c) have a high efficiency to convert the substrate into biomass (Tate and Lyle, 1975). So, for the two last points, the upgrading processes of the organic residues must be done under aerobic condition, since the anaerobic biological conversion consumes several times more carbohydrate to gain energy.

### 2.4.1. a) Raw materials

Although in principle, any organic residue is suitable for upgrading, the selection of suitable organic wastes must be done according to a rule, by which the producer of the organic residue and the consumer of the upgraded product are either the same person or persons well known to each other and organized in some sort of cooperative. The reason for this lies in the cost of a quality label and will be further discussed below under 2.4.2. and 2.4.4.

For these reasons, the contractor believes that upgrading processes, can only deal with animal residues and organic residues from food industries, and will deal with other organic residues only in particularly favourable cases (Tomlinson, 1975 ; Pope, 1975). A survey on utilization of utilization of residues in animal feeds was recently done by Blair (1975).

#### 2.4.1. b) End products

Untill now (Ifeadi and Brown, 1975 ; Yen, 1974), when aerobic bioconversion was used for the treatment of organic residues, only two extreme cases have been considered :

(a) a very rough treatment to yield a product of low quality : e.g. the aerobic bioconversion of poultry residues to fertilizer (Ostrander, 1975) or the aerobic bioconversion of cow dairy waste, in oxidation ditches, also to fertilizer (Dab et al., 1975) ;

(b) a sophisticated treatment to yield single-cell protein of high grade purity. A comparison of chemical composition and nutritional value of single-cell protein obtained from various organic residues as well as from pure raw materials was recently published by Schulz (1975).

Semi-solid organic residues, such as animal residues or food industry residues deserve a better lot than a rough treatment to make low quality products, but are not very suitable to make high grade single-cell protein. One sufficient reason would be the complicate task, either to have a 100 % bioconversion or to remove unconverted solid residues from the produced single-cells. Another sufficient reason is to gain an end product with a quality label, from a hazardous organic residues. There are a few brilliant exceptions where the organic residues were of good reproducible quality and mainly soluble : molasses (Lefrançois, 1964 a, 1968) and spent sulfite liquor from paper mills (Ingman, 1976).

Therefore, a partial bioconversion would be most suitable for semi-solid organic residues if the produced product had such definite advantage over the starting raw material that bioconversion costs are covered. These advantages are listed below :

- (a) increased content in protein,
- (b) better balance of amino-acid, i.e. increased net protein utilization factor,
- (c) better storage of capacity, i.e. removal of easily fermentescible compounds,
- (d) increased dry matter content, e.g. because bioconversion also resulted in the

- conversion of soluble organic material into insoluble biomass,
- (e) increased digestibility of the protein moiety of the mixture,
  - (f) increased digestibility of the polysaccharide moiety of the mixture (bio-conversion of cellulose into starch),
  - (g) increased physical handling, i.e. easier removal of insoluble organic matter by mere decantation,
  - (h) better balance of polysaccharide to lipid to protein ratio,
  - (i) reduced content in metal contaminants (e.g. Cu (II)),
  - (j) reduction in nucleic acid content.

#### 2.4.2. Commercial processes : possible technologies

In the high grade single-cell protein market, the producer of the raw material and the consumer of the end product are usually unrelated. This requires that the end product carries a label of constant quality. The obtention of a label of constant and high-grade quality usually requires rather a sophisticated technology, which in turn can only be organized in large-size industries with a well trained specialized staff. This is still enhanced by the fact that the raw material is an organic residue of the worst quality for its producer not directly interested in its fate or in its recycling.

As will be discussed under 2.4.4., the economics of these large-size industries look at present unfavourable. Furthermore single-cell protein production from animal residues does not lend itself easily to a process leading to a constant quality high-grade end product.

Therefore, the upgrading processes should be restricted to small-size agricultural industries. E.g. medium-size farms of say 2000 capita could recycle their animal residues after upgrading within themselves. Health hazards could be taken in charge by the veterinarian usually attached to such medium-size agricultural exploitations.

If these processes are to be handled in small size agricultural industries, they should involve as simple as possible technologies. Particular attention should be given to air-lift fermentors, since long known and used, and patented by an European Company (Lefrançois, 1964 b, 1968). In these air-lift fermentors, oxygen injection provides adequate agitation and oxygen transfer is maximal (hence, energy requirements minimal). A tower fermentor has been proposed for waste process water upgrading by mould growth, by Imrée and Greenshield (1973).

It should be recalled that organic residues such as animal or food industry residues have already been utilized as animal feed (see also 2.4.1.) but after some kind of crude physico-chemical upgrading, mainly grinding and mechanical fractionation (Cereco Process : Ward et al., 1975, and Ward and Seckler, 1975).

Moreover, an aerobic upgrading bioconversion could successfully be completed by an appropriate silage, an anaerobic lactic slow fermentation, e.g. with corn, which process would not only improve the nutritive value of the mixture, but could result in a benefic dilution or destruction of hazardous compounds (metal ions, antibiotics) or pathogens. Silage without upgrading has already be proposed by Fontenot et al. (1975) and by Vezey and Dobbins (1975).

#### 2.4.3. Status of R&D

No R&D program is known to the contractor within the Benelux.

One R&D program has been developed in the U.K. (Tate and Lyle, 1975), and includes besides the technological data, a tentative evaluation of the process.

One R&D programm has been developed in the U.S. (Animal Science Department, University of Minnesota, St. Paul) for upgrading of beef slurry into animal feed using oxidation ditches (Hegg et al., 1975).

#### 2.4.4. Economic evaluation

In 1975, the upgrading process production costs were evaluated at F.B. 7,000 x ton<sup>-1</sup> bioconverted dry product in a 500 ton x year<sup>-1</sup> plant and F.B. 13,000 x ton<sup>-1</sup>, in a

100 ton x year<sup>-1</sup> plant, when the raw material was solid agricultural waste (Tate and Lyle, 1975), which the authors claim to be within acceptable limits, compared to the 1975 U.K. prices of soya protein (F.B. 7,000 x ton<sup>-1</sup>).

It should be recalled that single-cell production costs in large size industries are very high due to :

- (a) a sophisticated technology due to the high grade finished product, namely in post treatments (see (2.4.2.) ,
- (b) the transport costs of organic wastes. Large size industries require expensive collection of organic wastes from a relatively large geographical area ,
- (c) the fact that the marketable end product should be of constant high quality (see 2.4.1 b).

These three factors of production costs increase are not to be taken into consideration for small size upgrading production units.

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### 3. COMPETITIVE PROCESSES

It seemed interesting to the contractor to oppose to the developments made by the pilot country and the other co-pilots, a detailed review of the possible uses of hydrocarbon and simply related compounds for protein or metabolite recovery. According to the draft Table of content, adopted at the meeting in Dublin, this review will be classified as 3.1. and subdivided into :

- 3.1.1. 1) Raw materials.  
2) End products
- 3.1.2. Commercial processes
  - 1) Possible technologies
  - 2) Existing processes
- 3.1.3. Status of R&D
- 3.1.4. Economic evaluation

Preliminary research proposals will be classified under 4.4. Protein recovery.

#### 3.1. Possible uses of hydrocarbon and simply related compounds for protein and metabolite recovery

##### 3.1.0. Introduction

The present world gross potential protein market, resulting from the gap between availabilities on the one hand, and human nutritional requirements and/or human demand on the other hand, can be evaluated at  $14 \times 10^6$  tons N.P.U. 100<sup>(1)</sup> protein per year (MICHOLT et al., 1973). Projections for 1975 and 1985 show the constancy or even a slight increase in this market.

What are the chances of hydrocarbon-grown S.C.P. to compete successfully on the protein market for animal and/or human consumption ?

Yeast are known to assimilate the *n*-paraffins from crude oil since 1939 (TAUSSON, 1939). But it took until 1963 before CHAMPAGNAT realized the potential industrial importance of this phenomenon (CHAMPAGNAT et al., 1963). There are two main steps from the raw material to marketable end products : the production of a protein concentrate by a petroleum fermentation industry and its manufacture into a marketable end product by a food industry. This is well exemplified in the collaboration between Esso and Nestlé (AN., 1967 ; KIHBERG, 1972). As well mastered and appropriate technology should lead to a product of good nutritive value, acceptable by the customer and competitive in price.

A well mastered and appropriate technology requires cheap sources of hydrocarbons in sufficiently large amounts, actively growing microorganism strains, low investments and maintenance costs, and low operation and production cost.

### 3.1.1. 1. Raw materials

Various raw materials have been proposed as carbon substrates for hydrocarbon fermentation : *n*-paraffins or gasoil (SHACKLADY, 1967), methane (HAMER et al., 1967 ; NORRIS, 1968 ; BELOT and BARAT, 1969 ; KLASS et al., 1969 ; BEWERSDORFF and DOSTALEK, 1971), or paraffinic hydrocarbon-rich crude oils (IYENGAR et al., 1967). More recently methanol, produced by the catalytic oxi-

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(1) Abbreviations : N.P.U. 100 : net protein utilization 100 ; S.C.P. : single-cell protein ; F.A.O. : United Nations Food Organization ; F.A.O. : United Nations Food and Agricultural Organization ; W.H.O. : World Health Organization ; U.N.I.C.E.F. : United Nations International Children Fund.

dation of methane or by a new unexpensive low pressure process (BOLTON, 1969) was proposed as a new substrate for yeast, though it was since long known as a classical substrate for bacteria (ASTHANA et al., 1971 ; STIEGLITZ and MATELES, 1973 ; HÄGGSTRÖM, 1969).

The potential of the *n*-paraffins and gasoil substrates, for which a mature industrial yeast fermentation technology exists, is illustrated by the fact that the diversion of the *n*-paraffinic fraction of a mere 15-20 % of the world production of crude oil, could meet the entire protein requirements of the world inhabitants (JOHNSON, 1967). Crude oil contains up to 10 % *n*-paraffins. The economic yield, i.e. the weight of protein obtained from 1 kg of *n*-paraffins is roughly 0.5 kg. Table 1 compares the oil production, the refining capacity and the gas production on the one hand, with the gross potential protein market on the other hand for the 23 countries for which the largest gross potential protein markets were calculated (MICHOLT et al., 1973). The major 16 out of the 23 countries possessed either their own oil fields or their own refining capacities, sufficient to provide the basic *n*-paraffins to satisfy their own protein demands by S.C.P. obtained through fermentation. A protein production of  $30 \times 10^3$  tons  $\times$  year<sup>-1</sup> could be obtained from a refinery with a modest capacity of  $2 \times 10^6$  tons  $\times$  year<sup>-1</sup> (CHAMPAGNAT, 1966). According to DJAVANMARD and GATELLIER (1969), the optimum plant size lies between  $40$  and  $80 \times 10^3$  tons of protein  $\times$  year<sup>-1</sup>, i.e.  $60$ - $120 \times 10^3$  tons of biomass.

Recent information points to the following facts.

Gas-oil or paraffinic hydrocarbon rich crude oils seem to be less desirable as raw material for single-cell protein production because :

a. the costs of post-treatment (removal of adsorbed gas-oil after bioconversion),

TABLE 1.  
Localization of crude oil wells, refineries, and natural gas sources in countries  
with a gross potential protein market exceeding 30,000 tons × year<sup>-1</sup>.

| Country                   | Oil production (1)<br>(10 <sup>6</sup> l × year <sup>-1</sup> ) (4) | Refining capacity (2) | Gas production (1)<br>(billions cu ft × year <sup>-1</sup> ) | Gross potential protein market (3)<br>(1000 tons × year <sup>-1</sup> ) |
|---------------------------|---|-----------------------|--|---|
| India . . . . .           | 6,565   | 21,261                | 61.6   | 2,729   |
| China (Pekin) . . . . .   | 22,134  | (6)                   | 140.1  | 2,443   |
| Indonesia . . . . .       | 46,087  | 18,435                | 137.4  | 681   |
| Pakistan . . . . .        | 391   | 3,500                 | 129.0  | 531   |
| Thailand . . . . .        | 1   | 6,774                 | —  | 188   |
| Burma . . . . .           | 826   | 1,100                 | 3.6  | 171   |
| Nigeria . . . . .         | 79,000  | 2,609                 | 32.4   | 160   |
| Zaire . . . . .           | (5)   | 702                   | —  | 139   |
| Philippines . . . . .     | —   | 12,013                | —  | 129   |
| Iran . . . . .            | 218,304   | 26,957                | 1,429.7  | 99  |
| Vietnam (North) . . . . . | (6)   | (6)                   | —  | 85  |
| Vietnam (South) . . . . . | —   | —                     | —  | 84  |
| Ceylon . . . . .          | —   | 1,626                 | —  | 65  |
| Colombia . . . . .        | 8,609   | 7,843                 | 117.6  | 60  |
| Malaysia . . . . .        | 12,696  | 2,748                 | (7)  | 45  |
| Algeria . . . . .         | 46,435  | 2,641                 | 107.8  | 44  |
| Mozambique . . . . .      | —   | 739                   | (5)  | 43  |
| Afghanistan . . . . .     | 1   | —                     | 94.1   | 43  |
| Nepal . . . . .           | —   | —                     | —  | 40  |
| Ghana . . . . .           | (5)   | 1,157                 | —  | 39  |
| Angola . . . . .          | 5,821   | 696                   | 38.4   | 38  |
| Morocco . . . . .         | 1   | 2,546                 | 319.7  | 33  |
| Peru . . . . .            | 2,957   | 4,412                 | 1.1  | 32  |

- (1) Figures for 1972, (AN., 1973b).
- (2) Figures for 1972, (AN., 1972e).
- (3) 1969 figures.
- (4) 10<sup>6</sup> l × year<sup>-1</sup> = 23 barrels × day<sup>-1</sup>; 2.0-2.6 × 10<sup>6</sup> l will yield 1000 tons S.C.P.
- (5) Offshore wells, not yet on production.
- (6) Existing, quantity unknown.
- (7) Reserves estimated at 7,500 billions cu ft (AN., 1972E).

b. the previously unsuspected treatment costs of the biologically deparaffinized gas-oil to transform it into domestic fuel. During the bioconversion process, the gas-oil dissolves some by-products of the fermentation, namely fatty acids which are deliterious, e.g. by their emulsifying properties.

Methane also seems to be less desirable as raw material for single-cell protein production because of :

a. the hazards of the fermentation process, due to the explosive character of methane air mixture,

TABLE 1.  
Localization of crude oil wells, refineries, and natural gas sources in countries  
with a gross potential protein market exceeding 30,000 tons  $\times$  year<sup>-1</sup>.

| Country                   | Oil production (1)<br>(10 <sup>6</sup> l $\times$ year <sup>-1</sup> ) (4) | Refining capacity (2)<br>(4) | Gas production (1)<br>(billions cu ft $\times$ year <sup>-1</sup> ) | Gross potential protein market (3)<br>(1000 tons $\times$ year <sup>-1</sup> ) |
|---------------------------|--|------------------------------|---|--|
| India . . . . .           | 6,565  | 21,261                       | 61.6  | 2,729  |
| China (Pekin) . . . . .   | 22,134   | (6)                          | 140.1   | 2,443  |
| Indonesia . . . . .       | 46,087   | 18,435                       | 137.4   | 681  |
| Pakistan . . . . .        | 391  | 3,500                        | 129.0   | 531  |
| Thailand . . . . .        | 1  | 6,774                        | —   | 188  |
| Burma . . . . .           | 826  | 1,100                        | 3.6   | 171  |
| Nigeria . . . . .         | 79,000   | 2,609                        | 32.4  | 160  |
| Zaire . . . . .           | (5)  | 702                          | —   | 139  |
| Philippines . . . . .     | —  | 12,013                       | —   | 129  |
| Iran . . . . .            | 218,304  | 26,957                       | 1,429.7   | 99   |
| Vietnam (North) . . . . . | (6)  | (6)                          | —   | 85   |
| Vietnam (South) . . . . . | —  | —                            | —   | 84   |
| Ceylon . . . . .          | —  | 1,626                        | —   | 65   |
| Colombia . . . . .        | 8,609  | 7,843                        | 117.6   | 60   |
| Malaysia . . . . .        | 12,696   | 2,748                        | (7)   | 45   |
| Algeria . . . . .         | 46,435   | 2,641                        | 107.8   | 44   |
| Mozambique . . . . .      | —  | 739                          | (5)   | 43   |
| Afghanistan . . . . .     | 1  | —                            | 94.1  | 43   |
| Nepal . . . . .           | —  | —                            | —   | 40   |
| Ghana . . . . .           | (5)  | 1,157                        | —   | 39   |
| Angola . . . . .          | 5,821  | 696                          | 38.4  | 38   |
| Morocco . . . . .         | 1  | 2,546                        | 319.7   | 33   |
| Peru . . . . .            | 2,957  | 4,412                        | 1.1   | 32   |

- (1) Figures for 1972, (AN., 1973b).
- (2) Figures for 1972, (AN., 1972e).
- (3) 1969 figures.
- (4) 10<sup>6</sup> l  $\times$  year<sup>-1</sup> = 23 barrels  $\times$  day<sup>-1</sup>; 2.0-2.6  $\times$  10<sup>6</sup> l will yield 1000 tons S.C.P.
- (5) Offshore wells, not yet on production.
- (6) Existing, quantity unknown.
- (7) Reserves estimated at 7,500 billions cu ft (AN., 1972f).

b. the previously unsuspected treatment costs of the biologically deparaffinized gas-oil to transform it into domestic fuel. During the bioconversion process, the gas-oil dissolves some by-products of the fermentation, namely fatty acids which are deliterious, e.g. by their emulsifying properties.

Methane also seems to be less desirable as raw material for single-cell protein production because of :

a. the hazards of the fermentation process, due to the explosive character of methane air mixture,

- b. the fact that chemical oxidation of methane to methanol seems to be cheaper than the biological oxidation of methane to methanol,
- c. the easier transportation of methanol vs. methane. A possibility exists that the presently burned methane on the oil fields, could be chemically transformed to methanol and, as such, transported to any eventual customer.

Pure n-paraffins seem more promising because :

- a. the post treatment (removal of adsorbed n-paraffins after bioconversion) is cheaper than is the case with gas-oil, and, furthermore, in cases of animal feed, could eventually be suppressed
- b. the large scale production processes (molecular sieves) of n-paraffins from crude oils or gas-oils, seem now well at hand and the production costs of n-paraffins, excluding cost of raw materials, reasonably low. A market price fluctuation may however arise from competition with other potential uses of n-paraffins, namely biodegradable surfactants obtained by oxidation and sulfonation of n-paraffins.

The most promising raw material, presently, seems to be methanol because :

- a. no peculiar post-treatment for the removal of adsorbed raw material seems necessary.
- b. the economics of the raw material, both as compared to other hydrocarbon raw materials (see below) and as compared to more classical raw materials (to be discussed elsewhere under point 3.1.5.)

### 3.1.1. 2. End products

#### 3.1.1 2.1. Microbial single-cell protein

Various microorganisms have been proposed for hydrocarbon fermentation ; bacteria such as *Micrococcus* (GUENTHER and PERKINS, 1968), actinomycetes such as *Nocardia* (RAYMOND and DAVIS, 1960), yeast such as *Torula* (CHEPIGO, 1969) or

*Candida* CHAMPAGANT, 1963) or fungi such as *Trichosporon* (UENO et al., 1970). The choice criteria included a high protein content (50-80 %) of appropriate biological value, a low maintenance coefficient<sup>(1)</sup> and a fast growth rate (e.g. a mean generation time of 0.4-4.0 hrs), as a result of which aseptic fermentation conditions were sufficient and sterility could be avoided. This high generation rate, a thousand times faster than of cattle, allowed the artificial induction of rapid genetic changes and thus offered a high selection potential of strains containing "tailor-made" proteins, better adapted to general or local needs. Analogous results could be obtained by influencing growth conditions, e.g. an increase in methionine content of 45 % after changing the nitrogen source, and an increase in lysine of 95 % after changing the pH of the growth medium (ERTOLA et al., 1971).

A question which arises is whether bacteria, yeast or fungi should be used in the production of single-cell protein from hydrocarbon conversion.

Bacteria contain the highest protein concentrations (up to 65 %) but are more difficult to recover after fermentation. When a few years ago, methane was thought to be a possible good raw material, bacteria had to be used because no yeast or fungi would decently grow in on methane. Due to the particular nature of this raw material, the recovery of methane from bacteria was facilitated. Bacteria are presently no longer favoured.

Fungi have often and on been proposed by industries at international meetings. But the contractor has never had the feeling that fungi were promising, or offered peculiar advantages.

.....

(1) The maintenance coefficient measures the amount of substrate used up during fermentation for other reasons than growth.

Yeasts are presently greatly favoured, because :

- a. socio-psychological reasons. Mankind seems more prepared to accept yeasts, some species of which have been used in feed and food since the origin of mankind, than bacteria or fungi ;
- b. yeast strains were found in the last 10 years which grew easily and well on methanol, long thought to be a general biological poison and also when present in large amounts ( $\sim 1\%$ ) in culture media.

### 3.1.1. 2.2. Single-cell protein or Secondary metabolites

A first question is whether hydrocarbon fermentation should result in the production of single-cell protein or in the production of secondary metabolites. Quite curiously, from various sources, the contractor has heard more opinions in favour of secondary metabolites, i.e. from chemicals, than single-cell protein. This is clearly illustrated by the example of Japan, where attempts were made to replace the more conventional carbohydrate sources of raw materials by hydrocarbons in the biological production of various chemicals, namely amino-acids, vitamins, nucleic acids, organic acids, such as citric acid, etc ... The example of Japan cannot be, as such, applied to the E.C. as in the E.C., at least some conventional carbohydrate sources are available.

### 3.1.1. 2.3. Food or Feed

A second question is whether single cell protein should be used as feed or as food.

Recently animal feed protein came in tight supply, largely as a consequence of low fish protein concentrate production in Peru, i.e.  $1.2 \times 10^6$  tons in 1972, against  $1.8 \times 10^6$  tons in 1971 and  $2.3 \times 10^6$  tons in 1970 (AN., 1972 a, b, c, d ; AN., 1973 a). The hot "el Nino" current drastically reduced the ancho-

veta fishing stocks. According to some experts, no return of these stocks to their original level is to be expected in a foreseeable future (HOLT, 1973).

Adding to this first factor of international protein feed shortage, is a second factor arising from the sudden massive exportation of U.S. soy proteins to the S.S.S.R. and mainland China (CARTIER, 1973). A third factor lies in the increasing demand for meat, arising from higher living standards in Japan, Europe, the U.S. and the S.S.S.R. (F.A.O., 1970 b), in the last two decades.

The Western European demand for protein feed amounted roughly to  $7 \times 10^6$  tons during 1970 (AN., 1971 b). The average yearly increase in production of formula feed in France was 12.7 % between 1960 and 1966 (DJAVANMARD and GATELLIER, 1969). In Japan, the demand for formula feed is expected to rise from  $17 \times 10^6$  tons in 1972 to  $28 \times 10^6$  tons in 1978, which implicates a demand for S.C.P. of  $1.4 \times 10^6$  tons in 1978 (HOSHIAI, 1972). Hence, the animal feed protein market is currently the first aim of S.C.P. (AN., 1971 a).

As far as the human food protein market is concerned, until now only a soy sauce called Petein has been produced on an experimental scale in Taiwan (HUMPHREY, 1969). Recently, the F.A.O. officially stated that "in fact, there is no such thing like a protein problem" (SUKHATME, 1973). As a matter of fact, there is a convincing evidence from dietary surveys in India that more people are below calorie requirements than below protein requirements : practically all protein deficient diets are also calorie deficient. In such circumstances, the first thing to do is to meet the calorie requirements to prevent the organism from burning dietary proteins solely for energy. Further, in the average Indian diet, consisting of rice and vegetables, proteins account for more than 5 % of the total calories, so that a sufficient bulk of food will automatically provide a sufficient quantity of proteins.

However, one should not overlook the tendency for Indian farmers to replace the culture of vegetables by the commercially more rewarding high yield rice. One should not overlook either the fact that the Indian data may only be extrapolated to other countries with similar average diets. It would be improper to extrapolate these data e.g. to countries where cassava is the staple food.

Therefore, a new increase in staple food production will not suppress the gross potential protein market. In MICHOLT et al.'s paper (1973), the substantial increases in staple food availabilities expected by the Indicative World Plan (F.A.O., 1969 a and b), were taken into account for the estimation of the gross potential protein market projections for 1975 and 1985, and resulted in a positive increasing gap between supply and demand in human protein food.

Quite clearly, the original idea of the Petroleum Companies, intensively involved in these R&D projects, was to produce food quality products. The contractor is now under the impression that the tendency has reversed and the the output of feed should be first if not the main goal, followed eventually by the output as food.

#### 3.1.1. 2.4. A remarkable breakthrough : complementation

Hydrocarbon-grown dried yeast is of limited nutritional value as such. The observed biological value of its protein was usually rather low : around 64, the two limiting amino acids being methionine and cysteine (CHAMPAGNAT, 1969 ; NARAYANASWAMY et al., 1971). The same appeared for a hydrocarbon-grown *Pseudomonas* (KO and YU, 1968) and for the unclassified methane-grown microorganism IGT 10 (KLASS et al., 1969). Disruption of the cell wall by heating increased the digestibility of the yeast, rising the absorbed nitrogen from 52 to 94 %. Excessive heating, however, affected adversely the digestibility by enhancing Maillard's reaction between sugar moieties and protein free-amino groups (ADRIAN and FRANGNE,

TABLE 2 : Protein content of the most common starchy food and grains and of their mixtures with 10 % hydrocarbon-grown dried yeast.

|                                  | Protein content<br>(g protein x g <sup>-1</sup> dry matter) |  |
|----------------------------------|---|--|
|                                  | Commodity alone (1)   | Mixture of commodity and 10 % (final concentration) of hydrocarbon-grown dried yeast (2) |
| STARCHY FOODS                    |   |  |
| Cassava flour . . . . .          | 1.5   | 7.4  |
| Plantain . . . . .               | 1.0   | 7.0  |
| Potato . . . . .                 | 2.0   | 8.4  |
| Sweet potato . . . . .           | 1.8   | 8.2  |
| Yam . . . . .                    | 2.4   | 8.8  |
| GRAINS                           |   |  |
| Barley (pearled light or dark) . | 9.2   | 14.9   |
| Maize (whole) . . . . .          | 9.2   | 14.9   |
| Millet (ragi) . . . . .          | 6.2   | 12.2   |
| Oats . . . . .                   | 14.2  | 19.4   |
| Rice . . . . .                   | 7.6   | 13.4   |
| Rye . . . . .                    | 12.1  | 17.5   |
| Sorghum . . . . .                | 10.1  | 15.7   |
| Wheat flour (whole) . . . . .    | 10.3  | 18.6   |
| Wheat flour (white) . . . . .    | 10.5  | 16.1   |

(1) F.A.O. (1964).

(2) With a protein content of 66 % in g protein x g<sup>-1</sup> dry yeast.

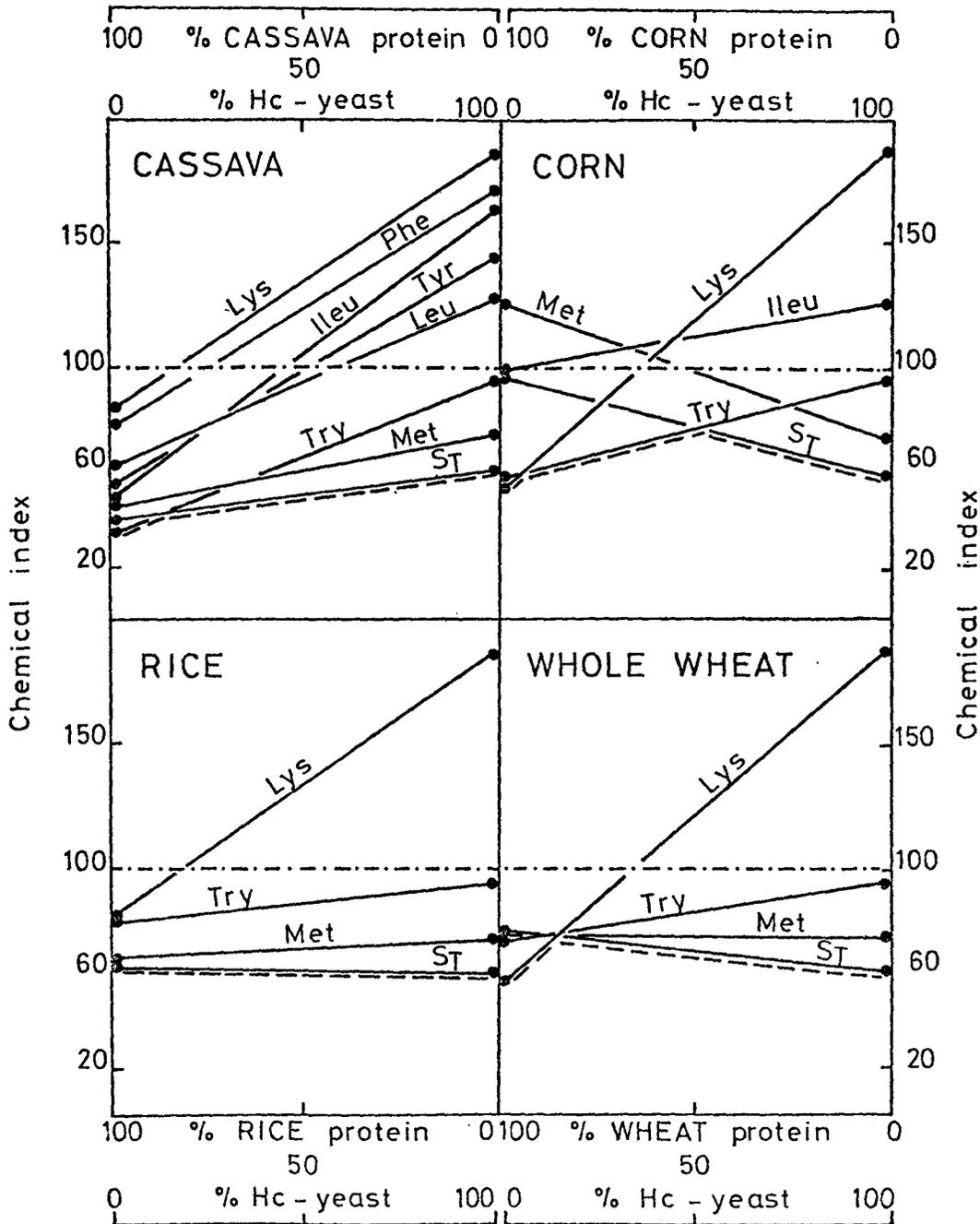


Fig. 1. - Influence of the progressive addition of S.C.P. from hydrocarbon-grown yeast on the chemical index of some staple food proteins.

1970). Only *in vivo* digestibilities should be relied upon as large discrepancies between *in vitro* digestibilities were reported, e.g. experimentally observed digestibilities *in vitro* of 69 and 95 for a yeast with a true *in vivo* digestibility of 85 (BIROLAUD, 1971). Occasional digestive intolerance to yeast (VON LOESECKE, 1946) was attributed by BIROLAUD (1971) to the introduction of live cells into

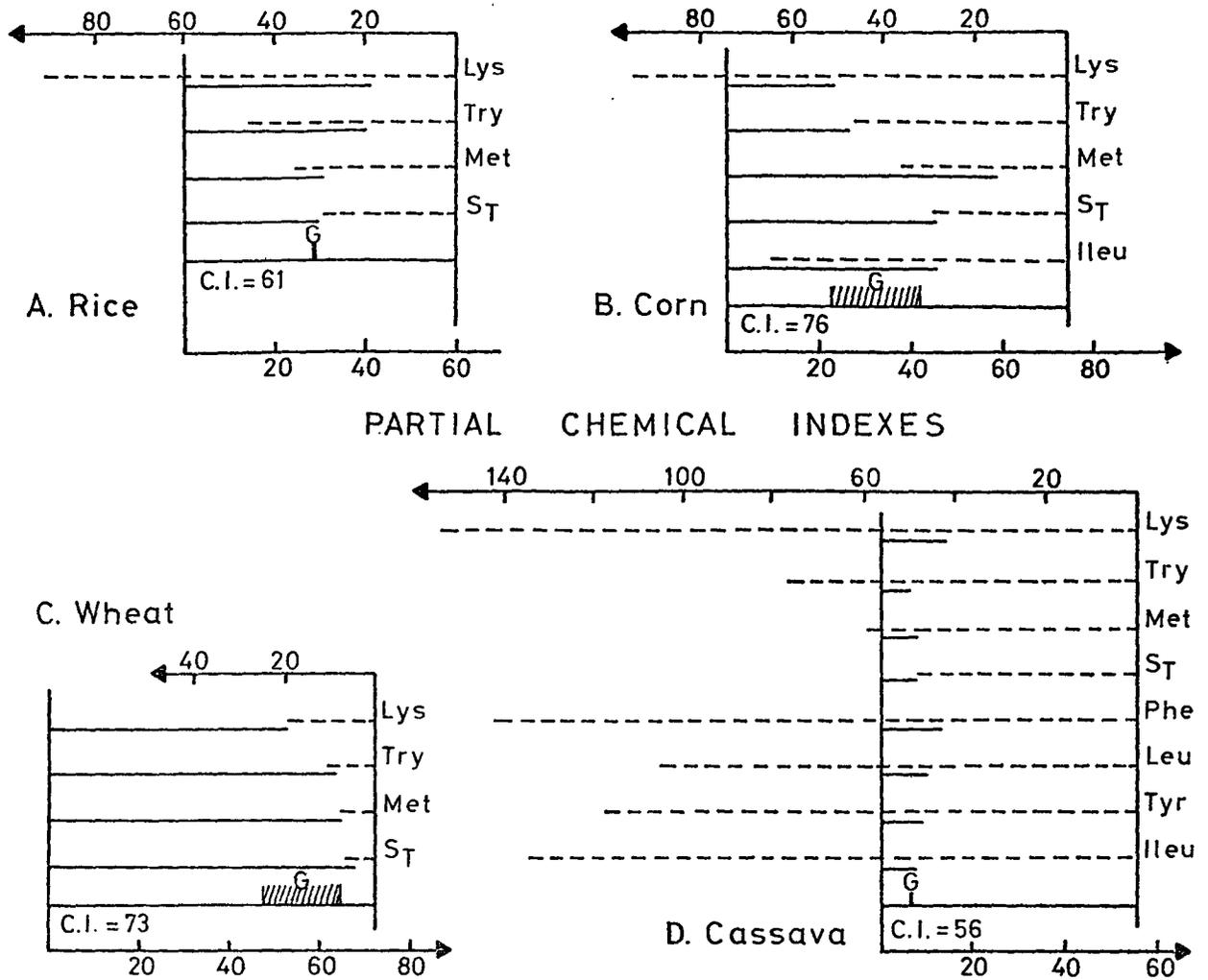


Fig. 2. - Increase in chemical index of optimum mixtures of hydrocarbon-grown yeast and staple foods.

the digestive tract. Even experimental avitaminosis could be induced in this way. No such troubles were reported after ingestion in physiological quantities of killed yeast. An impressive list of references of studies on the digestive tolerance and nutritive value of various S.C.P. materials, as feed or food, was reviewed by KIHLEBERG (1972).

A remarkable breakthrough of the potential nutritional uses of hydrocarbon-grown yeast appeared once they were looked upon not as a food or a feed *per se*, but as a supplement to classical protein sources.

First, because of their high protein content (50-70 %), a 10 % (wt x wt<sup>-1</sup>) addition of hydrocarbon-grown single cells resulted in a manifold increase in protein content of starchy foods and in a substantial increase in protein content of the most commonly used grains (table 2). A 10 % addition was the best compromise between a maximum protein content and minimum acceptability problems. In any event, in the case of government-sponsored compulsory staple food complementation, providing a quantity of N.P.U. 100 protein exceeding 5 % of the total calories, would be a waste of proteins.

Secondly, a close comparison of the amino acid composition of common staple food proteins and of hydrocarbon-grown yeast protein revealed their complementary character. Selected additions of hydrocarbon-grown yeast, not exceeding 10 % of total, often resulted in a better balanced amino acid composition of the mixture. 4 typical examples are given in fig. 1. Hydrocarbon-grown yeast protein and e.g. wheat protein have different limiting amino acids, respectively sulfur-containing amino acids and lysine. Complementation occurred when these proteins were ingested as a mixture, *i.e.* more nitrogen was assimilated by the organism from the mixture than from the proteins, each ingested separately. Maximum assimilation could be expected, *i.e.* complementation was optimum, when the chemical index of the binary mixtures, as depicted in fig. 1, reached a maximum.

Thus, in some case, a substantial gain in chemical index might be obtained and this is illustrated in fig. 2, where the complementation effect appeared positive in the case of corn or wheat supplemented with hydrocarbon-grown yeast but nought in the case of cassava or rice supplemented with hydrocarbon-grown yeast. In studies of wheat supplemented by yeast, the *in vivo* observed increase in biological value proved even superior to the chemical-based prediction (CREMER et al., 1951). Various studies on rats and dogs fed on yeast-supplemented diets confirmed this observation (BRESSANI, 1968).

### 3.1.1. 2.5. Nutritional and psycho-sociological aspects of hydrocarbon-grown single-cell protein

Because they were tasteless and odorless, a 10 % addition of hydrocarbon-grown yeasts to most commodities with the exception of some fruity or citrus flavoured products (GRIMM, 1968) did not alter their organoplastic properties nor did it modify their traditional culinary uses or the palatability of the prepared food, two most important conditions for acceptance (BACIGALUPO, 1968). As yeasts have been classified in the plant kingdom, their introduction as potential food would not affect the religious patterns in eating habits, another very important condition for acceptance (BLAIR, 1968). Hence hydrocarbon-grown yeast meets the basic requirements of sociological and psychological acceptance (McKENZIE, 1968).

In Asian countries, where autolyzates are used as protein sources, cheaper substitutes with identical taste could be manufactured from hydrocarbon-grown yeast lysates, obtained through autolysis, plasmolysis or hydrolysis. These methods for yeast lysis seem presently too expensive to permit the production of low cost foods. Cheaper enzymatic substitute processing or mechanical rupture of the cell wall, using a ball-mill, a freeze-press or ultrasonic waves, are being studied (TANNENBAUM, 1968 ; KIHLEBERG, 1972 ; HEDENSKOG and MOGREN, 1973).

The present knowledge of safety evaluation involves a careful evaluation of the eventual toxicological properties of the hydrocarbon-grown cells (OSER, 1968). It has already been shown that the extraction procedures lowered the content of residual carcinogenic hydrocarbons, such as benzopyrene, which showed a tendency to accumulate in the interspace between the cell wall and the cell membrane (MUNK, 1966), below the accepted value for common foods (LAINE, 1969). Extensive studies demonstrated the general harmlessness of hydrocarbon-grown yeasts (SCHACKLADY, 1969 ;

POKROVSKY, 1968 ; BARBER et al., 1971). Toxicological and nutritional testing of B.P.'s S.C.P., involved 40,000 rats during five years, and its use in formula feed received Government approval in France since 1971 (CHAMPAGNAT, 1971).

Yeast contain from 2.5 to 8 % nucleic acids (COOK, 1958 ; NESMEYANOV et al., 1971). The end product of purine catabolism in man is uric acid, since man and higher apes lack uricase which catalyses the oxidation of uric acid to the more soluble allantoin. EDOZIEN et al. (1970) and WASLIEN et al., (1970) demonstrated that the daily intake of S.C.P. nucleic acid should not exceed 2 g, which corresponds to 20-30 g dry biomass, since regular higher intakes caused serum and urine to contain an abnormally high amount of uric acid. Gout attacks were reported after a two weeks diet on 150 g dried algae x day<sup>-1</sup> (KONDRATIEV et al., 1966) and the formation of urate stones in the urinary tract could be expected in subjects with acid urines.

Among the processes for reducing the nucleic acid content of S.C.P., in view of its human consumption, a three-step heat treatment (MAUL et al., 1970), protein precipitation by heating at an alkaline pH, and NaCl extraction of nucleic acids (HEDENSKOG and BBINGHAUS, 1972) were claimed to be rather unexpensive processes. The extracted nucleic acids might be valorized, e.g. as flavour enhancers (NESMEYANOV et al., 1971).

Fatty acids of uneven number of carbon atoms were frequently encountered in microorganisms grown on *n*-paraffins (DUNLAP and PERRY, 1967). These fatty acids are removed in the extraction steps and should not cause any toxicological problem.

The major chance of success lies in the political decision to complement traditional protein-deficient staple foods, with S.C.P. on a national scale, to start with the promotion of the use of appropriate complemented mixtures as transitional or weaning food, for which there is the highest need (JELIFFE, 1968) or in public schools where their state-organized distribution is easy. To give

but one example, in traditionally cassava-eating countries, the consumption of bread by the urban population is rapidly increasing and so is the importation of wheat. Attractive loaves could be baked from cassava flour into which an appropriate quantity of S.C.P. had been incorporated. Not only did the nutritive value improve, but this improvement was made without importation of foreign wheat and thus did not impair the international balance of payments. Acceptability of these loaves was comparable to that of "modern bread", i.e. wheat supplemented with lysine, groundnuts or soybeans, vitamins and minerals. "Modern bread" is being purchased even by the poorest segment of the Indian population and a potential market of  $50 \times 10^6$  loaves  $\times$  year<sup>-1</sup> currently exists (AN., 1972 g).

### 3.1.2. Commercial processes

#### 3.1.2. 1. Possible technologies

The insolubility of *n*-paraffins is not a major problem in aqueous fermentation. Fatty acids are obligatory intermediates in hydrocarbon metabolism (STEWART et al., 1959) and tend to accumulate to a small extent in the fermentation medium (IERUSALIMSKY and SKRIABIN, 1966). These fatty acids promote and maintain a well-emulsified multiphase system throughout the fermentation medium.

A 60,000 tons cells  $\times$  year<sup>-1</sup> production plant using gasoil or *n*-paraffins required a total investment estimated in 1969 at  $10 \times 10^6$  U.S. dollars and a working capital estimated at  $1 \times 10^6$  U.S. dollars (DECERLE et al., 1969 ; P.A.G., 1970). This supposed fixed charges of 3.5 U.S. cents per kg of cells, i.e. 19 %  $\times$  year<sup>-1</sup> of investment + 9 % of working capital. A plant using methane as carbon source for S.C.P. production by fermentation, would be about twice as expensive (ELLWOOD and LAW, 1970).

Operating costs per kg cells produced are mainly the sum of the substrate

cost per kg of cells produced, the oxygen transfer cost per kg of cells produced, the heat removal cost per kg of cells produced (ABBOTT and CLAMEN, 1973), the mineral salts of the fermentation medium and other chemicals (BENNET et al., 1969), the cell harvesting and drying energy, and the operating labor costs (DJAVANMARD and GATELLIER, 1969).

The oxygen transfer cost is directly related to the power expenditure kg of oxygen transferred to the medium, and inversely related to the oxygen yield coefficient ( $Y_0 = \text{g cells produced} \times \text{g}^{-1} \text{ oxygen consumed}$ ). MATELES (1971) established an equation for calculating  $Y_0^{-1}$ . The oxygen demand for the conversion to cells of *n*-paraffin substrates was roughly three times greater than the oxygen demand for the conversion to cells of conventional carbohydrates, i.e.

$$Y_{0, n\text{-paraffins}} = 0.50 ; Y_{0, \text{carbohydrates}} = 1.47.$$

Similarly, the oxygen demand for the conversion to cells of methanol was 3.5 times greater ( $Y_{0, \text{CH}_3\text{OH}} = 0.44$ ), and 7 times greater for methane ( $Y_{0, \text{CH}_4} = 0.20$ ) (ABBOTT and CLAMEN, 1973). The power expenditure per kg of oxygen transferred depends upon the type of aeration used. It was definitely the lowest in the process of airlift fermentation:  $0.106 \text{ kWhr} \times \text{kg}^{-1} \text{O}_2$  at an  $\text{O}_2$  transfer rate of  $50 \text{ moles O}_2 \times 1^{-1} \times \text{hr}^{-1}$  (WANG et al., 1971).

The heat removal cost is inversely related to the heat yield coefficient ( $Y_{\text{kcal}} = \text{g cells produced} \times \text{kcal}^{-1} \text{ evolved}$ ). The latter could be computed from the difference in combustion heat of equivalent quantities of substrate and cells (GUENTHER, 1965). The heat removal cost ranged for 1.8 U.S. cents per kg of cells grown on glucose to 2.8 U.S. cents per kg of cells grown on *n*-paraffins, 3.8 U.S. cents per kg of cells grown on methanol and 7.4 U.S. cents per kg of cells grown on methane (ABBOTT and CLAMEN, 1973). If some way could be found to conserve this wasted energy and to couple it to the assimilative production of biomass, an increase in  $Y_S$  of up to 30 % could be achieved, besides the heat removal economy (BELL, 1972).

Correlation between  $Y_S$ ,  $Y_O$ ,  $Y_{kcal}$ ,  $\mu$  (the growth rate expressed in  $hr^{-1}$  or in % of  $\mu_{max}$ ) and  $m$ , the maintenance coefficient (as g substrate consumed  $\times g^{-1}$  cells  $\times hr^{-1}$ , when  $\mu = 0$ ), were published by ABBOTT and CLAMEN (1963). An economy of up to 14 U.S. cents  $\times kg^{-1}$  cells produced would be realized by growing a microorganism with a low maintenance coefficient, at a high growth rate ( $\mu > 50\%$  of  $\mu_{max}$ ). Improved technology, e.g. continuous multistage fermentation with recycling of used fermentation medium (HUMPHREY, 1968 ; MATELES, 1968), or perforated plate column fermentation (KITAL and YAMAGATA, 1970) resulted in reduced production costs, namely as a consequence of increased oxygen transfer, higher substrate yield and lower power requirements.

The cost of the mineral salts of the fermentation medium and of various chemicals was evaluated at 2-3 U.S. cents  $\times kg^{-1}$  cells produced, and the cost of total labor in a 60,000 tons cells  $\times year^{-1}$  production plant at 1 U.S. cent  $\times kg^{-1}$  cells produced (DECERLE et al., 1969).

Total production costs could be computed as early as in 1968 in the following way : 30 % of the production costs in B.P.'s yeast-on-gasoil fermentation process, were due to the mineral salts of the fermentation medium (LAINE and HONDERMARCK, 1968 ; BENNET et al., 1969) ; 90 % of these salts was accounted for 2/3 by phosphate and 1/3 by ammonia. The production of 1 kg dry yeast required 100 l fermentation broth containing 10 % of gasoil, 100 l fermentation broth contained 200 g phosphate and required 50 g  $NH_3$  during the fermentation (FILOSA, 1968). From marked prices of the latter two compounds, the production cost could then be evaluated at 25 U.S. cents per kg dry yeast. prices of 13 U.S. cents per kg dry yeast, i.e. 20 U.S. cents per kg protein, were published since (VILENCHICH and AKTAR, 1971).

Harvesting by multiple step centrifugation, eventually followed by evaporation, was sufficient to yield a crude yeast base product when the substrate

was methane or *n*-paraffins. However, when the carbon source was a crude oil fraction, a rather stable emulsion of water in oil, into which the yeast cells collected at the end of the fermentation, did for some time hamper proper cell crop harvest. The addition to the oil-in-water emulsion, separated from the bulk of the aqueous fermentation broth by a mere decantation, of various substances such as non ionic tensioactive agents (LAINE, 1968), crude oil with or without added organic solvents (GHOSH et al., 1968), chlorinated organic solvents (BERNHEIMER and FRIEDMAN, 1970), ketones (GATELLIER and GLIKMANS, 1969), allowed a good separation of the cells in adapted centrifuges (WESTFALIA, 1965). Hydrocarbon-grown yeast contained up to three times more lipids than carbohydrate-grown yeast (18 % vs. 6 % wt x wt<sup>-1</sup> dry cells)(NYNS et al., 1968). An extraction step with hexane-isopropanol mixtures yielded defatted, hydrocarbon-free yeast cells (EVANS, 1968). Lipid extraction and emulsion resolution could be combined in a single step (BONAVITA, 1969).

The cost of yeast recovery was estimated at 1 U.S. cent x kg<sup>-1</sup> biomass (WANG, 1968b). Total cost of bacteria recovery was 3 to 4 times larger than the total cost of yeast recovery, due to the smaller size of bacteria (1-2 μ vs. 6-10 μ, WANG, 1968a).

Finally, the hydrocarbon cell crop could be dried by conventional methods. Provided the moisture degree was kept below 7 %, a condition easily fulfilled, the storage stability of the dried product was very satisfactory (CHAMPAGNAT, 1966). The cost of drying was estimated at 1 U.S. cent x kg<sup>-1</sup> produced biomass (WANG, 1968b).

Different cost computations were published by RAMIREZ (1970), BENNET et al., (1969) and WANG (1968b). Detailed discussion of yield factors were published by PAYNE (1970) and BELL (1972). Cost and yield factors for methane fermentation were discussed by KLASS et al. (1969).

The technological aspects of the bioconversion of hydrocarbons into single-cell protein are thus well at hand. Even the harvesting of the cells produced by fermentation from oil-water mixtures, and the removal of the last traces of high molecular weight hydrocarbons adsorbed by the cells during the fermentation process, are well at hand since many years.

One problem remains costly to solve. Yeast single-cell proteins contain too large amounts of nucleic acid to be used per se. The technology of nucleic acid removal, based on extraction processes, remains very expensive.

The existence of oil refineries and natural gas reserve in countries where a potential protein market exists (table 1) locates the protein production plant in the very country where this market exists. The influence on the international balance of payments should be negligible. Further, the investment necessary for a protein production plant and a final product distribution system is limited : the protein production plant can be located nearby the oil refinery and take advantage of its staff, its services and its distribution system. The investment may even originate as a reinvestment of oil refineries local benefits. The limited number of fairly centralized oil companies is well adapted for vast international development plan to be fulfilled in a relatively short period of time.

### 3.1.2. 2. Existing processes

Several industrial plants are already operating, under construction or planned for a near future (table 4). Furthermore B.P.'s process of yeast production from *n*-paraffins (Tropina, registered trade name) has promoted over 200 inquiries from 42 different countries interested in setting up protein production plants.

TABLE 4.

Hydrocarbon-grown S.C.P. industrial production plants in operation, under construction, or planned.

| Capacity<br>(tons × year <sup>-1</sup> ) | Location                 | Microorganism | Hydrocarbon<br>source | Trade name | Company                    | Reference                                     |
|--|--------------------------|---------------|-----------------------|------------|----------------------------|---|
| <b>A. In operation</b>                   |                          |               |                       |            |                            |   |
| 4,000 . . . . .                          | Grangemouth, Scotland    | Yeast         | <i>n</i> -paraffins   | Toprina    | B.P. Co, Ltd.              | AN., 1971c                                    |
| 16,500 . . . . .                         | Lavera, France           | Yeast         | Gasoil                |            | B.P. Co, Ltd.              |   |
| 1,000 . . . . .                          | Japan                    | Yeast         | —                     |            | B.P. Co, Ltd.              |   |
| 120,000 . . . . .                        | Japan                    | Yeast         | <i>n</i> -paraffins   |            | Dai Nippon Ink & Chemicals |   |
| 60,000 . . . . .                         | Japan                    | Yeast         | <i>n</i> -paraffins   |            | Dai Nippon Ink & Chemicals |   |
| 350 . . . . .                            | India                    | Yeast         | Gasoil                |            | Reg. Res. Lab.             | KIHBERG, 1972<br>HUMPHREY, 1969<br>AN., 1971a |
| —  | Taiwan                   | Bacteria      | Fuel oil              |            | Chinese Petroleum Corp.    |   |
| 20,000 . . . . .                         | Ukrain, S.S.S.R.         | Yeast         | Crude oil             |            | —                          |   |
| —  | Chicago, U.S.A.          | —             | Methane               | IGT 10     | I.G.T.                     |   |
| <b>B. Under construction</b>             |                          |               |                       |            |                            |   |
| 60,000 . . . . .                         | Takasago, Japan          | Yeast         | <i>n</i> -paraffins   |            | Kanegafuchi Chemical       | TAKASHI, 1969                                 |
| 100,000 . . . . .                        | Japan                    | —             | —                     |            | B.P. Co, Ltd.              | KIHBERG, 1972<br>CHAMPAGNAT, 1971             |
| <b>C. Planned</b>                        |                          |               |                       |            |                            |   |
| 100,000 . . . . .                        | Cagliari, Sardain, Italy | Yeast         | <i>n</i> -paraffins   |            | B.P. Co, Ltd and A.N.I.C.  | AN., 1971c                                    |
| 100,000 . . . . .                        | Japan                    | Yeast         | <i>n</i> -paraffins   |            | Kyowa Hakka Kogyo          | KIHBERG, 1972                                 |
| 10,000 . . . . .                         | Chicago, U.S.A.          | —             | Methane               |            | I.G.T. and Nigas           | AN., 1970                                     |

Three European multinational Companies seem to remain actively engaged in single-cell protein productions : B.P., *n*-paraffins and gasoil, Grangemouth (U.K.) and Lavera, France, I.C.I., methanol, deep shaft process, and Shell, methane and methanol, Sittingbourne, U.K.). No industrial projects or realization, within the Benelux are known to the contractor. The contractor has gained the impression :

- a. that most major Petroleum Companies do have at hand the "know-how" to produce single-cell proteins by hydrocarbon bioconversion, but are in the expectative.
- b. that the major chemical industries, although having at hand a R&D group in biotechnology, see little if any favourable prospect in industrial single-cell protein production by bioconversion of whatever raw material.

### 3.1.3. Status of R & D

There does not seem to be any public R&D group actively involved in bench to pilot to semi-industrial scale research on the bioconversion of hydrocarbons to single-cell protein within the Benelux. Two public groups are actively engaged in R&D projects on the bioconversion of methanol to single-cell protein in the German Federal Republic :

- a. Gesellschaft für Biotechnologische Forschung, m.b.H.,  
Mascheroder weg, 1  
3300 Braunschweig (Prof. Dr. F. WAGNER)  
Bench to Industrial scale R&D.
- b. Institut für Mikrobiologie der Universität Münster  
Tiburstrasse, 7-15  
4400 Münster, Westf. (Prof. Dr. H.-J. REHM)  
Bench to pilot scale R&D.
- c. One public group is actively engaged in a R&D project on the bioconversion of gasoil to single-cell proteins in France :

Laboratoire de Génie Biochimique

Institut National des Sciences Appliquées

Avenue de Rangueil

(Dr. G. GOMA and Dr. G. DURAND)

31 Toulouse 04

### 3.1.4. Economic evaluation

Untill late 1972, the cost of carbon substrates (ABBOTT and CLAMEN, 1973) ran for 2 U.S. cents per kg of substrate for methane to 4 U.S. cents for methanol and 8 U.S. cents for *n*-paraffins. Taking into account the substrate yield coefficients ( $Y_S = \text{g cells} \times \text{g}^{-1} \text{ substrate}$ ), the substrate cost per kg of cells was respectively 3.2 U.S. cents for methane, 10 U.S. cents for methanol and 8 U.S. cents for *n*-paraffins. When gasoil was the substrate its cost of 2.5 U.S. cents per kg cells was balanced by an increased value of the gasoil dewaxed by fermentation, resulting in a net gain of up to 2.5 U.S. cents per kg of cells (DECERLE et al., 1969). The crude oil price increases in 1973 deeply modified those figures, a fact which will be discussed below.

Until late 1972, hydrocarbon-grown single cells, when compared to other new protein sources, appeared to be competitive in wholesale price (table 3). Several factors might even improve the situation. Complementation of staple foods, whenever feasible, as is the case with hydrocarbon-grown S.C.P., is inexpensive. A 10 % addition of hydrocarbon-grown yeast to rice resulted in a retail price increase of the latter of less than 4 %. The agricultural lands can be kept in use for locally more profitable commodities than the cheap protein sources (SCRIMSHAW, 1968). In Taiwan, e.g. three rice crops per year are to be preferred to soybean production. A 1967 U.N. prediction that the price of S.C.P. would fall and that the price of competing protein sources would rise (U.N. Economical and Social Council, 1967b) became true beyond expectation : the price of soybean meal rose from U.S. \$ 100 to U.S. \$ 400 per ton,

TABLE 3 : Wholesale prices of proteins from various new sources

| Protein sources  | Price of protein sources<br>(US \$ per ton<br>pure protein) |
|--|---|
| Soybean meal . . . . .   | 312 <sup>(1)</sup> - 800 <sup>(9)</sup>                     |
| Alfalfa meal . . . . .   | 316 - 405 <sup>(1)</sup>                                    |
| Groundnut flour . . . . .  | 264 <sup>(2)</sup> - 920 <sup>(10)</sup>                    |
| Cottonseed meal . . . . .  | 316 <sup>(1)</sup>  |
| Cottonseed flour . . . . .   | 220 <sup>(2)</sup>  |
| Linseed meal . . . . .   | 356 <sup>(1)</sup>  |
| Fish meal . . . . .  | 471 <sup>(1)</sup>  |
| Egg . . . . .  | 440 - 880 <sup>(3)</sup> - 900 <sup>(9)</sup>               |
| Dried skin milk . . . . .  | 950 <sup>(4)</sup>  |
| Id. (including state subsidies) .                                  | 1320 <sup>(5)</sup> - 1200 <sup>(10)</sup>                  |
| Sugar-grown yeast . . . . .  | 550 <sup>(6)</sup> (7)                                      |
| Sulfite liquor-grown yeast . . .                                   | 1320 <sup>(6)</sup>   |
| Whey-grown yeast . . . . .   | 528 <sup>(7)</sup>  |
| Esso-Nestlé hydrocarbon-grown<br>single-cell concentrate . . . . . | 792 - 990 <sup>(2)</sup>                                    |
| Hydrocarbon-grown yeast . . . . .                                  | 770 <sup>(2)</sup>  |
| "Toprina" (BP yeast protein con-<br>centrate) . . . . .            | 260 - 500 <sup>(8)</sup>                                    |
| I.G.T. and NIGAS (methane-grown<br>S.C.P.) . . . . .               | 330 <sup>(11)</sup>   |
|  | 180 - 300 <sup>(12)</sup>                                   |

(1) AKESON and STAHPAN (1966) ; (2) AN. (1967) ; (3) AUTRET (1962) ; (4) CHAMPAGNAT (1968) ; (5) PEPLER (1968) ; (6) F.A.O. (1966) ; (7) U.N. ECONOMICAL and SOCIAL COUNCIL (1967a) ; (8) As calculated in the text ; (9) AN. (1973c) (protein content 50 % for soy flour and 66 % for fish meal) ; (10) VIX and DECOSSAS (1969) ; (11) AN. (1971c) ; (12) AN. (1970).

the price of fish meal from U.S. \$ 130 to U.S. \$ 600 (AN., 1973a). The recent oil price increase was solely an increase in exportation royalties. S.C.P. price should fall because of technological improvements. E.g., breakthrough in aeration, heat removal and cell harvesting of methane-grown bacteria were claimed to cut production costs from 24 U.S. cents to 14 U.S. cents x kg<sup>-1</sup> produced cells (ELLWOOD and LAW, 1970).

The economics of the hydrocarbon-type raw materials can be discussed by comparison with other types of raw materials (see elsewhere 2.4.), or by itself. Two facts have recently dramatically reduced the industrial efforts or outlooks, e.g. described in Table 4 :

- a. the increase in price of crude oil
- b. the extensive production of soya proteins in U.S.A.

It clearly appears that, at the present time, the price of single-cell proteins from hydrocarbon bioconversion is higher than the price of proteins of soya. Detailed information on the fate of existing plants and projects, if available, will be found in the next reports.

Special attention should however be reserved to methanol which can be produced from European methane or from commercially useless methane in the Middle East, suggesting greater competitiveness. This may explain presently continuing promotion of R&D projects of single-cell proteins production by methanol bioconversion in the German Federal Republic. No full scale industrial production unit of methanol bioconversion to single-cell protein is presently in steady state activity, so that no definite production costs are at hand. Market price fluctuations could, however, arise from competition with other possible industrial uses of methanol, namely in polymer chemistry.

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#### 4. PRELIMINARY RESEARCH PROPOSALS

##### 4.1. Anaerobic digestion

- 41.1. The most obvious lack of knowledge in the bioconversion of organic residues into methane biogas, is the black box defined above under 21321.5 : the mechanism by which products of acidogenesis are converted to methane with energy conversion for the growth and maintenance of methanogenic bacteria.

Methanogenesis is at present time like a car of which nothing should be known about the principles of functioning of its engine.

Therefore, the contractor is strongly persuaded that no substantial progress in methanogenesis will be done before definite knowledge is gained on the basic mechanisms of methanogenesis.

A suitable research program would consist of three small teams (one senior and one technical assistant each) in three different member countries of the E.C. which would have as joint target to elucidate the basic mechanisms of methanogenesis in mixed populations in a period of 5 years. One of these teams should be engaged in bioenergetics and familiar with Mitchell's chemiosmotic theory, one of the teams should be familiar with anaerobic electron transporting chains, and one of the teams should be familiar with anaerobic microbiology.

It is well known to the contractor, that a number of laboratories are being engaged, in an unorganized way, in the study of anaerobic energy

conservation by microorganisms with projects not necessarily related with methanogenesis. The three teams will have a chance to be in contact with these laboratories, to obtain information before publication (and hence with a gain of one or more years) and utilize this information to solve the specific problem of methanogenesis.

41.2. A number of technical improvements of the process of bioconversion of organic residues into methane biogas, are at present being studied. Expert for the International Agency for Energy, the contractor has recently made a survey of these projects among member countries of the I.A.E. Would the Commission be interested, the contractor could ask for permission to incorporate this survey in his final report.

It is therefore felt to the contractor that the Commission should pay attention to avoid useless duplication in granting projects which are already funded by other national or international authorities.

However, points of interests are the study of the pretreatment (see 21321.1) and the study of two stage biological conversion units (see 21321.4 and 2132.2).

41.3. Trials on the semi-industrial scale will become important and should be tested on particular small size pilot geographical area. It is felt to the contractor that at this time only theoretical approaches can be of interest and that one should await the results of the studies defined sub 41.1 and 41.2 before granting such projects. Here also, the Commission should pay attention to avoid useless duplication with existing or planned projects funded by other national or international authorities.

### 4.3. Carbohydrate hydrolysis

#### 4.3.1. Upgrading of ligno-cellulosic wastes : protein production

Feedstuffs based on lignocellulosic materials have been envisioned until now only on the basis of pretreatment of the material to enhance its digestibility. Hence, an alternative process which would constitute an upgrading of the cellulosic residue would be most valuable. A variety of techniques have been developed so far to hydrolyze cellulose to sugars. Particularly the microbial or enzymatic methods appear the most promising. By means of microbial processes the sugars produced can further be converted either into microbial proteins (SCP) or other microbial metabolites. But a direct conversion of the cellulosic materials to microbial biomass in one step would be more interesting and has been shown to be possible.

Furthermore, animal nutritionists indicate the increasing prices of animal feedstuff on the world market, and our growing dependence upon imported protein (soya). In addition they point out that in the near future the E.E.C. Countries will be put under increasing pressure by the Third World not to use high quality grains for ruminant feeding but low-quality carbohydrates and protein sources instead. In view of this information, the development of a process to convert waste cellulosic materials into animal feedstuff, accompanied by the transformation of residual lignin into commodity chemicals and the utilization of residual hemicelluloses, seems both economically and ecologically warranted.

For the research concerning the bioconversion of lignocellulosic materials to feedstuff, a team of one senior scientific, one junior scientific and two technicians with funds for equipment and animal nutrition trials seems a minimum.

#### 4.3.2. Future prospects for pretreatment of ligno-cellulosic materials

Very fine grinding and ball milling are the most common pretreatment used today. Milling is very effective with both crystalline and heavily lignified materials when it sufficiently decreases the average particle size. But it is very energy-intensive and, as a consequence, expensive.

Alternatives have to be found or ball milling should be made less costly.

A study of ball-milling has indicated that the increase in digestibility is apparently a result of decrease of particle size more than a result of reduced crystallinity, smaller particle having a larger available surface. This suggests that treatments that would cause fracture of the cells might also be effective; splitting of the cells parallel to their axis would probably require less energy than fracture perpendicular to the fibrils axis. Uses of sonic energy, cryogenic grinding and explosive depressurization should be envisaged. It must be pointed out that it might not be necessary to modify the crystallinity of cellulose.

Uses of swelling agents in combination with grinding or ball-milling might also enhance the decrystallization of cellulose.

However, the greatest challenge lies in the development of methods able to decrease the protective effect of lignin by dismantling the lignin-carbohydrate complex in ligno-cellulosic materials. Since almost all potential substrates for cellulose bioconversion or hydrolysis are heavily lignified, the overcoming of this barrier is a must before developing economically viable procedures.

The goal of such a treatment is to eliminate the protective effect of the lignin. Not all the lignin needs to be removed or changed to significantly increase susceptibility to enzymes and chemicals. According to the material employed and the final use, only 20 to 70 % of the lignin has to be modified.

Physical methods include grinding and ball-milling and ionizing radiations such as high energy electrons and photolysis with UV. Cryogenic grinding and explosive depressurization with the eventual use of swelling agents or lignin solvents might achieve a better cellulose accessibility.

Chemical methods for delignification are numerous. But they have been optimized in view of keeping the cellulose fibrils to get the best yields of optimal pulp quality. This should be reviewed with the goal of disrupting the lignin-carbohydrate complex just enough to obtain optimal cellulose digestibility. The degree of depolymerization of the carbohydrate would not matter as long as no sugar, or a minimum amount, would be lost. Such a treatment could certainly become much less costly than conventional pulping methods.

Most promising methods include those which could allow no loss of substance while disrupting lignin deeply enough; gaseous treatments (gaseous sulfur dioxide) and treatment with volatile solvents are among them. But a review of the pulping methods need to be done with this new goal in mind.

An economic evaluation of some methods already well investigated, should be done (gaseous sulfur dioxide). This review should be made on the basis of our knowledge of the chemistry of lignin.

Biological methods of degradation of lignin need to be pushed ahead, both for search of faster enzymatic systems and in intelligence of exact sequence of reactions that results in depolymerization of lignin. It is possible, in the first line of research, to use ligninolytic microorganisms (fungi) or the enzymes they produce. Since lignin-degrading enzymes would probably be a complex mixture of enzymes and co-enzymes, a pretreatment with whole organisms would be easier to develop than one based on isolated enzymes. A team of one senior scientific, one junior scientific and one technical assistant during three years with some equipment funding would be a critical minimum.

Finally, it should be mentioned that cellulose constitutes only 50 % of most lignocellulosic materials and that any integrated treatment should use also, as far as possible, the remaining 50 %, that is lignin and hemicelluloses.

Within this context, research on possible transformations and uses of lignin or lignin degradation products should be promoted.

#### 4.3.3. Chemical hydrolysis

##### 4.3.3.1. Reaction kinetics

The kinetics of wood saccharification at high temperature and pressure and low acid concentration need to be further studied to verify and refine, on this material, earlier findings made with paper.

This program should be of short duration since its aim is to pave the way for further development. We propose a team of a senior scientific with two technical assistant during two years.

##### 4.3.3.2. Chemical engineering

Supposing confirmation of the kinetic findings, a reactor for saccharification at high temperature and pressure should be developed, taking into account acid-resistant materials, problems of wood handling at these extreme conditions, short reaction time, etc...

A team of one senior scientific, one junior scientific and three technical assistants with adequate equipment funding seems necessary.

#### 4.3.4. Production of chemicals from hydrolysis lignin

##### 4.3.4.1. Pyrolytic hydrogenolysis

The Noguchi-CZ process is the only lignin process to have been rationally examined both commercially and technically. This process might well be economic today as it stands, but the development should be revised in two respects :

- a) the process, developed to use lignosulfonates, should be adapted to hydrolysis lignin.
- b) the hydrogenation technology should be updated.

A suitable program should tightly combine researches with knowledge in lignin chemistry and in catalysis, both being very important. A working force of one senior scientific, one junior scientific and two technical assistants with adequate equipment funding seems appropriate.

#### 4.3.4.2. Hydrolytic hydrogenolysis

It has the advantage that water needs not to be removed and that some hydrogen can be recuperated from cheap produced alcohol. The knowledge is much less developed and the process is then of less immediate application. However, it looks as the second technique which deserves further study.

A team of senior scientific, a junior scientific and a technical assistant seems sufficient in a first period.

#### 4.4. Protein recovery

##### 44.1. Upgrading

Upgrading processes should be favourably considered by the C.C.E. for research funding for the following reasons :

- (a) these processes concern small size agricultural industries, which are not able to finance these researches by themselves (progress in agriculture through research can only occur through public authority funding),
- (b) at present, no industry will be interested in funding researches in this field because a preliminary evaluation need to be done before the risk of success can safely be evaluated,
- (c) the deficit in animal feed protein in the E.C. is well known and common to all member countries,
- (d) the disposal of animal residues is a major problem in the E.C. (it may be recalled that there are  $30 \times 10^6$  pigs in the E.C.).

A suitable research proposal would best have to be presented by a team consisting of an academic group devoted to applied biology, and an industrial partner for the pilot-plant construction. The research proposal should aim at evaluating for a period of 3 years not only at the bench scale but also at the  $0.1 \text{ m}^3$  level, the feasibility and the yield of a process by which animal residues are upgraded to animal feed. This proposal should concern about 4 man x year but will most probably involve large equipment cost for a suitable pilot plant.

It would be up to the C.C.E. to decide whether, before such research proposals be taken into consideration, a preliminary study concerning the importance of the benefits to the E.C. of the outputs of such research proposals, by evaluating the theoretical number of such processes which could be built within the E.C. and the theoretical amount in tons of upgraded products that could be produced within the E.C. This preliminary study would require one man during 6 months, provided detailed agricultural statistics in the E.C. be made available to him.

#### 44.2. Hydrocarbon fermentation

Technological development of the bioconversion of hydrocarbons or simple derivatives of hydrocarbon into single-cell proteins : little knowledge is available about the amount of "know-how" in the possession of the major Companies involved in the field. Little cooperation is possible between public R&D groups and these Companies where the secrecy of the "know-how" is traditional. Therefore, the contractor feels, in a first approach, that no priority for efforts of the C.C.E. should be granted to this aspect.

Much research is carried out on the fundamental aspects of the assimilation of methane and methanol on the one hand, and on the assimilation of lower ( $C_6-C_{10}$ ) and higher ( $C_{12}-C_{18}$ ) paraffins. The contractor feels however that although the major Companies involved in the field remain interested in these fundamental researches, it is however not clear whether C.C.E. support of this research would be of priority need for the development of the industrial processes and factories.

An eventual gap to be filled is a "human science" one and deals with legal and socio-psychological aspects : mainly the legal definition of the norms to be fulfilled by single-cell proteins from hydrocarbon- or methanol-grown yeast to be accepted as food or feed and their appraisal, and the acceptance by all members of the E.C. of these legal definitions so that a well defined and recurrent market could be created.





COMMISSION OF THE EUROPEAN COMMUNITIES  
(Directorate General XII - Research, Science, Education)

FERMENTATION - HYDROLYSIS PROCESSES  
FOR THE  
UTILISATION OF ORGANIC WASTE MATERIALS

Report by

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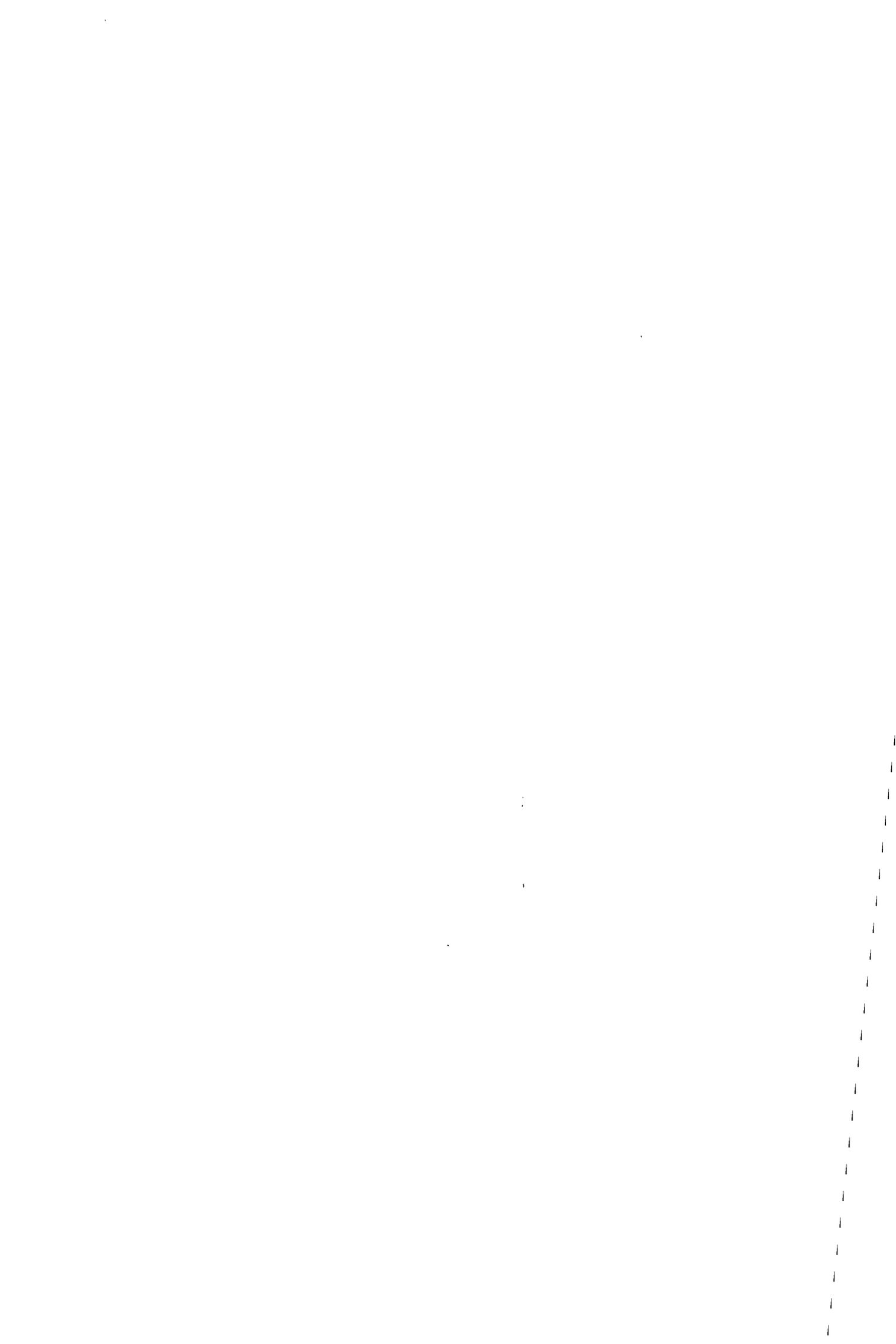


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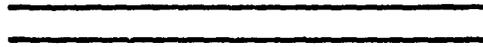
**Part 2 : Organic waste utilization processes**

- |  |                       |
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| 2.1. Anaerobic digestion                       | Ir. Ir. P. Pipyn      |
| 2.2. Composting                                | Ir. Lic. L. Van Acker |
| 2.3. Carbohydrate hydrolysis and fermentation  | Dr. Ir. E.J. Vandamme |
| 2.4. Protein recovery from organic waste water | Dr. Ir. W. Verstraete |

**Part 3 : Competitive processes**



## 2.1. ANAEROBIC DIGESTION



## ABSTRACT

A lot of wastes, liquid and solid, are known to be fermentable to methane. The different sources for this potential energy are given. Anaerobic fermentation can be executed by different procedures. The techniques are summarised and a listing of the most important plants in the Flanders and in the Netherlands is given. A typical example of a plant in the Flanders is described and the production of methane gas calculated. An economic evaluation of the fermentation of wastes in in the Flanders and the Netherlands is done. It is concluded that methane digestion deserves to be re-investigated in great detail, particularly with regard to two new process-technological concepts; i.e. the thermophilic treatment to optimize the digestibility and energy production for secondary waste sludges and the upflow methane reactor for concentrated highly fermentable waste waters.

### 2.1.1. Raw Materials and End Products.

A lot of wastes, liquid and solid, are known to be fermentable to methane, e.g. :

- sewage sludge from water treatment plants
- animal wastes such as manure
- night soil
- high strength waste waters from sugarbeet factories, potato starch industries, dairy industries, etc.

The most important source which will become available in the Flanders and the Netherlands will undoubtedly be the sewage sludges from domestic waste water treatment plants. For every inhabitant for whom the waste water is treated, some 10 kg sludge ( dry matter ) is produced per annum. Hence, if by 1980 some 50 percent of the domestic waste waters would receive primary and secondary treatment, some 75.000 tons of sludge ( dry matter ) will be produced per year in the Flanders and the Netherlands.

The second most important source is to be found in the animal wastes. For the Flanders alone, the yearly production of manure is estimated at 50 million tons/year. At least 5 percent of these wastes are not used for soil fertilisation purposes. As such, some 70.000 tons of organic matter ( dry weight ) per year are available in this sector. For the Netherlands, this amount is possibly still larger.

The third source can be found in the starch and sugar industry. For this branch, the total quantities are hard to estimate but there is no doubt that the quantity of organics available approaches also tens of thousands of tons.

The end product of the methane digestion process is the so called biogas. It contains 60-90 percent methane and has a calorific content of  $\pm 8.000 \text{ kcal/m}^3$ .

### 2.1.2. Commercial Processes.

Different techniques for the anaerobic treatment of wastes exist, such as :

- conventional low-rate digestion
- high rate digestion
- thermophilic digestion
- two-stage digestion ( acidification + methanogenesis )
- anaerobic filter digestion
- anaerobic contact digestion
- anaerobic upflow digestion

A listing of the most important methane plants in the Flanders and in the Netherlands is given below :

-Municipal plants at St.-Niklaas, Lokeren ( 15000 I.E. ), Turnhout ( 35000 I.E. ), Hamme ( 15000 I.E. ), Duffel ( 15000 I.E. ), Antwerp ( 350000 I.E. ).

A typical example is the plant at St.-Niklaas. This is a municipal water treatment station using trickling filters and anaerobic digestors. The capacity of this water treatment plant is  $3.200 \text{ m}^3/\text{h}$ . The organic loading represents about 100.000 inhabitant equivalents ( = I.E. ). The methane fermentation of the sludge happens in one tank of  $2.850 \text{ m}^3$  volume. This digester is maintained at a temperature of  $33^\circ\text{C}$ . The sludge is afterwards stored in a tank of  $2.500 \text{ m}^3$  volume which is not warmed. The latter tank serves also as a gas holding tank. A volume of  $50 \text{ m}^3$  sludge per day is treated by anaerobic digestion. This means that the retention time is about 50 days. The gasses are used to generate electricity. The quantity of gas produced is about  $600-700 \text{ m}^3/\text{day}$ . This represents the equivalent of 300-350 liter gasoil.

-Industrial and semi-industrial plants :

a. C.S.M.-Breda ( Netherlands )

Experiments are done on a semi-technical scale (  $30 \text{ m}^3$  reactor ) and on a technical scale (  $200 \text{ m}^3$  ) for the treatment of sugar-beet waste waters ( upflow reactors cfr. the design of Dr. Lettinga ).

b. Rixona-Warfum ( Netherlands )

Five plants of  $40 \text{ m}^3$  for treatment of the waste waters of a potato industry.

-Laboratory and pilot plants :

Fundamental and applied research with regard to methane digestion is done at the following locations :

- a. Agricultural Faculty-Wageningen ( Netherlands )  
Methane fermentation is applied on concentrated and diluted forms of waste waters from sludge till domestic waste waters.
- b. A.F.M.-Megista project ( Netherlands )  
Anaerobic fermentation is applied on stabilisation of pig manure.
- c. Technische Hogeschool-Delft ( Netherlands )  
The chemical and technical aspects of anaerobic fermentation for application on treatment of waste waters are studied.
- d. G.W. Amsterdam ( Netherlands )  
The acidification of some dissolved substrates is studied for development of the two stage anaerobic fermentation.
- e. Zuiveringschap W.-Brabant. ( Netherlands )  
Studies are made on the treatment of the filtrate of heat treated sludge.
- f. Provinciaal Waterstaat Utrecht ( Netherlands )  
Treatment of fresh-drinks industry waste waters.
- g. Instituut voor Bewaring en Verwerking van Landbouwprodukten ( = IBVL )-Wageningen ( Netherlands )  
The waste waters of a potato industry ( C.A.B.-wezep ) are treated on a 6 m<sup>3</sup> pilot plant.
- h. Instituut van Aardappelverwerking-Groningen ( Netherlands )  
The treatment of potato industry waste waters has been studied.
- i. Faculteit van de Landbouwwetenschappen, University of Ghent ( Belgium - The Flanders )  
Studies on the fermentation of sewage sludge, distillery waste waters, straw and manure are made.

### 2.1.3. Status of Research and Development.

The digestion of domestic sewage sludges has been developed as a reliable and efficient process. However, currently major attempts are made to increase the rate of the process. The process would become a complete success if the hydraulic retention time ( currently 10-20 days ) could be reduced to less than 5 days. In a recent paper, Haug ( 1977 ) evaluated some methods of sludge processing to optimize digestibility and energy production. He pointed out that thermal pretreatment followed by anaerobic digestions offers the following advantages : improved biodegradability ( more gass, less residual sludge ); improved dewaterability; no separate sidestream treatment; increased net energy production; odor control and sterilization. In view of all these

potential advantages, it is felt that a thorough evaluation of the thermal pretreatment-anaerobic digestion process is fully warranted.

The production of methane from animal wastes has hardly received attention and merits thorough investigation.

The anaerobic treatment of highly concentrated industrial waste waters is currently studied intensively in the Netherlands. The method of the anaerobic upflow reactor appears most promising both from the point of view of water purification and energy recovery.

#### 2.1.4. Economic Evaluation.

Anaerobic digestion has to be looked upon from two sides :

- Firstly, it qualifies as a low-energy requiring treatment process for concentrated wastes. Hence, it reduces the quantities of wastes and furthermore makes the wastes more susceptible to dewatering and final treatment techniques.
- Secondly, it produces energy, i.e. about 500 l biogas/kg organic matter removed.

As a consequence, anaerobic digestion is undoubtedly becoming more and more economically attractive. At the worst, it produces just enough energy to be self-supporting. At the best, it produces a large excess of energy which not only permits to bring the treatment costs of the wastes to nihil but which also gives rise to a considerable bonus on the nations energy balance.

If in the Flanders and the Netherlands some 200.000 tons organic matter would be digested per year, the total net-energy recovery would amount to  $\pm$  450 Teracal/year.

Documentation

For more information with regard to methane digestion, the following organisations can be contacted :

- for the Netherlands :

G. Lettinga, Afd. Waterzuivering, Landbouwhogeschool, Wageningen.  
R. Van de Meer, Afd. Chem. Technologie, Technische Hogeschool, Delft.  
R. Soetemeyer, Afd. Chem. Technologie, Universiteit Amsterdam.  
Th. M. van Bellegem, Instituut voor Aardappelverwerking, Groningen.  
P. Ten Have, RAAD, Arnhem.

- for Belgium ( The Flanders ) :

P. Pipyn, Faculteit van de Landbouwwetenschappen, Rijksuniversiteit, Gent.  
W. Verstraete, Faculteit van de Landbouwwetenschappen, Rijksuniversiteit, Gent.

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## 2.2. COMPOSTING

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## ABSTRACT

A variety of wastes can be composted e.g. crop residues, animal manure, garbage, etc. The composting process can be achieved in so-called windrows or in specific mechanical constructions such as for instance the Dano-biostabilisator.

Regular compost is used mostly in horticulture as an organic substrate for plant growth. In addition special types of compost are used for mushroom growing and for animal feeding.

The most important composting plants in the Benelux are listed. In addition the status of R&D with regard to the process is outlined. It is felt that composting of highly organic waste water sludges such as e.g. from the paper industry deserves special attention in view of the specific problem these sludges constitute at one hand, and the rising demand for organic fertilisers due to the increase of the so-called alternative agriculture on the other hand.

### 2.2.1. Raw Materials and End Products.

Various substrates can be composted e.g. paper, wood ( bark, leaves ), manure, food residues, crop residues, night soil, sewage sludge, animal manure, garbage, etc.

Two types of substrates are of extreme importance in the Benelux, i.e. household refuse ( + 6.0 million ton/annum ) and the bark wastes (  $\pm 100.000 \text{ m}^3/\text{annum}$  ).

Three types of end products can be obtained from the composting process i.e. regular compost for " gardening " and horticulture, high quality compost to be used as a mineral source in animal fodder and finally mushroom compost.

### 2.2.2. Commercial Processes.

The composting process is essentially aerobic and includes 4 phases : a mesophilic phase, a thermophilic phase, a cooling phase and maturation phase. The composting process is a function of the nature of the microbial populations and of the efficiencies of these populations. The principal environmental factors influencing the biological activity are moisture, temperature, pH-level, oxygen concentration, nutrient concentration and availability.

Municipal refuse ( + sewage sludge )

Composting municipal refuse involves four steps : sorting, grinding, composting and storage. Sorting can proceed composting or it can follow it.

There are several composting systems, but essentially there are two categories : a. " open " or windrow-systems.

b. mechanical systems.

#### a. windrow systems

The windrow-systems are characterised by having the composting taking place in the open air by placing the refuse in elongated piles ( 1-2 m high and 2-3 m wide ). Aeration is accomplished by periodically turning the piles. After a few days, there is an intensive fermentation, with a temperature reaching 70°C. After 10 weeks the product is stable and hygienic, e.g. Van Maanensystem, Wyster ( Netherlands ).

b. mechanical systems

Mechanical digestors are equipped to ensure adequate temperature and moisture control. Most of them are based on providing aeration by some type of tumbling or stirring action in the digester; e.g. the Dano-biostabiliser ( Ghent ). After sorting metals, rags, etc., refuse comes in the biostabiliser. This is a steel cylinder ( 26 m long and 3,5 m wide ), which rotates around a horizontal axis. Refuse has an average retention time of 2 to 4 days. The fermentation can be influenced by blowing air or injecting water. By opening a valve in the endwall of the biostabiliser, stabilised refuse is discharged. It is screened and stored in piles. Before sale, compost has to undergo a second fermentation for about 3 months on the compostyard.

Mushroomcompost

Mushrooms need compost as a substrate. The usual composition is 75% horse manure, 12,5% chicken manure, 10% straw and 2,5% gypsum. This mixture placed in piles ( 2 m high, 2 m wide ), undergoes a fermentation process during 9 days, with 3 periodical turnings.

Manure

Solid manure can be transformed into compost in the same way as refuse ( alone or together with other organic wastes ) ( e.g. ADA-system ). In the LICOM-system ( Alfa-Laval ), liquid manure is aerated in isolated reactors and undergoes a composting process at a temperature of 50°C.

Bark wastes, garden wastes, etc...

In the organic Farming and Gardening ( e.g. Howard-Balfour method, Biological-dynamic method, Lemaire-Boucher method ), compost is the most common soil conditioner and nutrient source. All the manure and organic waste is composted and recycled, usually on a small scale. Bark and other wood processing residues can also be used.

The most important plants in the Benelux are listed below :

Municipal refuse

- Stadscompostbedrijf Gent  
Ottergemsesteenweg zuid, 705  
B-9000 Gent, Belgium  
Dano-system; production 15.600 ton/year.
- Intercompost Bilzen  
starting period 1977-78 : production prognoses 32.000 ton/year.
- V.A.M. ( Vuilafvoermaatschappij )  
Jacob Obrechtstraat, 67  
postbus 5380  
Amsterdam 7, The Netherlands
- Van Maanensystem : production ( at Wyster and Mierlo )  
120.000 ton/year.
- Other plants e.g. Dano-system in Haarlemmer Meer, Soestbaren,  
Venlo and raspwasteprocessing in Arnhem, The Netherlands.

Mushroomcompost

- Jan Sterckx p.v.b.a.  
Roeselarestraat, 39  
B-8701 Kachtem, Belgium  
production : 50.000 ton/year.
- J. Ackermans  
Wonkeweg, 12  
Zichen-Zussen-Bolder, Belgium  
production : 12.500 ton/year.
- Coöperatieve Nederlandse Champignonkwekervereniging ( CNC )  
Driekoningenstraat, 1a  
Attersem, The Netherlands  
production : 220.000 ton/year.
- Firma Theeuwen  
Bliterswijck ( Venlo ), The Netherlands  
production : 20.000 ton/year.

### Bark compost

- Denayer N.V.  
B-2660 Willebroek, Belgium  
production capacity 50-100 m<sup>3</sup>/day.
- Papeteries de Belgique  
B-9000 Langerbrugge, Belgium  
production capacity 150-250 m<sup>3</sup>/day.

### 2.2.3. Status of Research and Development.

The following topics require investigation :

- Development of an integrated composting process for several types and combinations of organic wastes such as domestic refuse, plant wastes, sewage sludge, industrial waste, including inventarisation of the wastes.
- Screening for a C-source to optimise the composting of materials with a low C/N ratio ( e.g. liquid manure ).  
Such a C-source can e.g. replace horse manure in the preparation of mushroomcompost.
- Market study of compost; a comparative study of compost with chemical fertilizers and not-composted manure, on different crops, vegetables, flowers and for land reclamation ( field studies and economy ).
- Study of the economy and total planning of composting, in comparing with other treatment systems, cost benefit analysis, system analysis, energy analysis, including the social-economic, ecological and planological aspects ( Vanacker, 1977 ).

For Flanders, priority should actually be given to the study of the composting of waste water sludges from the paper factory at Langerbrugge. This firm produces each year a total quantity of 8,000 ton of sludges ( 20-40 percent organic matter ). Currently, the firm does not know how dispose these large quantities of wastes. Preliminary trials have indicated that the sludge combined with bark has a potential to compost to a highly valuable organic substrate. In view of the steadily increasing demands for organic fertilizers by the so-called alternative agriculture in Flanders ( all ready some 250 ha ! ), it is felt it would be most interesting to stimulate a thorough investigation into the possibilities of composting and marketing the latter materials from the paper industry, rather than burning them at the expense of a considerable amount of fuel as is currently considered.

#### 2.2.4. Economic Evaluation.

Initially, one expected that the sale of salvage products and of the compost product itself would pay for a substantial portion, if not all, the costs of the processing of the wastes. Unfortunately, only 25 to 50% of the total costs can thus be recovered. However, when composting is compared with sanitary landfill and moreover with incineration, its balance is highly attractive. The latter processes can both be considered as giving rise to a loss of the material treated and their respective costs are estimated at 350,- B.F./ton domestic refuse and 700,- B.F./ton domestic refuse while those of composting vary around 250,- B.F./ton domestic refuse. There is however one major drawback : only 50% of the domestic refuse can be composted. Therefore, composting must for heterogeneous waste flows be coupled to an incineration plant. Such a combination is currently in construction for the city of Gent ( 200.000 inhabitant equivalents ).

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### **2.3. CARBOHYDRATE HYDROLYSIS AND FERMENTATION**

## ABSTRACT

The interest of applied research has been concentrated lately towards the utilization of hitherto largely unused renewable resources by means of fermentation.

Carbohydrates are such a totally renewable natural resource.

Although pure carbohydrates and conventional carbohydrate rich substrates such as molasses and starch have been used since long in many classical fermentation processes, it is now felt that especially crude carbohydrate materials and carbohydrate rich wastes should also find a use in microbial biomass or metabolite production processes.

Various processes to convert carbohydrate-substrates into sugars and/or useful microbial compounds are reviewed here.

It is felt that renewed attention must be paid to single cell protein ( SCP ), alcohol and organic solvent fermentations from sugary and starchy wastes. Photosynthetic bacteria can also be applied to these processes with economic profit. Such dilute wastes can also be recycled in immobilized enzyme or cell reactors to yield sugar-concentrates, which can then be fermented further on. Cellulose, as the most abundant renewable substrate, can be hydrolysed by microbial cellulases into glucose syrups, which in turn can be used as a substrate for SCP or other microbial products. The conversion of garbage paper and cellulosic agricultural waste into feed yeast or ethanol seems to be a priority problem all over the E.E.C. and is now receiving worldwide interest.

### 2.3.1. Raw Materials and End Products.

Carbohydrates are a totally renewable natural resource. In assessing them as raw materials for the ( bio )-chemical industries, we first have to consider the agronomic characteristics of their production ( Table 1 ). Furthermore, carbohydrates can be classified as three main types : the readily utilized mono- and disaccharides, particularly glucose, maltose, sucrose ( molasses ) and lactose ( whey ), the readily hydrolysed polysaccharides, notably the starches and the difficultly-hydrolysed structural polysaccharides like cellulose, hemicellulose and lignin. Renewable sources are compared with fossil resources as fermentation substrates in Table 2.

If carbohydrate wastes are to be used for hydrolysis and fermentation, pretreatment might be necessary, the sugar pattern might be unusual as well the C/N ratio and toxic materials might be present. The costs to convert the waste into a microbial growth substrate, together with its fluctuating distribution and availability in time and space will greatly influence its economical use in fermentation processes. Single cell protein ( SCP ) production seems to be the most efficient process, although production of ethanol and organic acids or solvents, looks also promising. A general production scheme is represented in Scheme 1.

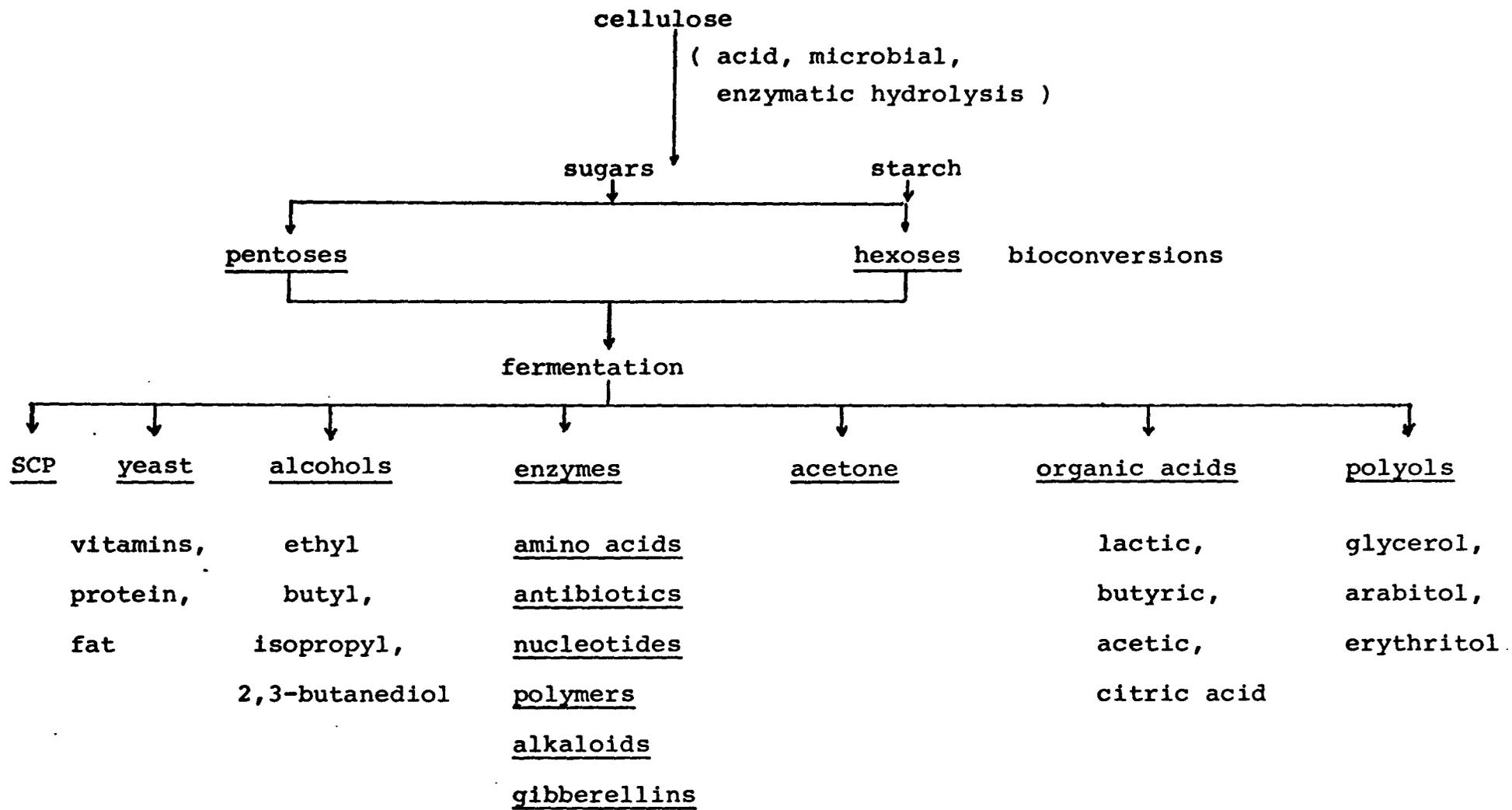
The microbial strains to be selected for these SCP-fermentations should conform to characteristics such as : a high growth rate at relatively high temperature ( thermophilic ), high yield, high protein content, optimal amino acid pattern, no toxicity and a wide acceptability. Also, cell separation should be easy to perform by centrifugation, flocculation, foam flotation or filtration.

The applied process characteristics can also vary widely : continuous fermentation processes can give a high productivity per unit volume, but this requires a high dilution rate, which will result in rising cell separation costs. Such sophisticated ( and expensive ) continuous processes also have to deal with a

TABLE 1 : Available Carbohydrates for Hydrolysis and Fermentation

|                        |   |   |
|------------------------|---|---|
| <u>Sugars</u>          | Cane ( bagasse, molasses )<br>Beet ( molasses )<br>Sulfite waste liquor<br>Milk ( whey )<br>Fruit and vegetable waste<br>Process wastes from above  | Surplus production may be temporary and local. Prior treatments minimal.                                |
| <u>Starches</u>        | Cereals<br>Rice<br>Cassava<br>Manioc, Coconut<br>Potato, Corn<br>Process wastes from above  | Surpluses local but seasonal. Prior treatments minimal. Fermentation may conflict with food uses.       |
| <u>Polysaccharides</u> | Corncoobs, oat, hulls<br>Straw, soybean, bagasse<br>Wood waste , peat<br>Paper waste, manure, sewage<br>Cellulosic materials in general :<br>( agricultural, food processing, domestic & municipal wastes ) | Large surpluses, but some local and seasonal. Low cost but bulky in transport. Prior treatment maximal. |





Scheme 1 : Fermentation products of carbohydrate hydrolysis

discontinuous supply of variable wastes, so that discontinuous or low level technology-processes could gain equal importance.

#### 2.3.1.a. Sugars

Fermentation of sugars ( glucose, sucrose, maltose, lactose ) into microbial biomass ( Single Cell Protein, SCP ) or metabolites is well known and has a worldwide application.

Glucose, molasses, malt, sulfite waste liquor and whey are indeed typical carbohydrate-carbon or energy sources, used in a wide variety of fermentation processes, including the production of food and feed-SCP, organic solvents or acids, ethanol, amino acids, antibiotics, vitamins, enzymes, polymers, alkaloids, gibberellins, nucleotides, toxins, vaccines, pigments or bioinsecticides. These product-fermentations will not be discussed here.

Crude sugar wastes such as sulfite waste liquor, molasses and whey, are easily used for biomass ( SCP ) production and some industrial exploitations are summarized in Table 3.

Conversion of molasses or malt to ethanol by anaerobic yeast fermentation processes is common practice. However, ethanol in turn can be converted into acetic acid by Gluconobacter ( Acetobacter ) species, and both these products can be used as a substrate for SCP production as shown in Table 4.

Ethanol can also be considered as a basic material for the chemical industry and as an energy source.

Lactose, recuperated from whey, can be hydrolysed by microbial  $\beta$ -galactosidases into glucose and galactose. Whey can also be fermented to lactic acid.

#### 2.3.1.b. Starches

Hydrolysis of starches to sugars and subsequent fermentation of sugars into useful microbial compounds or cells ( Single Cell Protein, SCP ) are also well documented fields in fermentation science, though still liable for further improvement and sophistication. Some examples of starch-SCP production plants are given in Table 5.

The enzymatic hydrolysis of starches into glucose, using microbial amylases and amyloglucosidases is gaining worldwide

TABLE 3 : SCP production from sugar waste.

| Substrate      | Microorganisms   | Company  | Country |
|----------------|--|--|---------|
| Sulfite liquor | yeast (10.000 ton/year)<br>( <u>Candida utilis</u> ,<br><u>Paecilomyces variotii</u> ) | Finnish Pulp<br>& Paper Co<br>(Pekilo pro-<br>cess)  | Finland |
|                | yeast (10.000 ton/year)  | Khabarowsk   | USSR    |
|                | yeast (10.000 ton/year)  | Boise Cascade,<br>Portland, Ore                      | USA     |
|                | yeast  | Thorold,<br>Ontario                                  | Canada  |
|                | yeast (20.000 ton/year)  | Japan Pulp,<br>Jiyo Pulp &<br>Paper                  | Japan   |
| Whey           | yeast (10.000 ton/year)  | Milbrew,<br>Wisconsin                                | USA     |
|                | yeast ( <u>Saccharomyces</u><br><u>fragilis</u> )                                      | Silverwood<br>Industries<br>London,<br>Ontario       | Canada  |
| Molasses, Malt | yeast  | Anheuser-<br>Busch Inc.,<br>St. Louis, Mo.           | USA     |
|                | yeast (10.000 ton/year)  | Kojin License  | Cuba    |
|                | yeast (20.000 ton/year)  | Kyowa Hakko<br>License                               | Mexico  |
|                | yeast<br>( <u>Saccharomyces</u> ,<br><u>Candida</u> )                                  | many countries<br>in Europe, Asia,<br>Japan, America |         |



**TABLE 5** : Single cell protein production from starches and starchy wastes.

| Starch source  | Microorganisms  | Company   | Country                               |
|--|---|---|---------------------------------------|
| - Starches   | fungi<br>( <u>Fusarium</u> , <u>Aspergillus</u> ,<br><u>Penicillium</u> , <u>Neurospora</u> ,<br><u>Alternaria</u> , <u>Trichoderma</u> ) | Rank-Hovis-Mc Dougall-<br>Du Pont, High Wycombe<br><br>University of Guelph<br>Nestlé, Geneva | England,<br><br>Canada<br>Switzerland |
| - Carob waste<br>( <u>Ceratonia si-</u><br><u>liqua L.</u> ) | fungi<br>( <u>Aspergillus niger</u> )   | Tate & Lyle, Reading  | England<br>Bélize ( C.A. )<br>Cyprus  |
| - Potato starch  | mixed yeast culture<br>( <u>Endomycopsis fibuliger</u><br>and <u>Candida utilis</u> )   | Swedish Sugar Cy.,<br>( SYMBA-process ), Eslov  | Sweden                                |
| - Citrus waste   | <u>Candida tropicalis</u>   |   | Israel                                |
| - Coconut waste  | yeast   |   | Philippines                           |

interest and can now easily compete with chemical hydrolysis procedures. Furthermore, bioconversion of the obtained glucose into fructose by microbial glucose isomerases is a rapidly expanding field and has recently resulted in the marketing of pure- and high-fructose corn syrups ( HFCS ). Glucose can also be converted to gluconate. Starch is also-since centuries- the starting material for brewing and distilling industries.

The potential of fructose to replace sucrose, when used on an equisweetness basis has become a reality. Pure fructose syrups, as opposed to high fructose syrups, have hitherto only attained wide utilisation in W. Germany ( Flarom Nahrungsmittel GMBM, 6800, Mannheim ), France ( Applexion, Roquette ) and Finland ( Finnish Sugar Cooperation, Helsinki ) but not yet in the Benelux.

Since cyclamate and saccharin are virtually rejected from food use, fructose is the only alternative left as a general purpose sweetener to sucrose. It can also find application in medicine for diabetic use and as wine sugar.

#### 2.3.1.c. Cellulose

Attention has recently been concentrated on cellulose as a carbon and energy source for growth and product formation by microorganisms.

As the primary means of harnessing the sun's energy, the photosynthetic process results in an estimated production of  $10^{11}$  tons of cellulose ( = 24 tons of cellulose per person per year ). Cellulose is the only organic material that is annually replenishable in such very large quantities.

We can look to the vast, annually renewed, amount of cellulose as a source of food for man, as a substrate for single cell protein and as a raw material for product fermentation. It is astonishing that cellulose has been practically unused for these purposes except as part of the fodder for ruminants and its use as paper, wood and a source of chemicals.

Enormous amounts of cellulose are readily available in the form of waste. Indeed cellulose is a major compound of

- agricultural wastes :

straw, stubble, leaves & stalks

hulls, peanut waste

shells, corncobs, bagasse, rice waste

- food processing wastes :  
fruit peels, pulp, coffee grounds  
vegetable trimmings, wine making residues  
slaughterhouse waste
- wood waste :  
brush, chips, bark, sawdust, papermill fines,...  
sulfite waste liquor
- municipal and domestic wastes :  
paper waste, urban refuse

The utilization of these resources is greatly simplified if cellulose is first hydrolysed into its monomer, glucose.

This conversion could be accomplished by either acid or enzymatic hydrolysis.

Acid hydrolysis procedures require expensive corrosion proof equipment and need a high temperature. Simultaneously, decomposition of the resulting sugars occurs so that yields of glucose are low and the syrups contain unwanted by-product and reversion compounds. The microbial or enzymic hydrolysis on the other hand is specific for cellulose and occurs in mild conditions.

However, the commercial application of cellulose fermentation systems is virtually non-existent at present. This dilemma can be traced back to the difficulty of satisfying process criteria for these systems at the technology level. A survey of pilot scale processes is given in Table 6.

#### a. Cellulase production

Cellulase-production ( 300 ton/year ) is so far mainly concentrated in Japan and the U.S.A.. Submerged as well as Koji-culture principles are used with Rhizopus, Trichoderma, Penicillium and Aspergillus strains.

Indeed, costly cellulase preparations from Trichoderma viride, Rhizopus and Aspergillus niger are commercialized as a digestive acid, and an additive in ruminant feed. Cellulases are up to now mainly used for the removal or weakening of unwanted cellulosic fiber from other materials such as protoplast

TABLE 6 : Pilot scale fermentations on cellulosic substrates.

| Cellulose source   | Microorganisms  | Product                               | Country  |
|--|---|---------------------------------------|--|
| Paper waste  | <u>Trichoderma viride</u><br><u>Clostridium thermocellum</u>  | Cellulase,<br>Glucose, SCP            | Natick Laboratories, Mass., USA;<br>Mass. Inst. Technol., Mass., USA |
| Rice straw hydrolysate                                   | <u>Trichoderma viride</u> ,<br><u>Candida utilis</u> ,<br><u>Saccharomyces</u><br>(Two step process)  | SCP & ethanol                         | Univ. California, Berkeley, Calif., USA                              |
| Bagasse  | <u>Cellulomonas flavigena</u> and<br><u>Alcaligenes faecalis</u>  | SCP                                   | Bechtel-Louisiana State Univ., USA                                   |
| Agricultural waste                                       | <u>Thermomonospora fusca</u> ,<br><u>Thermoactinomyces</u>  | SCP                                   | General Electric Cy, USA;<br>Univ. Pennsylvania, Pa., USA            |
| Milled Mesquite Wood                                     | <u>Pseudomonas JM 127</u>   | SCP                                   | Texas, USA   |
| Lignocellulosic waste                                    | <u>Sporotrichum pulverulentum</u><br>and <u>Candida utilis</u>  | SCP                                   | Swedish Forest Product Research Lab., Sweden                         |
| Coffee wastes  | <u>Trichoderma viride</u> ,<br><u>Myrothecium verrucaria</u>  | SCP                                   | ICAITI, El Salvador  |
| Manure   | Thermophilic actinomycete   | SCP                                   | Casa Grande, Arizona; General Electric Cy, USA                       |
| Corn, soy and pea processing waste<br>Straw & Wood waste | <u>Trichoderma</u> ,<br><u>Gliocladium</u><br><u>Agaricus bisporus</u> , <u>Pleurotus ostreatus</u> , <u>Volvariella</u> &<br><u>basidiomycetes</u> | SCP<br>Cellulose predigestion         | Green Giant Cy, USA<br>Tropical regions, Malaysia                    |
| Cellulosic waste   | Anaerobic mesophiles & thermophiles   | Methane, SCP, organic acids, solvents |  |

preparation of yeasts, fungi and higher plants, the controlled digestion of amorphous cellulase in wood pulp and the improvement of extraction procedures ( protein from soya, coconut, bean, yeast, or starch from potato or maize, invertase extraction from Daucus carota, oils and aromas from plants, clarification of citrus fruit juices, agar extraction from sea weed ) and for use in septic tanks and blocked pipes.

b. Metabolite production

Only a decade ago, attention has been directed towards the conversion of cellulose into useful compounds, such as glucose, ethanol, SCP or microbial products.

a.a. Methane

Methane can also be produced from cellulosic wastes ( see part 2.1. ) and used as an energy source or further converted into SCP, as seen from the following ( industrial ) realisations :

|          |  |   |   |
|----------|--|---|---|
| Methane  | - bacteria<br>( 1000 ton/year )<br><u>Pseudomonas</u> ,<br><u>Methylomonas</u> ,<br><u>Methylococcus</u> | Shell U.K.,<br>Sittingbourne  | U.K.                                    |
|          | ↓  |   |   |
| Methanol | - bacteria, yeast<br>( 100.000 ton/year )<br>- yeast   | Imperial Chemical<br>Ind., Teeside<br>Mitsubishi Gas,<br>Mitsui Toatsu,<br>Kyowa Hakko Kogyo,<br>Danippon Inc.,<br>Indemitsu<br>M.I.T., Mass. | U.K.<br><br>Japan<br><br><br><br>U.S.A. |

b.b. Glucose

At the U.S. Army Natick Laboratories, Mass., U.S.A., recent efforts to develop a practical process for microbial enzymatic saccharification of waste cellulose to produce cheap technical glucose are very promising. This development has its origin in

the prevention of deterioration of cellulosic army materials. Now, the concept has been extended to intentionally transforming municipal, agricultural and industrial cellulosic wastes into usable basic materials, stimulated by the new awareness of our depleting fossil resources ( oil & minerals ).

The produced crude glucose syrups can be used as a carbon source for a wide range of fermentation processes, such as SCP production ( food ), ethanol production ( fuel ), or the synthesis of useful microbial metabolites ( chemicals ) in general. The overall process is schematically represented in the figure 1.

At the Natick Laboratories, U.S.A., pure cellulose could be hydrolysed by a concentrated cellulase preparation from Trichoderma viride QM 9414 to yield 90% glucose syrups.

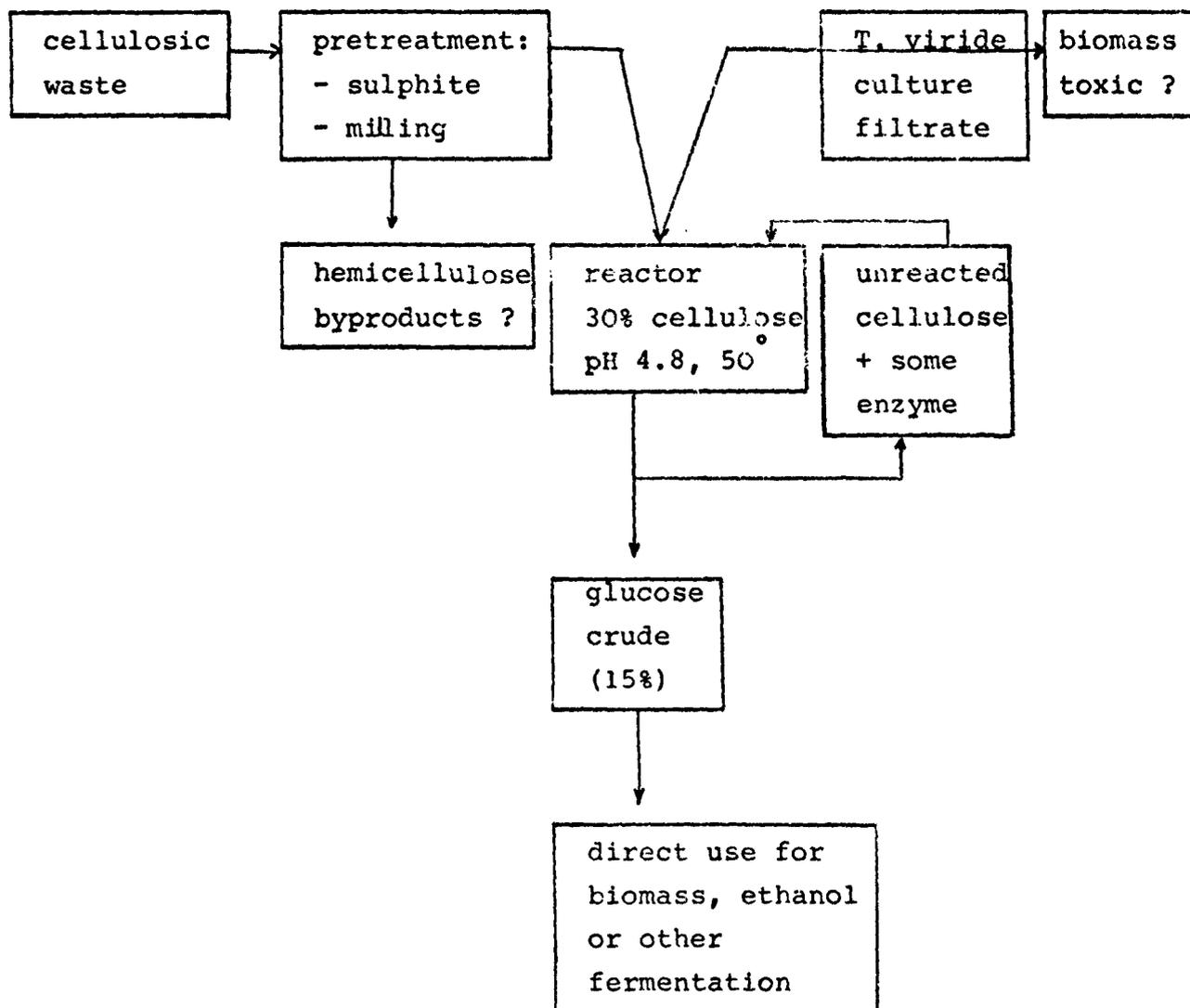
The bioreactor contained a 7 to 10% cellulose slurry and was anaerobically stirred ( 200 rpm ) at 55°C and pH 4.8, which avoided contamination.

In an evaluation study of crude cellulosic substrates, the Natick researches discern 4 cellulosic waste groups, as represented here below :

| Group | substrate evaluation | relative saccha-<br>rification (48 hrs) | example of<br>waste                        |
|-------|----------------------|---|--|
| I     | excellent            | > 0,85                                  | sulfite pulps                              |
| II    | good                 | 0,85-0,5                                | rice & sugar<br>cane waste                 |
| III   | fair                 | 0,5-0,9                                 | agricultural<br>wastes, corn,<br>lemon,... |
| IV    | poor                 | < 0,5                                   | coffee bean<br>hulls,...                   |

By far, the largest category of available cellulosic materials, are the agricultural wastes. Acceptable reactivity appears to be obtained only after pretreatment.

Natick Laboratory process



Natick Laboratory data, 1974

Substrate (5%) stirred in T. viride filtrate at 50° C

|                               | % saccharification in |                 |
|-------------------------------|-----------------------|-----------------|
|                               | <u>4 hours</u>        | <u>48 hours</u> |
| cotton fibre                  | 2                     | 10              |
| filter paper                  | 10                    | 60              |
| cellulose                     | 5-15                  | 62-91           |
| bagasse (27% lignin)          | 3                     | 6               |
| alkali-treated                | 23                    | 50              |
| milled newspaper (27% lignin) | 12-32                 | 27-55           |

Fig. 1 : Natick Laboratory process for cellulose hydrolysis.

Ball milling seems to be the best pretreatment of crystalline cellulosic materials as it reduces crystallinity, increases the surface area and bulk density; it is however an expensive procedure, disturbing the overall economy of the process.

So far, it seems that agricultural wastes, newspaper and municipal refuses require ball milling, while papermill wastes are readily available for microbial conversion.

Studies to find an inexpensive chemical or physical pretreatment method to reduce crystallinity remain an important priority. Removal of lignin or conversion of lignin is also inherent to this aspect. The pilot studies now being carried out should help to elucidate the economics of the overall process.

c.c. SCP  
...

Direct production of SCP from cellulosic wastes has been the subject of intense research but has not reached the industrial scale level yet. Some research projects are summarized in Table 6 and relative production costs of SCP plants are compared in Table 7. High protein prices might make several processes of economic interest:

- enzymatic hydrolysis followed by yeast or fungal fermentation
- growth of mesophilic bacteria ( mixed cultures ) on pretreated cellulose
- fermentation by thermophilic actinomycetes or basidiomycetes.

d.d. Other products  
.....

As there seems to be no correlation between cellulase activity and antibiotic production capacity in microbial strains, mixed fermentations were performed using fungal strains with cellulolytic activity and strains, producing antibiotics on soluble carbohydrates. In this way, penicillin could be produced in a cellulose mixed-fermentation ( Penicillium + Trichoderma or Aspergillus ).

In addition to cellulose degradation and cellulase production, many other enzymes can be produced such as :..amylases, proteases,.. from cellulosic materials, but also vitamins, amino acids, etc...

Undirect methods, using enzymatic cellulose-hydrolysates, and converting glucose into microbial protein or ethanol are only recently gaining full attention.

TABLE 7 : Relative Production Costs of 100.000 Metric Ton SCP/year.

|  | N-Paraffin | Gas Oil | Methanol | Methane | Cellulose<br>(Thermo-<br>actinomyces) |
|--|------------|---------|----------|---------|---------------------------------------|
| Capital $\$ \times 10^6$                 | 90.5       | 10.7    | 66       | 72      | 75                                    |
| Raw Material<br>c/kg                     | 11.4       | 9.0     | 13.5     | 5.6     | 3.1                                   |
| Utilities                                | 4.4        | 3.0     | 3.4      | 4.6     | 4.3                                   |
| Operation                                | 1.7        | 2.1     | 1.5      | 2.0     | 3.1                                   |
| Overhead                                 | 6.1        | 7.2     | 4.7      | 5.4     | 6.2                                   |
| Production cost<br>US $\$/\text{kg}$ SCP | 0.90       | 0.40    | 0.23     | 0.18    | 0.17                                  |

Pretreated rice straw ( 2 mm particles + 4% NaOH ) has been enzymatically hydrolysed with crude Trichoderma enzymes at 50°C. After deactivation of the cellulases, glucanases and chitinases in the crude hydrolysate, Candida utilis-SCP could be produced. A 500 ton per day plant could be profitable.

Newspaper hydrolysate has recently been converted into yeast and/or ethanol. Optimization of operational parameters and economic evaluations of such systems is in progress at the University of California, Berkeley, U.S.A. and at M.I.T., Mass., U.S.A.

### 2.3.2. Commercial Processes ( in The Netherlands and in Belgium ( The Flanders ) ).

#### 2.3.2.a. Sugars

- Gist Brocades N.V., Delft, N.

- molasses → yeast ( SCP )  
ethanol

- sugars → enzymes → β-galactosidase  
antibiotics cellulase  
solvents amylases  
steroids invertase  
nucleotides proteases  
vitamins glucose isomerase  
solvents

- Zuid Nederlandse Spiritusfabriek, Postbus 6, Bergen-op-Zoom, N.

- molasses → yeast  
ethanol

- Alcoholfabriek, Melasseweg 1, Delfzijl, N.

- molasses → yeast  
ethanol

- Coöperatieve Condensfabriek, Leeuwarden, N.

- whey → lactose → glucose + galactose

- Koninklijke Nederlandse Gist & Spiritusfabriek, Brugge, B.

- molasses → yeast  
ethanol

- La Citrique Belge, Tienen, B.

- molasses → citric acid  
( 120.000 ton/year )

- Bruggeman, Gent, B.
  - molasses → yeast  
ethanol
- Fromageries, Gervais, Jauche, B.
  - whey → SCP
- R.I.T. ( Smith Kline & French ), Rixensart, B.
  - starch → antibiotics
  - sugar

### 2.3.2.b. Starches

- Numerous brewing and distilling companies
- Gist Brocades N.V. Delft, N.
  - starches → glucose enzymes, ...  
ethanol
- Koninklijke Nederlandse Gist & Spiritusfabriek, Brugge, B.
  - starches → enzymes ( proteases )  
alcohol
- Corn Products Company, Westkade, 119, Sas van Gent, N.
  - corn starches → glucose → gluconaat  
fructose
- AVEBE, Herengracht, 209, Postbus 3661, Amsterdam, N.
  - potato starch → glucose → gluconaat
- Koninklijke Scholte Honig ( KSH ), Koog aan de Zoon & Groningen, N.
  - potato starch → glucose → High Fructose Corn Syrup (HFCS)
  - corn starch → HFCS
- Bruggeman, Gent, B.
  - starches → ethanol
- AMYLUM, Van Wambekekaai, 13, 9300 - Aalst, B.
  - corn & wheat starch → glucose → fructose  
( 400.000 ton/year )

### 2.3.2.c. Cellulose

Industrial exploitation of cellulose hydrolysis-processes has to await further fundamental and applied research. Only laboratory scale cellulose-hydrolysis and conversion studies are being performed at this moment at the Universities of Gent, Louvain ( B ) and Wageningen ( N ).

### 2.3.3. Status of Research and Development.

#### 2.3.3.a. Sugars

This part falls rather within the field of the classical ( industrial ) fermentation science, and will not be discussed here in detail.

A big problem with all molasses fermentations is the disposal of residues. Evaporation, secondary anaerobic fermentation, protein separation and desalination of these residues are now intensely tried out.

The use of thermophilic yeast strains with a high and fast flocculation capacity will greatly improve the overall economics of these SCP-processes.

The microbial production of solvents from wastes, rich in sugars, should gain renewed impetus with increasing oil prices.

#### 2.3.3.b. Starches

The use of immobilized enzymes, and recently immobilized intact cells as amylase, amyloglucosidase, glucose oxidase or glucose isomerase source for starch hydrolysis and glucose conversion has brought a revolutionary approach to this field.

Recycling of dilute starchy wastes in immobilized amylase reactors, coupled with ultrafiltration units, might yield glucose concentrates.

The limit of equilibrium with most glucose isomerase enzymes is situated around 55%, and the economical conversion is about 42-44%. It has been shown that addition of borate allows conversions up to 80%.

Inulin, a fructose polymer abundant in plants ( such as Jerusalem artichoke ), and levan, a fructose polymer in certain microbes, could be used as alternative fructose sources. Fructose is also a by-product of commercial microbial dextran production from sugar by Leuconostoc species.

Cultivating photosynthetic bacteria ( PSB ) as SCP on a mass-scale on food processing wastes or other agricultural by-products also appears to be a reasonable alternative. Preliminary studies have indicated that photoorganotrophic bacteria may be profusely cultured in crude extracts, prepared from bananas, potato starch waste, wheat and rice bran. A flow sheet of photosynthetic SCP and product synthesis from waste is represented in Fig. 2.

#### 2.3.3.c. Cellulose

The microbial conversion of cellulose to glucose has long been known as a very slow and unefficient process, but recent developments have changed this negative view into an optimistic one. Due to pretreatment of the cellulose, and the discovery and development of highly active enzyme preparations, the above described processes look promising for industrial application.

The preparation of concentrated cellulose-suspensions ( up to 50% w/v ) as a result of grinding, ultrasonic rupture, milling, chemical treatment,  $\gamma$ -irradiation or heating allowed the preparation of highly concentrated glucose-syrups, which in turn lowered the process costs. Highly active mutants of Trichoderma viride are by now the most promising extracellular cellulase producers.

Classical batch experiments, are soon to be followed by continuous procedures for the saccharification of cellulose and by the use of immobilized cellulase-reactors.

By coupling with an ultrafiltration unit, a continuous harvesting of glucose has become possible from anaerobic multi-stage continuous bioreactors. Adsorption of cellulase to a cellulose column allowed also for a continuous recycling, yielding 14% glucose syrups. Milled newspaper ( 10% w/v ) yielded up to 6,6% syrups in 48 hrs.

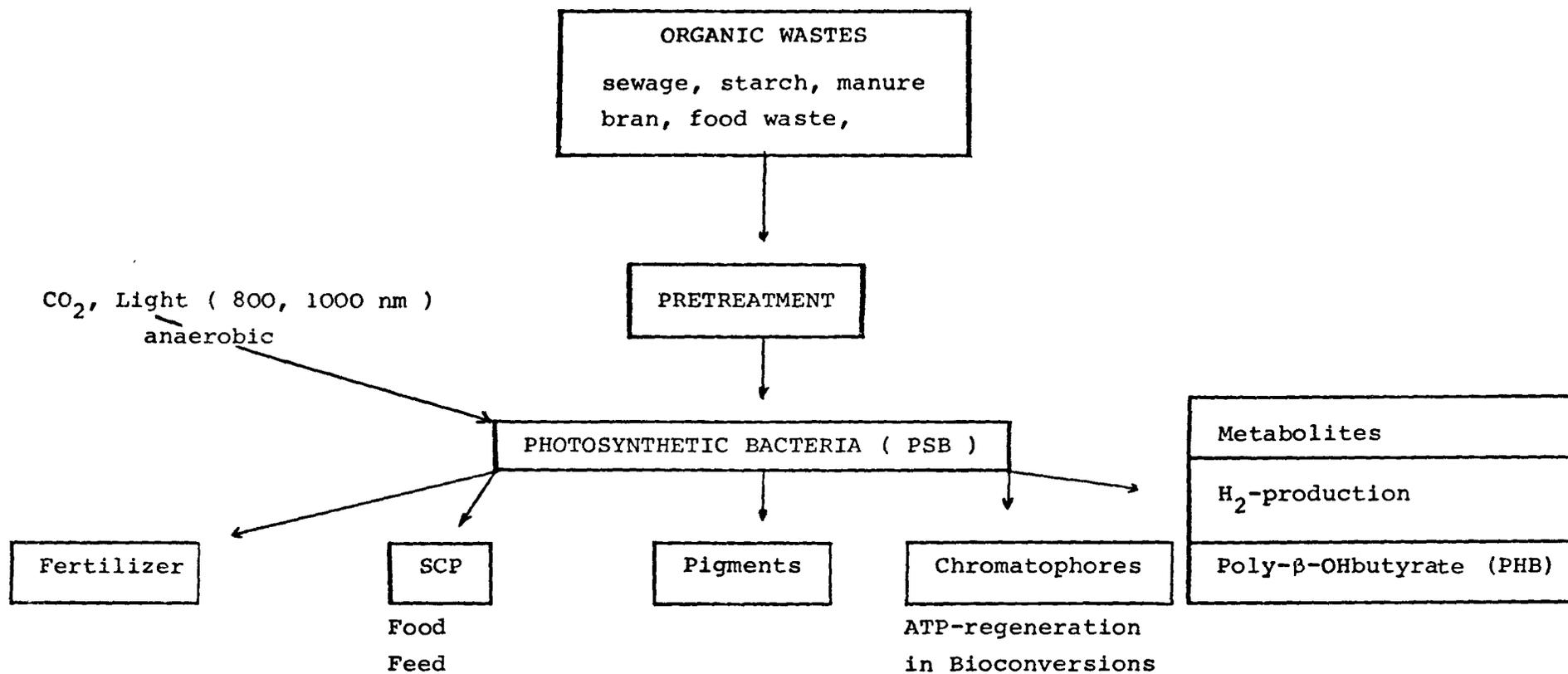


Fig. 2 : Flow sheet for photosynthetic bacteria-SCP and product synthesis.

Auto-saccharification of delignified wood-waste by fungi in surface cultures can help to reduce the high costs brought in by the use of commercial cellulase preparation.

In some materials ( vegetable and corn waste ), cellulose is accessible enough to allow direct fermentation with no pretreatment. An open ditch annular fermentor has been described in the U.S.A. to handle waste from processing corn, soy and pea. High BOD-wastes are attacked by fungi to yield SCP combined, with an efficient disposal system. This is in essence a low-technology system capable of adaptation to many countries.

Filamentous aerobic thermophilic actinomycetes ( Thermoactinomyces ) have only recently gained attention as cellulose digestors. These strains can utilize lignin or lignin-cellulose complexes, have a higher rate of digestion than mesophiles, and grow at " pasteurization " temperatures, ( eliminating pathogenic contaminants during fermentation ) and allow also an easier temperature control of the fermentors or reactors.

Many cellulase producing strains display a high maintenance requirement ( 0,05 g cellulose/g cells/hr ), suggesting that in many cases about 50% of the substrate is utilized for maintenance. So maintaining high growth rates - where then diffusional resistance to oxygen must be overcome - seems to be essential throughout the fermentation to improve the cell, product or enzyme yield.

Attention should also be paid to the use of mixed ( axenic ) cultures for SCP production from cellulose, where for instance cellobiose as an accumulating intermediate-causing catabolite repression of cellulase synthesis - is readily consumed by a second non-cellulase strain, thus relieving the repression effect.

The availability of stable and highly active cellulase preparations or producing strains is indeed a prerequisite for promising developments in this fascinating field.

#### 2.3.4. Economic Evaluation.

##### 2.3.4.a. Sugars

Fermentation of molasses, whey, sulfite liquor, malt and similar sugar wastes has proven already its economic viability as well for biomass ( SCP ) as ethanol and metabolite production.

Molasses has steadily increased in price lately, and can also be used directly as a cattle feed, however.

Recently, Brazil has started a nationwide program for ethanol fermentation from different types of sugars wastes, to be used as an energy source and as a feedstock for its chemical industry. The price of ethylene derived ethanol has now reached a level such that ethanol produced from cheap or waste carbohydrates should be competitive. In India, I.C.I. produces polyethylene from molasses-alcohol at a scale of 15.000 tons a year.

Whey - once considered as a waste product but now mainly spray dried - can easily be recovered economically by ultrafiltration. Fermentation plants handling 50 to 100 m<sup>3</sup> per day are already economical ( yeast yield on whey ± 2%, waste water-BOD : 1000. A continuous microbial process has been described for lactic acid production from whey and dairy wastes, using a fixed-film anaerobic upflow bioreactor.

#### 2.3.4.b. Starches

Microbial hydrolysis of starches into glucose and subsequent conversion into fructose or gluconaat are now well established processes.

As well the Koji-method ( Aspergillus, Rhizopus amylases on grain ) as the sophisticated enzymatic methods proof to be competitive for glucose production.

Ethanol production from starchy wastes is being realised on a big scale in Brazil as a feedstock and gains interest in many other countries.

For SCP-production from starches, Aspergillus, Penicillium, Fusarium or Rhizopus strains are mainly used, but one should switch to other fungi such as Mucor species, which are not known to produce toxins or antibiotics !

The Symba-SCP-process, when looked at it just as a water purification system, costs ± \$ 0,50/ton of starchy waste, compared with \$ 6-10/ton by the conventional processes. This process could in principle be adapted for SCP-production from any starchy waste, containing 0,5 to 2% starch. Alternatively, too dilute starchy wastes could be converted by low-grade amylases to sugars, which then can be fermented anaerobically to ethanol or organic acid-rich liquids.

#### 2.3.4.c. Cellulose

Few economic studies have been performed on cellulose fermentations. It is claimed that a 100 ton per day scale can be economically feasible for bioconversion of cellulose to glucose, to ethanol or to SCP. Glucose produced from paper waste would cost about \$ 2 per ton, which is indeed an economical price ! With Thermoactinomyces, it was found that a volumetric productivity of 2 g cells/1/hr, yields of more than 0,35 g cells/g cellulose utilized and a 90% total conversions of the cellulosic substrate were needed to be economically feasible.

Biomass ( SCP ) production is probably not economical as an end in itself with a scale of less than about 50.000 ton/year of product. Microbial metabolite production from polymer carbohydrates such as cellulose should therefore be a major area of interest in the coming years.

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**2.4. PROTEIN RECOVERY FROM ORGANIC WASTE WATER**

## ABSTRACT

A number of wastes either are very rich in proteinaceous matter or can easily be converted to proteins.

A variety of techniques to recover proteins from organic wastes have been developed. At present this stage, only the so-called Alprecin process applied to slaughterhouse waste waters, is economically attractive. Preliminary research data with regard to a special procedure to recover proteins from animal manure ( acidic precipitation of cellular proteins ) are presented.

From this, it appears that a number of other highly organic waste waters should be examined to see whether they can be treated by this process. However, in view of the fact that commercial production of proteins stands only an economic chance at a scale of 5000 tons/year, it is felt that the production of protein from waste should only be advocated in those situations where the protein directly can be refed within the boundaries of the waste producing plant.

As whole, the potentials to recover proteins from organic wastes seem to be majorly undeveloped.

#### 2.4.1. Raw Materials and End Products.

Waste waters which are rich in proteinaceous compounds can be treated so that a considerable amount of the protein is recovered. These low-quality proteins can then be used for animal feed.

In an attempt to assist planners and policy makers to obtain a clear view of the scope and limitations of a chosen process, Stanton ( 1977 ) has made up a list of factors to be considered. These factors relate to the type of waste and to the type of process considered. Of particular importance with regard to the problem of protein recovery from wastes are the following factors :

- i) what is the fraction of " crude "versus useable protein and what is the amino acid spectrum of the protein ?
- ii) is the product non-toxic, free of pathogens and acceptable to animals ?
- iii) is the technology involved relatively simple or does it require special sterilization, separation and handling techniques ?
- iiii) what are the socio-economic advantages/disadvantages of using the waste ? How does the negative environmental value of the waste compares to the overall treatment costs and the value of the protein recovered ?

In the sections which follow, some of the most promising processes for protein recovery from waste waters will be considered with regard to their current state of technology.

##### 2.4.1.a. The acidic precipitation of the proteins as such

The Alprecin Process for protein recovery from meat and fish industry waste waters is based upon protein recovery with ligno-sulphonic acid at pH 3, followed by dissolved air flotation; the flotated material is concentrated together with blood and dried with other products in a rendering plant. The safety of the recovered product in animal feed has been established and the efficiency of the system for effluent treatment has been demonstra-

ted at about 80% BOD removal.

The economics of the process are usually good for units above a minimum size - for example plants with a throughput of more than 300 cattle or 20,00 chickens per day.

#### 2.4.1.b. The acidic precipitation of cellular proteins

The waste compounds are transformed into microbial biomass. This biomass is harvested and concentrated by means of an acidification procedure. The acidic biomass slurry can be used to supplement animal feeding rations.

#### 2.4.1.c. The production of fungal protein from liquid waste

The liquid waste is treated in a continuous fermentor with a relatively short residence time under conditions which favor the development of fungal biomass. The fungal biomass is harvested dried and used as animal feed.

A variety of process schemes are known :

- a. Acidification of the liquid waste to pH 3.0-4.0 and treatment as such at pH 3.0-4.0 under conditions resembling those of classical activated sludge. Research at the University of Gent is currently under way with regard to production of yeast protein from piggery wastes by this process.
- b. Acidification of the liquid waste to pH 3.0-4.0, inoculation with Candida utilis at 10%, and fermentation during 15-24 hours. Tomlinson ( 1976 ) has published some preliminary results with regard to this process scheme. It must be considered as a pretreatment of the waste water.
- c. Acidification of the liquid waste to pH 3.0-4.0, inoculation with a thermophilic fungus and fermentation at a temperature of 45°C. Meyrath ( 1975 ) showed that various yeast strains converted 95% of the COD present in molasses to biomass. These yeasts were grown at a ML-concentration of  $\pm 15 \text{ g VSS l}^{-1}$  at a dilution rate of  $\pm 1 \text{ hr}^{-1}$ . The author mentioned that virtually no bacterial contamination occurred. Seal and Eggins ( 1976 ) found that pig waste could be upgraded by aerobic treatment at 50°C after acidifying it to pH 4-5. They worked at a hydraulic retention time of 10 days and could by means of this

process, convert the slurry to an odourless product which was usable as an animal feedstuff. Finally, it should be mentioned that in the Netherlands, the Megista Workgroup is looking along the latter lines to fractionate and convert animal wastes into a proteinaceous feedstuff.

#### 2.4.1.d. Extraction of protein from activated sludge

To avoid the hygienic and toxicological aspects inherent to the use of sewage sludge as animal feed, the sludge is subjected to an extraction procedure for proteins. Subsequently, the protein-concentrate is used as animal feed.

#### 2.4.2. Commercial Processes.

##### 2.4.2.a. The acidic precipitation of the proteins as such

- N.V. van het Fabriek van A. van Dijk, Lopik, N.
  - Margarine production
- Klaasen & Co., Ravels, B.
  - Poultry processing
- Exportslachthuis, N.V., Kosterstraat 1, Westrozebeke, B.
  - Slaughterhouse

##### 2.4.2.b. The acidic precipitation of cellular proteins

The University of Gent has, in collaboration with several private firms, constructed a treatment plant for piggery waste waters. This plant treats the manure daily produced by 1000 pigs. The slurry is first centrifuged to remove the coarse solids. The centrate is composted while the liquid phase is treated in an activated sludge basin ( $\theta_H = \theta_H = 15-30$  days, volumetric loading rate  $0,3 \text{ kg BOD/kg/m}^3 \cdot \text{day}$ , sludge loading rate  $0,05 \text{ kg BOD/kg/sludge per day}$ ). The mixed liquor of the activated sludge basin ( $6 \text{ m}^3/\text{d}$ ) is acidified with sulfuric acid to pH 3. In the sedimentation tank, the sludge is allowed to settle. The sludge is withdrawn and stored while the supernatant is neutralized with  $\text{Ca(OH)}_2$  and after final filtration upon a sand bed, is discharged.

The results of this treatment procedure are summarized in Table 1. The total BOD reduction amounts to 99%, while the N and P reductions amount to 95 and 96% respectively. Of the daily volume treated, 75 percent is discharged and 25 percent is retained as a protein slurry.

The acidic sludge slurry has a concentration between 4-5% dry solids. The composition of these solids is given in Table 2 and 3. It is utmost importance to note that this slurry, due to the aerobic pretreatment, is free of obnoxious odours and due to the acidic pH, is stable. The acidic conditions bring about a severe die-off of all residual faecal bacteria. The slurry is acceptable to animals. It is furthermore not toxic and it does not cause allergies or other negative effects during handling and feeding. The acidification has brought about a partial hydrolysis of the microbial cells and as a consequence the digestibility of the proteinaceous matter amounts to a relatively high level of 70 percent.

2.4.2.c. The production of fungal protein from liquid waste

None to our knowledge.

2.4.2.d. Extraction of protein from activated sludge

None to our knowledge.

2.4.3. Status of Research and Development.

2.4.3.a. The acidic precipitation of the proteins as such

The process as such is technologically well designed. The topics which require further research are :

- the concentration and drying procedures used to dewater the proteinaceous slurry collected from the Alprecin flotator.
- the digestibility of these dried sludges and the effect of the Alprecin additive there upon.

2.4.3.b. The acidic precipitation of cellular proteins

So far, the treatment procedure outlined is the only one which permits to obtain from highly concentrated organic waste waters ( BOD-levels of 10-20 g/l ) a dischargeable effluent.

TABLE 1 : Average analysis of the principal characteristics of piggery waste treatment plant

|                                     | Influent   | after<br>centri-<br>fugation | mixed<br>liquor | acid<br>effluent | surplus<br>sludge | neutral<br>effluent | final<br>effluent |
|-------------------------------------|------------|------------------------------|-----------------|------------------|-------------------|---------------------|-------------------|
| pH                                  | 6.5±0.86*  | 6.9±0.8                      | 7.5±0.3         | 2.7±0.6          | 2.8±0.6           | 7.05±0.4            | 6.7±0.3           |
| DW g/l                              | 31.8±5.9   | 19.8±4.2                     | 18.3±0.49       | 12.5±0.59        | 43.3±6.1          | 10.3±1.7            | 9.7±1.6           |
| SS g/l                              | 24.5±5.0   | 12.6±1.8                     | 12.3±1.2        | 0.3±0.15         | 35.5±4.7          | 0.37±0.25           | 0.017±0.010       |
| BOD ppm                             | 26600±9000 | 17500±2500                   | 13300±1800      | 906±160          | -                 | 638±49              | 530±110           |
| K <sub>J</sub> N ppm                | 2500±650   | 2050±500                     | 775±170         | 110±84           | 2060±220          | 90±16               | 85±17             |
| NH <sub>4</sub> <sup>+</sup> -N ppm | 1600±420   | 1230±410                     | 125±34          | 40±34            | 63±37             | 40±20               | 29±10             |
| NO <sub>2</sub> <sup>-</sup> -N ppm | -          | -                            | 15±1.2          | 0.8±1.0          | -                 | -                   | 0.7±0.4           |
| NO <sub>3</sub> <sup>-</sup> -N ppm | -          | -                            | 42±20           | 41±19            | -                 | -                   | 31±14             |
| SO <sub>4</sub> <sup>--</sup> ppm   | -          | 1960±400                     | 1790±290        | 6600±930         | 5900±1300         | 4700±890            | 4280±730          |
| tot P ppm                           | -          | 455±136                      | 472±45          | 430±41           | -                 | 60±24               | 18±7              |

\* 95% confidence limit

TABLE 2 : Composition of the acidic sludge slurry

|                    | <u>% on the dry solids</u> |
|--------------------|----------------------------|
| Crude protein      | 31                         |
| True protein       | 22                         |
| Fat                | 6                          |
| Carbohydrates      | 10                         |
| Cellulose + lignin | 24                         |
| Ash                | 11                         |

TABLE 3 : Amino-acid spectrum of the acidic sludge

| Amino-acid     | % of the dry matter<br>of the slurry | % of the true<br>protein |
|----------------|--------------------------------------|--------------------------|
| Leucine        | 2.07                                 | 9.2                      |
| Isoleucine     | 1.22                                 | 5.4                      |
| Valine         | 1.78                                 | 7.9                      |
| Phenylalanine  | 1.08                                 | 4.8                      |
| Glycine        | 1.86                                 | 8.3                      |
| Alanine        | 1.80                                 | 8.0                      |
| Proline        | 1.66                                 | 7.4                      |
| Threonine      | 1.26                                 | 5.6                      |
| Methionine     | 0.38                                 | 1.7                      |
| Serine         | 1.15                                 | 5.1                      |
| Cystine        | 0.32                                 | 1.4                      |
| Tyrosine       | 0.77                                 | 3.4                      |
| Asparatic acid | 1.78                                 | 7.9                      |
| Glutamic acid  | 2.36                                 | 10.5                     |
| Lysine         | 1.15                                 | 5.1                      |
| Arginine       | 1.31                                 | 5.8                      |
| Histidine      | 0.54                                 | 2.4                      |
| Total          | 22.49                                | 99.9                     |

Indeed, the sludge separation procedure makes it possible to obtain clear effluents from mixed liquors containing up and above 10 g/l suspended solids. The sludges obtained have been shown to be acceptable for animal feeding. Furthermore, the process appears to be economically viable.

The process outlined has not been examined in great detail. It certainly merits fine tuning in the near future. Attention should be focused upon the acceleration of the aeration phase and the technological improvement of the sludge separation phase. In particular, one should examine whether unit processes such as lamellar sedimentation and flotation could be used to harvest the acidic sludges. Finally, the practical aspects of the refeeding of the acidic proteinaceous sludges require more in depth studies.

From these considerations, it appears that a number of other highly organic waste waters should be examined to see whether they can be treated by this process. In particular waste waters from fermentation plants and food processing industries should be examined in this perspective.

#### 2.4.3.c. The production of fungal protein from liquid waste

- A variety of aspects urgently need further research, such as :
- the environmental factors which govern the conversion of the organic waste into yeast biomass.
  - the technological problems associated with the harvest of the yeast produced.
  - the quality of the yeast fodder.
  - the planological aspects of transport of waste materials to a full-scale treatment plant.

It is felt that this is a high priority research area.

#### 2.4.3.d. Extraction of protein from activated sludge

From an academical point of view, this process merits further research. From a practical point of view however, the implications of the " sludge residue after extraction " make it doubtful that this process will be economically feasible.

#### 2.4.4. Economic Evaluation.

##### 2.4.4.a. The acidic precipitation of the proteins as such

( Case-study, calculated after Voorburg, 1972 ).

The costs, for a relatively large installation with a capacity of 1000 m<sup>3</sup>/day can be estimated as follows :

|   |                  |
|---|------------------|
| Investment ( machinery, installment, etc... )               | 10.000.000,-B.F. |
| Yearly costs ( mortgage, chemicals, manpower, maintenance ) | 2.700.000,-B.F.  |

Such an installation, working on slaughterhouse waste water, reduced the BOD of the water from  $\pm$  1600 mg/l to  $\pm$  300 mg/l.

For every kg BOD removed, 0,8 kg sludge d.s. was produced.

Hence, the latter installation can produce about  $1.3 \times 0.8 \times 1000 \times 365 = 850.000$  kg sludge/year.

The composition of the sludge is given below :

|                     | Alwatech-<br>sludge | Meat<br>meal |
|---------------------|---------------------|--------------|
| Raw protein         | 59.4                | 60.3         |
| Raw fat             | 15.7                | 15.1         |
| Raw fiber           | 0.4                 | 0.0          |
| Other carbohydrates | 20.8                | 2.9          |
| Ash                 | 3.7                 | 21.6         |

Hence, the sludge compares well with meat meal. Its value is estimated at  $\pm$  650,- B.F./100 kg. As a consequence, the recovery of proteinaceous sludge by means of the Alprecin process appears attractive because it permits to recover a total value of  $\pm$  5.500.000,- B.F./year. It should however be understood that, to the yearly costs specified above, the costs for the concentration and drying of the sludge slurry ( usually 10-15% d.s. ) must be added. No data are known to us with regard to the latter process.

#### 2.4.4.b. The acidic precipitation of cellular proteins

The overall balance of the treatment system outlined above can be presented as follows :

- of every 100 kg of organic matter which are biologically treated about 15 kg are lost during the aeration process and 85 are retained in the form of sludge;
- the acidification procedure permits to recover 90 up to 100% of this sludge;
- of the sludge ( dry solids ), 25 to 30% is protein;
- this protein has a digestibility which is comparable to that of hay. Hence it can be considered to equal the economic value of the latter proteins, that is 12,- B.F./kg crude protein.

From this, it is clear that the treatment permits to recover for animal feeding ca 20 kg of raw protein or the equivalent of 240,- B.F. from the biological treatment of 100 kg of organic waste organic matter. The total treatment costs ( investment + maintenance + chemicals + electricity ) have been found to amount to  $\pm$  1200,- B.F./100 kg organic matter treated. Hence, the recovery procedure represents  $\pm$  20 percent of the total treatment costs.

#### 2.4.4.c. The production of fungal protein from liquid waste

On the basis of his laboratory results, Tomlinson ( 1976 ) tried to evaluate the economic feasibility of producing Candida yeast from highly organic waste waters. He noted that his process ( see b ) could " pay for itself " for industries as distillery, brewing, beet-sugar processing and potato processing. He also pointed out that only a large scale plant producing about 5000 ton yeast/year stands a economic chance.. Similary, Surucu et al. ( 1975 ) stressed the need for large scale approaches in order to make the process profitable.

#### 2.4.4.d. Extraction of protein from activated sludge

Verachttert and Houtmeyers ( 1976 ) have developed a method to extract proteins from activated sludge. They could extract about 30-40% of the protein available in sludges from biofilters, activated sludge basins, and oxydation ditches. The protein appeared to be of a high nutritional quality. The authors refrained from any detailed economic evaluations.

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#### Addresses

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## **PART 3**

### **COMPETITIVE PROCESSES**

No processes other than those outlined by the committee and treated above were considered competitive by the research team in the realm of utilization of organic waste materials.

COMMISSION OF THE EUROPEAN COMMUNITIES  
(Directorate General XII - Research, Science, Education)

FERMENTATION - HYDROLYSIS PROCESSES  
FOR THE  
UTILISATION OF ORGANIC WASTE MATERIALS

Report by

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1. INTRODUCTION

Growing environmental awareness is bringing waste materials from production and processing into the field of general public interest. In many instances, these are still considered as material for which conventional disposal is cheaper than further processing. The threat of a deterioration of the environment has slowly given rise to the belief that there exists a vital future task for technology either to develop low-waste production processes or to improve, by suitable methods, the low potential value of wastes to the point where recovery can be economically achieved. This development is stimulated further by the world-wide scarcity and increasing cost of raw materials of all kinds, necessitating optimal use of the available reserves.

A contract for the study of "Fermentation and Hydrolysis Processes for the Utilisation of Organic Waste Materials" was given out by the Commission of the European Communities in order to obtain a general overview of the practice and economic prospects of recovering organic waste materials with the aid of micro-biological processes.

The waste materials which enter into question with such processes originate from:

- vegetable and livestock production,
- the processing of vegetable and livestock products into food and related products,
- the cultivation and processing of wood.

Urban waste such as household refuse and sewage sludge represent a special case. The valuable materials contained therein are essentially:

- carbohydrate
- fats
- protein

The contribution below deals only with the circumstances in the Federal Republic of Germany.

2. RECOVERY OF ORGANIC RESIDUES

Currently four possibilities can be considered in relation to the treatment of biogenic residues by fermentation:

1. Anaerobic processes.
2. Aerobic processes (composting).
3. Protein recovery (Single Cell Protein).
4. Competitive processes.

With regard to 1:

This refers above all to the decaying of sludge to recover methane (biogas) (energy recovery). In addition, fermentation processes may be carried out, by which secondary raw materials can be won from biogenic residues.

With regard to 2:

With aerobic fermentation processes for the recovery of secondary products, composting processes are particularly important. The composting of organic residues well describes the oldest form of treatment. Notwithstanding the abundance of available experience, further intensive work in this area is taking place, but, as with methane recovery, composting offers only modest economic incentives until now.

With regard to 3:

Protein recovery from the raw materials under consideration, as a possibility for solving residue problems, is at an early stage of development in the Federal Republic of Germany. This form of exploitation is technically very variable and offers attractive economic possibilities.

With regard to 4:

Here, by competitive processes, is meant those processes which do not rely exclusively on fermentation, or use waste materials as a substrate.

These characteristically aim at resource conservation. Examples are:

- SCP recovery on an n-alkane or methanol basis.
- the cultivation of other rapid growth organisms based on organic residues.
- the recovery of metals with the aid of micro-biological processes (leaching-processes).

2.1. Anaerobic Reduction of Organic Residues

Anaerobic reduction processes are distinguished by the fact that they take place only with the rigorous exclusion of oxygen. The technology employed demands completely de-aerated nutrient solutions, air bubble-free, sealed reactors, oxygen-free gaseous atmospheres in vacuum desiccators, the application of oxygen absorption agents and the assistance of other agents. Generally, supplementary reduction agents are admixed to the carefully prepared nutrient solutions in order to immediately render harmless small quantities of in-flowing oxygen. This care is necessary as oxygen has a toxic effect on the anaerobic organisms (1,2).

From the microbiologically well-known anaerobic processes, only a few are suitable for the treatment of biogenic wastes. Both liquid and solid residues occur in the form of mixtures so that actual fermentation is only possible after extensive pre-treatment or, where these are allowed to mature as a complex substrate, the products must be extracted using suitable subsequent treatment. The production of methane by fermentation and the recovery of manure and supplementary feedstuff represent exceptions, where neither the input raw material nor the end product needs to be homogeneous.

### 2.1.1. Raw Materials and Products.

The initial materials for anaerobic processes are determined by the desired product and are more specifically set out in the description of technical processes.

This mainly refers to:

- organic sludges from the food industry
- sewage sludge
- sugar and starch-containing sludges and effluents
- molasses
- liquid and solid faecal matter from livestock production
- cellulosic residues

Essentially, the following products are recoverable from these organic residues with proven anaerobic processes:

- methane
- alcohol (Ethanol, butanol, glycerin)
- carbonic acids
- hydrogen

Under present circumstances, only methane and ethanol recovery appear to be of economic interest. Yet there is the disadvantage that some processes produce considerable quantities of sludge with a solids content of ca. 20%, the disposal of which creates new costs. Among other things, lower carbonic acids arise as intermediate products of the anaerobic reduction of biological sludges. Theoretically, it is possible to utilise these as a substrate for recovery of SCP, after draining from the system. However, such a process has until now not yet been developed.

#### 2.1.1.1. Agricultural Residues

##### Raw Materials

The conversion of organic materials to end-products which, under ordinary operational storage conditions, remain biologically and chemically stable, can be achieved by anaerobic reduction, of which the most important process stage is represented by methane-fermentation.

Biochemically determined process conditions enable an evaluation to be made of the organic matter in respect of its suitability for controlled anaerobic decomposition. Accordingly, in the agricultural field, the excreta (faecal matter and urine) of agricultural livestock and all vegetable residues and waste matter (e.g. straw and beet-leaves) are in principle suitable. There emerges, for the individual typical groups of materials, a high degree of variability in the quantities of gas attainable and in the corresponding periods needed for complete decay, in relation to their nature and the proportion of carbohydrate-containing components. The duration required for complete decay depends not only on the composition of the material, but also on the free surface area of the organic solids dispersed in the liquid, that is the degree of mechanical disintegration.

In principle, organic matter in solid phase will also ferment, as long as there is sufficient moisture present. As the distribution and close-mixing of the bacteria and substrate in the solid phase cannot be secured, the technical fermentation of solid mixtures of materials has no significance in practical terms. Solid organic matter needs to be dispersed in liquid in sharply comminuted form so that, apart from giving the maximum surface area possible for the individual particles, the viscosity of the overall suspension affords trouble-free degassing, mixing and hydraulic conveyance. Hence, vegetable matter is processed with cutting, shredding or crushing equipment in order that particle sizes less than 100 mm and extensive additional mechanical reduction of the haulm and leaf portions are achieved.

### Products of Fermentation

End products of methane fermentation are the solid as well as liquid fermentation residues (bio-sludge) and the fermentation gas (biogas).

#### Bio-sludge.

The bulk of the bio-sludge stems from the substrates supplied, which are reduced in quantity by the partial conversion of the organic mass into biogas (between 30% and 50%). Process conditions act against further losses in mass. The value of the bio-sludge as fertilizer depends on its material composition. With methane-fermentation, the available quantity of nitrogen is preserved in the final substrate. Phosphorous and potash are found again intact in the end-product. In contrast, the carbon content is reduced according to the degree of final fermentation. The limiting of the C/N ratio in this way means that no shortage of nitrogen occurs for the metabolism of bacteria in the soil. However, with long-term storage of the fermentation sludge in open containers nitrogen develops into waste gas in the form of ammonia.

The feed value of bio-sludge should not be overstated inasmuch as, with technical methane-fermentation, digestion is extensively carried out in the final material conversion process and consequently the feed-value of certain contents is reduced. To a large extent, the feed-value would seem to be mainly confined to the fixed-protein in the biomass.

#### Biogas.

The composition of biogas is dependant upon the contents of the original substrate as well as the degree of fermentation (and thereby the time for fermentation). The main components are  $\text{CH}_4$  and  $\text{CO}_2$ .

The complete fermentation of agricultural matter delivers up to 80%  $\text{CH}_4$  and the rest consists of  $\text{CO}_2$  and traces (respectively less than 1%) of  $\text{H}_2$  and  $\text{H}_2\text{S}$ . In the initial stage of fermentation, the  $\text{CO}_2$  component can amount to more than 50% and with continuous operation, less than 40%, with correspondingly lower values for

CH<sub>4</sub>. Of the trace components, H<sub>2</sub>S requires particular attention on account of its toxicity, odour intensity and corrosive effect. The CO<sub>2</sub> concentration adversely affects the heating value. The lower heating value of fermentation gas produced under varying technical conditions, lies between 20 and 22 MJ/m<sup>3</sup>. Biogas can be employed as heating gas as well as a fuel for combustion engines. In each case, it is necessary to remove the H<sub>2</sub>S content to reduce corrosion.

The gas yield depends on the technical conditions under which the process operates. The theoretically achievable quantity of gas per unit mass of dry, organic matter is determined by the material composition of the organic matter. Very different fermentation periods are required for the complete release of gas from individual groups of materials, in the course of which, for each fermentation period, the production of gas initially rises steeply and, after reaching a maximum, gradually diminishes. The main portion of the achievable gas quantity thus occurs in an appreciably shorter fermentation time. Accordingly, the technical fermentation period is reduced on economic grounds to about 30% of the time for complete fermentation. With agricultural matter, one obtains in this way, for fermentation temperatures of around 30°C, fermentation periods of 10 to 15 days with a 30 to 40 % degree of fermenting-out. In this regard, gas quantities of between 0.2 and 0.4 m<sup>3</sup> for each kg. of organic mass are released. The gas yield, for a given fermentation period, can be controlled by varying the loading of the fermentation chamber (organic dry mass supplied daily, per m<sup>3</sup> of fermentation volume). Normal loadings of the fermentation area lie in the region of 3 to 5 kg. organic dry mass/m<sup>3</sup>, daily, and proportionately lower in the case of substrates with a high N content (e.g. liquid faecal matter from poultry), on account of the required dilution.

**2.1.1.2. Residues from the Food and Food Processing Industries**

**Raw Materials and Products**

**Raw Materials**

Wastes predominantly of vegetable or animal origin are suitable for treatment using anaerobic processes, especially those from the processing of vegetable and livestock products. Theoretically, this can include all of the materials given in the following table (Table 1). However, in practical terms, many of these materials can only be employed in anaerobic reduction processes after initial, controlled treatment, i.e. chemical or physical break-down, as these can only run well in a liquid medium. Such breaking-down processes are generally costly as they require special technical facilities and energy. For this reason, the reutilisation of materials which are not already in liquid or well-dissolved form, is still not currently undertaken, and cheaper methods of waste disposal such as tipping or incineration are selected.

Table 1.

Wastes of Vegetable and Animal Origin, including Processing Residues

FOOD, BEVERAGE, TOBACCO AND RELATED WASTES (EXCLUDING WASTES FROM FAT PRODUCTS AND ANIMAL SLAUGHTERING)

Food Wastes

Kitchen wastes  
Overripe food  
Chaff and chaff dust  
Spice residues  
Sludge from raw sugar production  
Sludge from vinegar manufacture  
Residues from canned food production  
Molasses  
Dough wastes

Wastes from Beverage, Tobacco Production and Related Wastes

Over-stored luxury foods  
Tobacco dust, debris, veins, sludge  
Reject cigarettes  
Brewers grains, matt germs, matt dust  
Hop husks  
Spent and skimmed barley  
Fruit slops  
Grain slops  
Potato slops  
Trub  
Brewery sludge  
Sludge from wine production  
Distilling sludge  
Husks, fruit residue  
Coffee production residues  
Tea production residues  
Cocoa production residues  
Yeast and similar residues

Fodder Wastes

WASTES FROM VEGETABLE AND ANIMAL FAT PRODUCTS

Wastes from Vegetable and Animal Oils

Rape seed residues  
Spoilt vegetable oils  
Volatile oils

Wastes from Vegetable and Animal Fats and Waxes

Waxes  
Fat wastes  
Wire-drawing wastes  
Fatty acid residues

Emulsions and Mixtures of Vegetable and Animal Fat Products

Contents of fat separators  
Whey

Sludges from Vegetable and Animal Fat Products

Sludge from margarine production  
Sludge from edible fat production  
Sludge from oil production  
Centrifuge sludge (dairies)

Refining Residues from Vegetable and Animal Fat Products

Bleaching (Fullers') earth

WASTES FROM ANIMAL  
BREEDING AND SLAUGHTER

Slaughterhouse Wastes

Bristle and horn wastes  
Bone and skin wastes  
Offal  
Poultry wastes  
Fish wastes  
Blood  
Feathers  
Stomach and intestinal  
contents  
Game wastes

Animal Carcasses

Animal for experimentation  
Confiscated animals  
Carcasses  
Parts of carcasses

Animal Excreta

Poultry excrement  
Pig manure  
Cattle manure  
Dung

HIDE AND LEATHER WASTES

Wastes from Hides and Pelts

Leather glue  
Gelatine  
Pelts and hides

Tannery Wastes (excluding  
Tanning Agent Wastes)

Liming sludge  
Tanning sludge

Leather Wastes

Leather wastes from chrome  
tanneries  
Chrome leather wastes from  
fabrication  
Pelts and unchromed leather  
wastes  
Leather swarf sludge  
Waste from leather working

WOOD WASTES

Wood Wastes

Bark  
Flitch-wood  
Sawdust and chips  
Grinding dust and sludges  
Wood packaging  
Building and demolition wood  
Wood shavings  
Beams and buntons  
Wooden hurdles from coke gas cleaning  
Wooden hurdles from sulphur arrestment  
Railway sleepers  
Piles and masts  
Oil and solvent saturated sawdust

CELLULOSE, PAPER AND PULP WASTES

Wastes from cellulose manufacture  
(without reagents)

Sludge from cellulose production

Wastes from cellulose processing  
(without reagents)

Residues from paper recovery (rejects)  
Sludge from paper production  
Sludge from rayon production  
Sludge from cellulose fibre production

OTHER WASTES OF VEGETABLE AND ANIMAL  
ORIGIN, INCLUDING REFINING PRODUCTS  
(EXCLUDING RUBBER, TEXTILE, MUNICIPAL  
AND HOSPITAL WASTES)

Other wastes of animal and vegetable  
origin

Starch sludge from separators  
Sludge from gelatine factories  
Gelatine stamping wastes  
Residues from potato-starch production  
Residues from maize-starch production  
Residues from rice-starch production  
Sludge from catgut string production  
Soap suds  
Brewing kettle residues  
Sludge from soap-sieving  
Gut wastes

### Products

The possibilities for exploiting the materials in Table 1 depend on their composition. Fermentation to high value secondary materials is only achievable with homogeneous base materials. Mixtures may be used to a certain extent solely for the recovery of fermentation gas.

The most important products and biological principles of special fermentation processes are considered below.

### Methane (Biogas)

Apart from the agricultural wastes already separately referred to, those wastes which are suitable as raw materials for methane recovery, and for which recycling does not appear feasible, are generally every oxygen-free, predominantly organic, hydrous substrate mixture. In this regard, it is necessary to achieve a balanced nutrient ratio, in particular a sufficient nitrogen and phosphate content. The following ratio is regarded as a standard: organic dry mass: nitrogen: phosphorus = 100:5:1 (4,5). The gas yield varies according to the composition and condition of the material. With loose, comminuted and fresh materials, the yield is substantially better than with solid, coarse, lignified or already decayed materials.

The fermentation of waste water sludges in a digestion tower is a very complex process of which certain stages are not yet fully understood. Methane formation is the last part of a chain of fermentation stages in which, in the first instance, organic acids, alcohols, carbon dioxide and molecular hydrogen among other things are formed by other fermentative organisms from carbohydrates, proteins and fats. These are converted by the methane-formers. At the same time, conversion processes take place in which carbon disulphide compounds, hydrogen sulphide and ammonia arise as end products, which are not usable by methane-forming bacteria.

As mixed effluents and effluent sludge are generally substrates already in fermentation, they include all possible intermediate stages and, after transfer into the digestion tower, there commences a strong growth in all the types of micro-organisms present. The individual reduction phases therefore inevitably overlap, which in practice is desirable as only in this way can a continuous quantity of gas be produced.

Table 2 contains information on the known methane bacteria (1):

Table 2: Methane-Forming Bacteria

| Type  | Substrates<br>(H-donors)                  | Source of CH <sub>4</sub> |
|---|---|---------------------------|
| Methanobacterium (omelianskii)<br>Strain M.O.H. | H <sub>2</sub>                            | CO <sub>2</sub>           |
| Methanosarcina barkeri                          | H <sub>2</sub> ; CO; Methanol;<br>Acetate | CO <sub>2</sub> ; CO      |
| Methanobacterium formicicum                     | H <sub>2</sub> ; CO;<br>formiate          | CO <sub>2</sub>           |
| Methanococcus vannielii                         | H <sub>2</sub> ; formiate                 | CO <sub>2</sub>           |
| Methanobacterium ruminantium                    | H <sub>2</sub> ; formiate                 | CO <sub>2</sub>           |
| Methanobacterium suboxydans                     | Butyrate;<br>Valerate;<br>Capronate       | CO <sub>2</sub>           |
| Methanobacterium sohngenii                      | Acetate;<br>Butyrate                      | CH <sub>3</sub> - Group   |
| Methanosarcina methanica                        | Acetate;<br>Methanol;<br>Butyrate         | CH <sub>3</sub> - Group   |
| Methanococcus mazei                             | Acetate;<br>Butyrate                      | CH <sub>3</sub> - Group   |

This necessarily concerns anaerobic bacteria. In nature, they are found in the anaerobic sludge-zones of liquids, in the stomachs of ruminants and practically everywhere where organic material is decomposed under anaerobic conditions, inter alia in the digestion towers of sewage treatment works. As these bacteria in pure cultures die in the slightest concentrations of oxygen, screening is extremely difficult.

There are two groups of methane-forming bacteria which are distinguished by their needs in respect of incubation temperature (1):

1. The mesophile group of which the temperature range lies between  $30^{\circ}$  and  $45^{\circ}\text{C}$ , with an average temperature of  $38^{\circ}\text{C}$ .
2. The thermophile group of which the temperature range lies between  $45^{\circ}$  and  $55^{\circ}\text{C}$ , with an average temperature of  $52^{\circ}\text{C}$ .

These bacteria are very sensitive to fluctuations in temperature, particularly a fall-off in temperature. The permissible range for fluctuations in average temperature lies, in the mesophilic domain, at  $\pm 3^{\circ}\text{C}$  and, in the thermophilic domain, at  $\pm 0.5^{\circ}\text{C}$ .

Fluctuations which exceed these limits, lead to a drastic reduction in yield. The pH-optimum of methane bacteria lies around the neutral point; values below 6 or above 8 also lead to poor gas yields.

Animal wastes and yeast wastes can be used as a source of nitrogen for the fermentation of cellulosic material because of their high protein content. The same applies to algae.

The residues from anaerobic treatment can be employed in the following ways:

- as fertiliser; but one needs to consider that not all pathogens are killed by the fermentation. Likewise, putrified sewage sludge can be contaminated with traces of heavy metals and other toxic substances;
- as substrate for algae cultivation; the algae can then be employed again for methane fermentation. Limiting factors for such processes are the duration and strength of the solar radiation, i.e. the available amount of sunlight. They can only be employed to a limited extent in the Federal Republic of Germany;

- as substrate for anaerobic, phototrophic bacteria, whereby high value protein can be produced, inasmuch as the effluent from thermophilic bio-conversion is used. The need for thermophilic conditions is at the same time a limiting factor for this process in temperate climates (higher energy demand);
- as substrate for renewed fermentation; where the residues contain polymers that are difficult to break-down (lignin, cellulose, etc), this material can, after alkali/heat treatment, be re-fermented in a second bio-convertor. So in this way, the rate of reduction as well as the methane yield can be markedly increased.

### Ethanol

Micro-organisms can form a range of alcohols.

Ethanol recovery represents the oldest form of biotechnical recovery of organic substrates. Processes such as beer and wine production do not represent commercially or technically new areas. In this respect it is obvious that this technology also reinforces its use for the recovery of residues. Just how effective that can be is shown by the exploitation of the non-crystallisable fraction in sugar production, the molasses. The utilisation of molasses is a special area within the food and food processing industries.

The organisms for ethanol recovery are primarily the yeasts, which are to be found everywhere in nature on sugar-containing substrates. The most important kinds are the two strains of *Saccharomyces Cerevisiae*, the baking and beer yeasts. The yeasts belong to the fungi, which however do not form mycelia but multiply principally by vegetative germination. Like most fungi, the yeasts are aerobically respiring micro-organisms. However, under anaerobic conditions they ferment sugar to ethanol and  $\text{CO}_2$ . Besides ethanol and  $\text{CO}_2$ , further products appear depending on the strain of yeast and the composition of the medium. These are primarily glycerin, fusel oil (higher alcohols) and organic acids. In each case, this gives rise to an increase in solid yeast through cell growth.  $\text{C}_6$  - sugars are predominantly fermented from the yeasts (in particular from the *S. Cerevisiae*). The monosaccharides glucose

galactose, fructose, manose, the bi-saccharides maltose and sucrose, and the trisaccharide raffinose can be directly fermented from certain yeasts.

Influencing parameters and supplementary materials:

Yeasts require potassium, magnesium, phosphoric acid and nitrogen compounds for growth. Inorganic ammonium salts, e.g.  $(\text{NH}_4)_2\text{SO}_4$ ;  $(\text{NH}_4)_2\text{HPO}_4$  can be used as a source of nitrogen. Other possible sources of nitrogen are such organic compounds as urea, amino acids and peptides. Many yeasts require sets of vitamins, principally those such as the B group. The temperature optima for the individual types of yeast lie between  $30^\circ$  and  $40^\circ\text{C}$ . The maximum permissible temperature varies between  $38^\circ$  and  $45^\circ\text{C}$ . Below  $5^\circ\text{C}$  and above  $50^\circ\text{C}$ , fermentation is not possible. Should heat arise from the metabolic reactions, the reactors must usually be cooled. Temperatures in the upper range lead to the formation of froth and more intense  $\text{CO}_2$  production.

Yeasts are capable of living in a wide pH range. The range between pH 3-6 is interesting on account of the acid protection connected therewith; a pH value of 4 is most frequently employed. The partial compression of oxygen is a further important influencing factor. With this the plant can be controlled. A high redox potential leads to increased yeast growth, while a lower potential leads to increased alcohol formation (Pasteur effect). Conversely, alcohol formation in SCP processes is evidence of poor aeration.

With fermentation of alcohol, the sugar content of the solution must not be too high; 12-15% with *Saccharomyces*; other types (wine yeasts) can tolerate higher concentrations; the upper limit lies at 40% (6,7,8).

Essentially, four types of substrates can be used:

- sugar-containing substrates,
- starchy substrates,
- cellulose-containing substrates,
- other substrates.

Sugar-containing substrates can be fermented directly (molasses from sugar beet and sugar cane). According to nitrogen and phosphate content, ammonium sulphate and/or ammonium phosphate must be added. Corresponding to the glucose inserted, the theoretical yield of ethanol amounts to ca. 50%. The so-called residual wort, which contains betaine, causes problems for the exploitation of molasses. As betaine in fodder is not permitted in the Federal Republic of Germany, the residual wort represents a considerable residue problem.

Starch substrates are supplied by various kinds of grains and tuberous roots (rye, wheat, tapioca, manioc, potato, etc.) The residues from processing these materials contain starch in quantities sufficient for further use. The starch cannot be directly assimilated by yeasts; they must be previously split by amylases into glucose (see sugar exploitation).

Cellulose-containing substrates:

These materials are suitable, after an initial decomposition process, for bio-technical alcohol recovery. This concerns such residues as:

- sulphite liquors,
- paper,
- straw,
- wood wastes.

In sulphite liquors, the cellulose is already sugared because of acid treatment. But as the liquors contain a range of fermentation-restricting substances, they cannot be immediately fermented to alcohol. The most important inhibiting substance is the sulphite anion, but that can be very simply removed.

There are various possibilities available for the decomposition of the other substrates:

Acid decomposition of paper residues leads, at high temperatures, to rapid reduction. The yield corresponding to the theoretically possible quantity of alcohol lies, at 85-95%, above the values for the fermenting of molasses (80%). The process is, however, more expensive. Acid-resistant containers must be used, secondary reactions with other components of the residue can appear, and there

exists the danger of the sugar being reduced by the acid before it can be fermented by the yeast.

Enzymatic decomposition has the advantage of greater selectivity, which makes the processing of heterogeneous materials theoretically possible. Aerobic cultures occurring in the soil, such as fungi, myxobacteria and eubacteria which produce and release hydrolytic enzymes into the medium, appear to be particularly suitable for decomposition of this type. Trial tests have been successful in establishing, amongst others, mutants of *Trichoderma Viride*, with which a sugar level of ca. 60% was achieved with waste paper. In tests with crystalline cellulose, a sufficient sugar level has not so far been achieved using enzymatic decomposition, so that recovery of alcohol in this way is not yet technically possible.

Acid saccharification of wood wastes by decomposing ethanol-fermentation has long been practised in wood-producing countries such as Canada, Sweden and Norway. In the Federal Republic of Germany, this process has not been employed since the end of the war. The statements made for wood also apply to the use of straw on account of their similar composition.

Other substrates:

Fermentation to alcohol is basically possible with all substrates which contain components that are capable of being assimilated or which can be produced by controlled pre-treatment. So, for example, whey can also be fermented to ethanol if lactose-reducing yeasts (*Torula Chrenoris*, *S. Cerevisae*) are introduced. Nevertheless, until now no process of this type has been attempted, although over 50% alcohol could theoretically be obtained.

#### Butanol

Some *Clostridia* form butanol. Industrial applications have found two different processes (1,2,7,12,13):

acetone-butanol-fermentation

butanol-isopropanol-fermentation

The usable *Clostridia* are *C. Acetobutylicum* and *C. Butylicum*. Where the *Clostridia* form amylases, these are suitable for the direct fermentation of starchy raw materials.

The products of fermentation, besides  $\text{CO}_2$  and  $\text{H}_2$ , are primarily butanol, acetone, isopropanol, as well as acetic and butyric acids. Theoretically, the same substances could be used as for ethanol-fermentation, i.e. sugar-, starch- and cellulose-containing materials. However, there is the condition that the sugar content of 5-8% must plainly lie under that for ethanol-fermentation. Fermentation of molasses with *C. Acetobutylicum* gives rise to ca. 5% acetone, 12% butanol, 0.5% ethanol, 32%  $\text{CO}_2$  and 1%  $\text{H}_2$  (% by weight). Ca. 30% remains over as dry solids.

The following conditions are strongly limiting:

- the Clostridia work severely anaerobically,
- the fermentation must be completely sterile,
- process regulation is relatively expensive (constant control of pH, composition of acids and foreign germs),
- infections by bacteriophages are frequent.

Because of the high demands on the fermentation process and the related expense of the process technology, fermentation based on Clostridia is not suitable, at least at the present time, for the recovery of residues, as it would be too costly.

### Glycerin

With the fermentation of sugar using yeast, there always arise small quantities of glycerin. The yield can thereby be raised, so that by addition of sulphite or bisulphite, acetaldehyde, as the hydrogen acceptor in the system, is extracted. Instead of which, dihydro-oxyacetonephosphate is reduced to glycerin-phosphate, from which glycerin appears by phosphate separation. With addition of an alkali to the fermentation batch ( $\text{NaHCO}_3$ ,  $\text{Na}_2\text{HPO}_4$ ), one also arrives at an increased formation of glycerin because acetaldehyde dismutates to ethanol and acetic acid (1,13). This glycerin fermentation has become known under the name 'Protol-process'. In the Federal Republic of Germany, glycerin has been obtained since the end of the second world war by lipolysis.

Also here, a fermentation process is known which is lipolysis by lipases, particularly by rhizinucleic lipases. Oil seeds contain lipases, in order to be able to mobilise reserves of fatty material for the process of germination. Castor beans are particularly rich with this enzyme. Fermentation of this kind results in a fatty acid/glycerin mixture of 3:1 (13). On commercial grounds, the process has not been successful for the recovery of fatty acids.

### Carboxylic acids

Lower fatty acids occur as intermediate products of methane-fermentation (1,2). Theoretically, these can be intercepted and further fermented into Single Cell Protein. The interception of the acids would, for example, be possible by electro dialysis of the fermentation fluid. By using this intermediate stage, an increase in the yield of methane can be achieved, as the acids impede fermentation. Because of the high costs which such a process step would entail, the prospects for the process are currently poor.

### Lactic acid

With the assistance of lacto-bacteria, particularly thermophilic types such as: *Lactobacillus delbrückii*, *L. Leichmannii*, *L. Bulgaricus* and also special fungi (*Rhizopus oryzae*), glucose can be fermented to lactic acid (2 - Hydroxypropionic acid) (1,13). With homo-fermenting bacteria, a yield of up to 90% of the theoretical value can be obtained. In practice, the fermentation has been carried out with pure cultures of the bacterium *L. delbrückii*.

Molasses, or grain mash, which has been sugared beforehand with diastase of malt, serve as the primary materials, as can potato starch and maize which have been sugared either with diastase or by hydrolysis with dilute (0.1%) sulphuric acid. Decay products from proteins, bran, malt germ, yeast and yeast extract are introduced as a nitrogen source. Fermentation of the sterilised mash (10-11% maltose) follows with constant neutralisation of the acids formed with calcium carbonate at pH 5-6, as lactic acid concentrations over 1.8% hamper the fermentation.

The entire process is carried out at temperatures of 48-50°C and is concluded after 5 - 8 days.

After a separation process, the lactic acid is released from the slightly-soluble lactic acid precipitate through the addition of sulphuric acid and brought to the desired degree of purity by recrystallisation.

Apart from those fermentation substrates mentioned, raw sugar, raw sugar molasses, glucose and whey can be utilised. In the fermentation of whey, *L. Bulgaricus* and *Streptococcus Lactis* have proved successful. The fermentation process lasts only 50 hours.

Lactic acid finds application as an additive in the food processing industry, in breweries and bakeries, for decalcifying and swelling hides in tanneries, for manufacturing fizzy lemonade, in the textile industry for brightening silk, as an aid in printing and colouring technology, and for manufacturing silage products. Nevertheless, the quantities required are small.

The fermentation of pentoses is also possible using species of bacteria such as *Lactobacillus pentosus*, *L. casei* and *Leuconostoc mesenteroides*. Besides lactic acid, an equimolar quantity of acetic acid results. Corn cobs, oat husks, groundnut shells and other pentose-containing vegetable components can serve as a substrate.

#### Butyric acid

Butyric acid fermentation is carried out in some small-scale processes with *Clostridium butyricum* (1,7). Yields of ca. 40% represent the maximum, and are only achievable with greater fermentation periods. A by-product of this fermentation is always acetic acid. Butyric acid, in the form of an ester, finds application in the cosmetics industry.

## 2.1.2. Technical Processes

### 2.1.2.1. Technical processes for the anaerobic reduction of organic residues from agriculture.

Of the biogas systems developed in the Federal Republic of Germany from 1947 to about 1955,

|             |                           |
|-------------|---------------------------|
| "Muenchen"  | (Strell, Goetz, Liebmann) |
| "Darmstadt" | (Reinhold, Noack)         |
| "Allerhop"  | (Schmidt, Eggersgluess)   |
| "Hohenheim" |                           |
| "Berlin"    | (Ikonomoff, Gaertner)     |
| "Hannover"  | (Poetsch)                 |

only that from SCHMIDT and EGGERSGLUESS reached the stage of commercial application. 15 plants of this type were erected in the Federal Republic of Germany of which one is still operating today (Klostergut Benediktbeuren near Bad Toelz).

#### The SCHMIDT/EGGERSGLUESS System

The intermittently functioning plants consist of two (or more) fermentation containers with a pre-connected container for the collection and mechanical processing of the matter to be fermented (liquid faecal matter and possibly with addition of straw and other vegetable residues), containers for storing the bio-sludge and a gas storage reservoir. The heat insulated fermentation containers are batch-exchanged. Only one pump is required for moving all liquid flows. The destruction of the surface film in the fermentation containers and in the bio-sludge storage containers occurred with a jet of liquid from the axis of the container; this arrangement is part of the circulating pumping system. The contents of the fermentation chamber are heated by hot water via a heat-exchanger or through injection of steam (fermentation temperature 30 to 35°C). The plants constructed had fermentation containers with a total capacity of 100 to 960m<sup>3</sup> and were designed for processing the excrement from 25 to 220 head of large cattle (1 head of large cattle = 500 kg live weight) and varying quantities of vegetable residues.

The ROEDIGER/FERMENTTECHNIK System

With the process objective of recovering biogas, solid manure and a final effluent with a pollution loading so low that drainage into the primary settlement pond is possible, the firm Roediger/Fermenttechnik, Rheinfelden (Switzerland) has been offering for the last two years a continuously operating process with aerobic treatment of the bio-sludge subsequent to fermentation. Essential characteristics are:

- the return of activated sludge from the primary and secondary settling stages and measured admixing of fresh material prior to administering in the fermentation chamber (injection);
- the warming of the fermentation material prior to introduction into the fermentation chamber;
- avoidance of the formation of heavy surface films through separation of coarse solids prior to introduction.

These plants are envisaged for animal production operations which have no possibility of disposing of the large quantities of liquids that arise on agricultural land, but on the other hand aim at using the solids and the energy content of the wastes.

2.1.2.2. Processes for the anaerobic reduction of residues from the food processing industry

From the processes described in the preceding chapters, there exist large-scale plants in particular for the recovery of methane, ethanol and butanol from sludge. However, currently operating plants are not aimed particularly at the recovery of organic residues. One exception is the recovery of methane gas.

Methane arises inter alia from the decaying of municipal sludges. An aim of anaerobic handling of effluent treatment sludge is stabilisation and partial sterilisation. Apart from recovering methane as fermentation gas, a reduction of the sludge volume and a general improvement in sludge characteristics is also achieved. Currently, 34 million m<sup>3</sup> of sewage sludge arise annually in the Federal Republic of Germany.

The technological decaying of sludge takes place in specially developed "fermentation-towers" made of steel or concrete, the inside volumes of which can lie between some 100 and 10,000 m<sup>3</sup>. Trials for using the methane have been conducted. The quantities of gas arising could cover not only the energy demand of large sewage works but also deliver surplusses and thus contribute to the covering of costs (14).

From theoretical calculations, the following quantities of fermentation gas (ca. 2/3 methane, 1/3 CO<sub>2</sub>; with a calorific value of 21,000 KJ/m<sup>3</sup>) can be recovered from wastes, depending on the dry substance (15):

|                       |                         |
|-----------------------|-------------------------|
| Sewage sludge         | 0.35 m <sup>3</sup> /kg |
| Slaughterhouse sludge | 0.90 "                  |
| Household refuse      | 0.40 "                  |
| Stable manure         | 0.25 "                  |
| Straw                 | 0.35 "                  |
| Grass                 | 0.45 "                  |
| Foliage               | 0.25 "                  |
| Weeds                 | 0.20 "                  |
| Paper                 | 0.25 "                  |

It has been further calculated that, for example, communities with under 30,000 inhabitants, could recover about 10,000 m<sup>3</sup> of fermentation gas daily by using the wastes in their catchment area, which would be sufficient to supply the population. But in practice, the feeding of fermentation gas into the existing natural gas grid has not proved successful, and tests were again discontinued. Apart from a high CO<sub>2</sub> content, trace quantities of sulphur-containing compounds, inter alia H<sub>2</sub>S, are contained in the gas, which make additional cleaning stages necessary.

The industrial mass production of alcohol and alcoholic beverages is operated in a process linked in together with the manufacture of baking yeast through the fermentation of molasses under defined conditions. Alcohol formation occurs anaerobically and yeast formation through switching the system over to strong aeration in an aerobic operation, after the so-called running-in process. The size of the installed fermenter lies between 50 and 200 m<sup>3</sup> inside volume. The efficiency of the process is determined by the following parameters:

- OTR (according to the fermentation fluid, 30 to over 50 m<sup>3</sup> air/hour per m<sup>3</sup> must be inserted) (OTR-oxygen transfer rate);
- nutrient admixture (ammonium sulphate, phosphoric acid, etc);
- pH value (between ca. pH 4 and pH 5);
- temperature (between ca. 25 and 30°C);
- duration (ca. 10 to 12 hours);
- control of running-in (control of sugar content).

In the running-in process, yeast and alcohol production can be regulated in almost any desired proportion. Spirit-free fermentation is just as possible as a yeast/alcohol ratio of ca. 50/50.

Butanol is manufactured by Clostridia fermentation. Technical processes for this are based on patents of the Deutschen Hydrierwerke AG. Molasses primarily serve as the substrate, but since Clostridia form amylases, starch-containing substrates can also be directly introduced (Weizmann process).

From technical production one obtains:

- 60% n-butanol
- 30% Acetone, and
- 10% Ethanol

A separate industry has formed based on the fermentation of molasses, with for instance, the following production branches:

- molasses distilleries,
- baking yeast,
- citric acid,
- lactic acid,
- itaconic, oxalic and gluconic acid,
- dextran,
- acetone, isopropanol and butanol,
- antibiotics,
- vitamins.

For this reason, molasses can no longer be rated as a waste, even though they arise as a residue in sugar production (ca. 4% arising of molasses calculated for beet). One currently pays ca. 14-20 DM/100 kg for molasses at the sugar factory. (Composition of beet molasses: 50% sugar; 20% water; 20-30% other organic components; 5-6% inorganic salts).

From the point of view of the possible recovery of secondary products from waste materials by anaerobic fermentation, only methane recovery is practised on a large-scale. But even in the case of methane recovery, a qualification must be made. Methane arises as a 'by-product' of effluent sludge treatment. This means that the prime purpose of currently existing plants is sludge treatment and not the production of methane (15,16).

### 2.1.3. Status of Research and Development

#### 2.1.3.1. Anaerobic reduction of organic residues from agriculture

Arising out of the basic work of various German and foreign scientists on the micro-biology of methane fermentation, a research programme began in the Federal Republic of Germany in about 1947 into the technology of fermenting organic agricultural wastes. From the results of laboratory investigations and experience gained with various systems from testing numerous pilot plants, conclusions could be drawn about process operation and outcome and, from these, important data for the design and construction as well as for the economic assessment of biogas plants could be obtained.

In laboratory work, interest was aimed primarily at determining the relationships between fermentation duration and temperature, and gas yield and composition for various organic agricultural residues and mixtures thereof, in particular liquid manure and straw. Of those plant systems proven in practical trials, only the Schmidt/Eggersglüss system and the "Darmstadt" system were designed for processing a liquid substrate containing up to about 10% solid matter. All other proposed solutions directed their attention to the demand for processing straw-rich, liquid and solid manure. The introduction of these systems in practice foundered essentially on the difficulties in handling the solids and the correspondingly high technical and labour costs. In addition, controlled fermentation under almost optimal conditions was barely achievable with these systems. However, the Schmidt/Eggersglüss system did permit extensive gathering of knowledge of biological and process techniques as well as its introduction in livestock production operations of different sizes. Today's operating data for assessing biogas plants are founded essentially on knowledge gathered in those years with plants based on this system.

Numerous tests in relevant specialised institutes (e.g. Braunschweig-Völkenrode, Göttingen and Hebelheim) and experience gained from organisations operating biogas plants, gave more reliable conclusions about the fertiliser value of the biogas.

In the Federal Republic of Germany, research and development activities in the field of anaerobic fermentation of agricultural residues ceased towards the end of the 50's due to alteration in the price situation for agricultural operating materials (in particular electric power, heating oil, petrol, fertilizers). In recent years, a new impetus has arisen through developments in the areas of energy, raw materials and environmental protection. Specific research work in the field of anaerobic fermentation of agricultural materials has nevertheless not been taken up again to any great extent (so far as is known), although, for about the last 4 years, one can observe notable scientific activity abroad in this field.

In the area of research, one can simply mention here one piece of work "the clarification of liquid manure from poultry and pigs by phototrophic bacteria in conditions of light and absence of air", which is being undertaken by the Institute of Microbiology of the GSF Göttingen, as well as further work on "the composition of micro-flora and their significance for various treatment methods" at the Bacteriological Institute of the University of Munich.

Development work was conducted by the firm Steinmann and Ittig, Minden, on the basis of experience gathered earlier from plants with the Schmidt/Eggersglüss system, which has so far not been commercialised. Using their extensive experience in the field of sewage sludge treatment, the firm Rödiger, Hanau, have built a pilot plant for which no operating results are yet available. One must proceed from the assumption that technical progress in the field of sewage sludge fermentation can be extensively employed for the structural execution of modern biogas plants, whereby, particularly for agriculture, solutions that are cost-effective and modest with regard to operating and maintenance costs are required.

Expansion in this direction has become known very recently and isolated by agricultural practice; this nevertheless still requires careful specialised investigation and evaluation.

Future research and development work should be structured towards the following objectives:

#### Microbiological Research

- increasing the metabolic performance of methane-forming microflora, particularly at low temperatures;
- reducing the sensitivity of methane-forming bacteria with regard to disturbing environmental influences;
- improving the biochemical stability of biosludge;
- mortifying pathogenic organisms during the fermentation process;

#### Technical Research

- clarifying the relationships between fermentation time, sludge loading, temperature, metabolic rate, hygienic effect with different types of substrate;
- clarifying the influence of aerobic pre-treatment and subsequent treatment measures on the gas yield as well as the odour stability and fertiliser value of bio-sludge;
- increasing the metabolic performance by physical and chemical preparation of the substrate;
- establishing and respectively increasing the effective value of the bio-sludge by physical and chemical treatment.

#### Development

- reducing the energy requirement;
- simplifying operation (supervision, control regulation);
- increasing plant life (reduction of corrosion and abrasion damage by applying suitable building materials and construction principles);
- reducing the investment costs;
- developing models for defined process solutions with regard to characteristic operating conditions;

- erecting pilot plants based on selected models;

Assessment

- constructing cost-effectiveness analyses for selected models and pilot plants.

2.1.3.2. Anaerobic reduction of residues from the food processing industry

A key point in activities on this theme is the clarification technique and the associated sludge stabilisation. The work has as its objectives:

- a reduction in the quantities of surplus sludge;
- better stabilisation of the surplus sludge with the aim of being able to use it as fertiliser;
- improved de-watering characteristics of surplus sludge;

The projects cannot be set out in detail as their number is too large and only seldom do they specifically serve to recover residues. In part, the theme only represents one aspect of current research work:

- ca. 25 projects are currently engaged on the analysis of effluent and effluent sludges, etc.,
- ca. 100 projects are devoted to inquiring into the conditioning of sludge and effluent from municipal plants,
- ca. 75 are occupied with the treatment of industrial effluents, and ca. 20 with the treatment of agricultural effluents,
- ca. 65 projects can be counted in the field of sludge treatment

Only 5 projects are currently engaged on the precise theme, namely the possibilities for recovering valuable material from natural materials (17):

| <u>Project</u>   | <u>Institution</u>  |
|--|---|
| Carbohydrate reserves in wood waste, bark and spent liquors from the wood and pulp industry and possibilities for their use. | Institute for wood chemistry and chemical technology of wood of the Federal Research Institute for Wood and Forest Management, Hamburg. |
| Microbial utilisation of unconventional sources of carbon  | Institute for Microbiology, University of Munich.   |

Examination of various biological  
- technical systems for energy  
recovery.

Dornier System G.m.b.H.  
Friederichshafen

Recycling of wastes as poultry  
feed.

Institute for Small Animal  
Rearing of the Agricultural  
Research Institute, Celle.

Treatment of wastes from live-  
stock mass production

Institute for Urban Water  
Management of the Technical  
College of Hannover.

One should not expect that these projects will result, in the  
short-term, in technically usable or feasible processes.

2.1.4. Economic Evaluation

2.1.4.1. Anaerobic reduction of organic residues from agriculture

Examination of the economics of a biogas plant must vary in outcome, depending on the main purpose which the plant will fulfil. Three main types of objective can be mentioned:

1. Production of biogas as the energy medium - the bio-sludge as fertiliser is a by-product.
2. Recovery of high value fertiliser ("Biodung") and preservation of the nutrients contained in the residues of agricultural operation - biogas is a by-product.
3. Fulfillment of environmental obligations, e.g. reduction and avoidance of odour emissions, achievement of sanitary conditions.

A rough calculation can only be carried out for "1)". For the evaluation of the biodung and the environmental impact, until now only groundwork, which is usable under certain conditions, has been put forward. The technical expense for a plant to meet objective "2)" is probably lower; that for objective "3)" must however be higher than that for objective "1)".

Economic viability is only possible when the following condition is met:

Annual costs (K) of the biogas plant  $\leq$  freely available and usable quantity of gas (N) x Comparative Price (P) of a unit of energy

+ Value (D) of Biodung in relation to normal dung + Value (U) of the environmental Impact

From this, the permissible supply price (A) of a plant can be roughly ascertained. If the dung value (D) and the environmental value (U) are ignored, a biogas plant has a value, when considered solely as an energy-producing plant, of:

$$A = 10 (N.P-M.B-L)$$

|   |                    |
|---|--------------------|
| A = Plant Price   | DM                 |
| F = Fixed costs/year (with write-off over<br>10 years) = $\frac{A}{10}$ | DM/a               |
| K = Annual costs = F + M.B + L  | DM/a               |
| M = Gas quantity/year   | Nm <sup>3</sup> /a |
| B = Cost of operating material (0,2 KWh/Nm <sup>3</sup> )               | DM/Nm <sup>3</sup> |
| L = Annual labour costs (@ 12DM/hour)                                   | DM/a               |
| N = Freely available quantity of gas/year                               | Nm <sup>3</sup> /a |
| P = Comparative gas price (including costs<br>for supply)               | DM/Nm <sup>3</sup> |

With the average values for the producible quantity of gas mentioned in section 2.1.2.2., one obtains a permissible plant price, with the most favourable calculations for labour costs, of between ca. 25,000 and 100,000 DM, for a plant capable of processing the liquid manure (without addition of straw) from 100 head of large cattle; this depends on whether the biogas produced is substituted for heating-oil, natural- or propane gas, or electric power. But at this price, according to information from industry, there is no plant available, i.e. it is still not economic without valuing the dung and the environmental benefits. The industry quotes a supply price for the part of the plant for producing biogas of around 300,000 DM for operations with 100 head of large cattle (17).

#### 2.1.4.2. Anaerobic reduction of residues from the food processing industry

Of the processes described, only ethanol recovery currently leads to positive results. But this is so only because its manufacture can emanate from a relatively homogeneous substrate. With the replacement of molasses by, for example, cellulose-containing mixed residues or the like, one can expect that above all a modified technology would have to be installed on account of the expensive conditioning and the probably greater quantities of residues, and that the economics in terms of profitability would be questionable.

On the basis of molasses, for instance, currently the following data result:

Molasses ex the sugar-factory: price per 100 kg, DM 14,-- to 20,--  
Raw spirit (contract price per hl 1975/76) DM 180,-- to 190,--

The price of raw spirit is established by the Federal Monopoly Administration.

The yield of ethanol amounts to ca. 26 to 31.1 per 100 kg of molasses. The maximum value lies around 34 l. This means that from 100 kg. of molasses ca. 53,-- DM can be realised, and when the price of the raw product is removed, ca. 35,-- DM/100 kg. remain to cover all the occurring costs. With waste substrates, only the cost of recovery (transport etc) must be considered. Given the general experience also available with other substrates (sulphite spent liquors, newsprint, wood wastes, etc.), an economic method of handling these waste materials through fermentative processing cannot be excluded. The same goes for the decomposition processes (see chapter on hydrolysis processes), which also enable the further processing of difficult residues. Nevertheless, it should not remain unmentioned that the manufacture of synthetic alcohol is currently cheaper than its biotechnical counterpart.

The statements which were made for ethanol recovery cannot however be readily transferred to the other products.

#### Methane recovery:

A plant, the sole purpose of which is to manufacture methane from residues cannot be justified on commercial grounds. The recovered fermentation gas would have to withstand competition from natural gas (19,20):

|   |                                    |
|---|------------------------------------|
| The heating value of fermentation gas (corresponding to 60% methane) amounts to | ca. 5000 kcal/Nm <sup>3</sup>      |
| and that of natural gas to  | ca. 7000-8000 kcal/Nm <sup>3</sup> |

Apart from this, fermentation gas must be desulphurized, which involves additional costs.

Moreover, the production-influencing factors are numerous:

- high duration of mesophilic bacteria,
- high energy demand of thermophilic bacteria,
- poor sludge conditioning with thermophilic bacteria,
- substrate constraint through lignin,
- low mass-reduction
- non-bactericidal conditions in particular with mesophilic processes.

On the basis of these somewhat unfavourable conditions, large-scale methane recovery for resource conservation is currently uneconomic.

Butanol recovery:

The manufacture of butanol represents a substitute for the other processes mentioned. Indeed, this includes the processes which were used in the past on a large scale, but which have been unable to withstand competition from petroleum-based synthetic products. The conditions required, particularly for Clostridia fermentation, set high technical demands. Moreover, the products are not homogeneous but represent a mixture of different components (acetone, butanol, isopropanol, etc.) which then require further processing. This means furthermore that the connected products must be marketed together, in the course of which no quantity variation of the inter-connected products is possible. With that, the imponderables of the market increase.

2.2. Aerobic Reduction of Organic Residues

2.2.1. Raw Materials and Products

2.2.1.1. Organic residues from agriculture

Solid substrates

Of the wastes arising from agricultural production, only mixtures of straw and liquid manure are immediately suitable for composting. They contain, with a C/N ratio of 10 to 30 and easily available nutrients, sufficient free water (moisture content 70 to 80%) to act as a living area for the active, composting micro-organisms. The structure of the mixture ensures a rich accumulation of air to maintain the aerobic micro-organisms. Depending on the level of interspersion and blending with the excrement, parts of the mixture can be subject to a lack or an excess of water so that aerobic reduction does not take place.

Where the animal excreta are not combined through interspersion, they arise as liquid manure and cannot be composted. Without adding absorbent materials, which enable the development of a rich accumulation of air, the conditions necessary for composting cannot be achieved. Sawdust, peat, composted and dried manure and foam-flakes are additives which are used.

Composting of straw is only possible through the addition of water and a source of nitrogen, as the original C/N-ratio is too large for microbial reduction.

The materials contained in the manure, or manure mixture, are altered through composting. In particular, the nitrogen content decreases so that by separation of urea, dissolved ammoniac easily disappears into the gaseous phase. The waste-gasification of ammoniac is promoted by the temperature occurring during composting, up to a maximum of 85°C, and by a pH-value of over 7. The level of nitrogen loss amounts to 15-60%. Further losses occur for carbon, amounting to a reduction in the organic mass of up to 47%, and water. Easily reducible

components of the manure are partially present in the form of a bacterial mass. All other components of the manure remain preserved in so far as no free liquid is lost.

Composted straw is not immediately usable as fodder. On the other hand, mixtures of straw and manure are introduced, after composting, as a nutrient substrate for fungi cultures (mushrooms, etc.).

Feeding of composted and dried manure to agricultural stock is possible, but legislation has so far prevented the use of this type of material as fodder. A part of the valuable content is lost in the composting process; however on the other hand, the high temperatures make sterilisation possible.

Straw is employed in the production of soil compost because of its breaking-up effects. Besides its use in agriculture, straw can be applied as an absorbing medium in the composting of sewage sludge. Liquid excreta serve as a source of nitrogen and moisture in refuse composting.

### Liquid substrates

The liquid manure which arises from the keeping of beef cattle, pigs and poultry, ought primarily to be transformed into a low-polluting or pollution-free fertiliser by aerobic treatment. It is necessary to achieve as high a degree of dispersion as possible of the added air, in order to safeguard the oxygen requirements of the micro-organisms. Further conditions for high biochemical performance of the metabolism are large material-exchange surfaces, and orientation and renewal of these. The solids dispersed in the substrate should thus be mechanically broken up as extensively as possible to give a small particle size; the viscosity of the suspension should be low enough to ensure free movement of all liquid and solid matter. On account of their material composition, all types of liquid manure are suited to aerobic fermentation, including those occasional mixtures with vegetable matter from litter or fodder.

Steam,  $\text{CO}_2$  and, depending on process control, various quantities of  $\text{NH}_3$  are released by aerobic fermentation. The liquid end-product loses mass because of the conversion of the organic mass into gaseous or vaporous decomposition products. Nitrogen is present in the final liquid either in the form of ammoniac, nitrite or nitrate, or in the biomass, and fixed in undecomposed compounds. Phosphate and potash as well as other vegetable nutrients are also preserved. With storage of the final liquid under anaerobic conditions, the biomass undergoes autolysis and, furthermore, nitrogen oxide is reduced; there follows a reduction in fertiliser value through gasification of molecular nitrogen as well as an odour-nuisance in the form of  $\text{H}_2\text{S}$ .

2.2.1.2. Urban Wastes.

Apart from household refuse, including bulky refuse and similar trade wastes (total ca. 24 million t/a), there arises:

Sewage sludge

- from municipal plants, 1.7 - 2.0 million t. dry weight/year;
- from industrial plants, ca. 1.5 million t. dry weight/year;
- as well as production-specific wastes from industry and agriculture which, in total, considerably exceed the quantities of waste previously mentioned, but which nevertheless do not exactly fit in with what follows.

The suitability of different types of waste for treatment or disposal by various processes is portrayed in simplified form in the following table:

Suitability for treatment/disposal by:-

| Waste Type           | Process  |            |              |
|----------------------|----------|------------|--------------|
|                      | Landfill | Composting | Incineration |
| Domestic refuse      | +        | +          | +            |
| Bulky refuse         | +        | -          | +            |
| Similar trade wastes | +        | /          | +            |
| Sewage sludge        | /        | +          | /            |

+ = suitable,      - = not suitable,      / = partially suitable  
(under certain conditions)

2.2.2. Technical Processes

2.2.2.1. Organic residues from agriculture

As animal excreta in the form of manure mixtures do not represent a commercial product, no processes have been developed to compost excreta on a large-scale. Agricultural practice does not place any great significance on the controlled composting of manure during the storage phase, particularly since the labour cost is high. By contrast, the transport of the manure from the animal to the field is extensively mechanised.

For the composting of litter-free poultry manure, a process has been developed for manufacturing a commercial fertiliser (WaDa<sup>u</sup> process). In this process, 1 m<sup>3</sup> of poultry manure is mixed with about 7 bales of peat and is composted for 7 - 10 days in a 0.5m high pile. With temperatures up to a maximum of 70°C, the material is sterilised. This results in a high-value fertilizer, free from animal-specific odours. In practice, the process has not found widespread use. A temperature-controlled area is required for composting during the winter months.

The firm FAHR has developed a windrowing system for composting refuse, sewage sludge or animal excreta, which has worked successfully.

For the composting of fungi-substrates, various types of machinery and equipment are being offered, which have also found application in practice.

### Liquid substrates

Various types of treatment processes are being offered in the Federal Republic of Germany, which have also been introduced in practice. But not all can yet be described as fully developed.

One version is the oxidation trench under the divided floor of the stall. Excrement and urea pass automatically through slots in the floor into the fermentation area. The substrate is circulated by coil aerators (Fuchs, Mayen) or propeller aerators (Maintz, Swisttal-Ollheim) and supplied with oxygen. In order to reduce sedimentation of materials and the associated unfavourable influence of anaerobic zones, the dry-mass content of the substrate should not be very high so that proper circulation is ensured. The lower dry-mass content can be achieved either with the addition of water in the oxidation trench or by conveying part of the contents of the trench into a separate basin at intervals of a few days. After sedimentation, part of the remainder is conveyed back to the oxidation trench for dilution. The sediment can, if necessary, be composted after de-watering.

With the "Roxidation-Stall", a ring stall from the firm of Eberle and Frick, Bodahus (Liechtenstein), the aeration basin is likewise arranged under the divided floor. It takes in the whole floor area of the stall and is turned and aerated with a surface ventilator. The arrangement of the aeration basin under the floor of the stall can improve the air in the stall and so lead to reduction in odour emissions, except that there is a risk, if operation is interrupted, of foam and harmful gas developing, which endanger the animals. These risks don't exist when treatment of the liquid manure takes place outside the stall. In liquid manure storage containers, surface ventilators (Eisele, Sigmaringen; Rieber, Reutlingen) are continually or intermittently operated. The surface aerators are correspondingly equipped with scoops so that blockages do not occur. Odorous emissions during the process, particularly with intermittent operation, cannot be completely avoided.

For liquid manures which are rich in solid matter, the firm of Reck-Betzenweiler is offering a multi-stage propeller-agitator which introduces compressed air into the substrate. Whereas in general the substrate temperature in the processes mentioned lies in the region of the ambient temperature, other systems deliberately exploit the heat released through oxidation of the organic substance to raise the temperature of the substrate to the mesophilic or thermophilic range (warm and hot processes). Raising the temperature is done with a view to more rapid and further reduction of the organic substance and the killing-off of organisms that are a hazard to health. The heat losses should be kept low and therefore the oxygen in the air brought in must be fully utilised. Suction aerators are particularly suitable for this.

The suction aerator "Centrinator" from the firm of Alfa-Laval, Tumba (Sweden), patent Fuchs-Mayen, was the first apparatus of this type brought onto the market. A rotor functions in its lower section as a fluid pump, and sucks in air into the upper section. The hot process is operated in special heat-insulated containers with the aim of conferring in a few days or weeks, sufficient odour stability on the liquid manure for further storage. This process has become known as "Licom" (Liquid-composting). At temperatures over 40°C, strong emissions of ammonia result.

For the hot process, pipeline suction aerators are equally suitable. Such an apparatus was first offered by the firm of Peters-Schwarzenbeck; one can find on the market the IKA-aerator from the firm of Recentrec-Teningen, and being tested, is a pipeline suction aerator from the firm of Stelzer-Warburg.

For warm treatment (30 to 40°C) in storage containers, the firm of Alfa-Laval has further developed the "Centrinator" to the "Aldo" aerator and equipped it with a foam removal device.

Trickling filter systems for liquid manures are not installed in the Federal Republic of Germany.

#### 2.2.2.2. Urban Wastes

The organic fraction of refuse is composted either alone or together with sewage sludge and/or other suitable waste materials in many places in the Federal Republic of Germany. A total of ca. 3.5% of municipal waste is returned to the biological cycle in this way. The non-compostable components are first of all sorted out and taken to an associated landfill site. Before the actual composting, further processing steps must be carried out such as pulverising, mixing, moistening, etc.

The biological process, also known as retting, is a process of decomposition invoked by aerobic microbial organisms in which mainly the macromolecular components of the material are attacked and converted into smaller units. As with the anaerobic reduction of sludges, this process is assisted by a balanced ratio of carbon to nitrogen.

The processes employed until now in the Federal Republic of Germany can be divided into static and dynamic processes. A process is described as static if, during the first decay phase, the entire material undergoes fungal attack. With constant turning of the decay product and prevention of formation of the related mycelia, one speaks of dynamic processes.

#### Extent of Composting

##### Refuse

In the Federal Republic of Germany, the wastes of 2.08 million inhabitants are processed into compost in 19 composting plants. The refuse processed amounts to ca. 475,000 t and 110,900 t sewage sludge. Figure 1 shows the composting plants currently found in the Federal Republic of Germany. In 1975, the number of inhabitants served increased to about 2.08 million which, nevertheless, is only about 3.4% of the population. Thus, about 205,000 t of compost were produced. This shows that composting methods currently play a subordinate role. It is estimated that about two-thirds of the annual compost production is sold, while the rest is tipped (23).

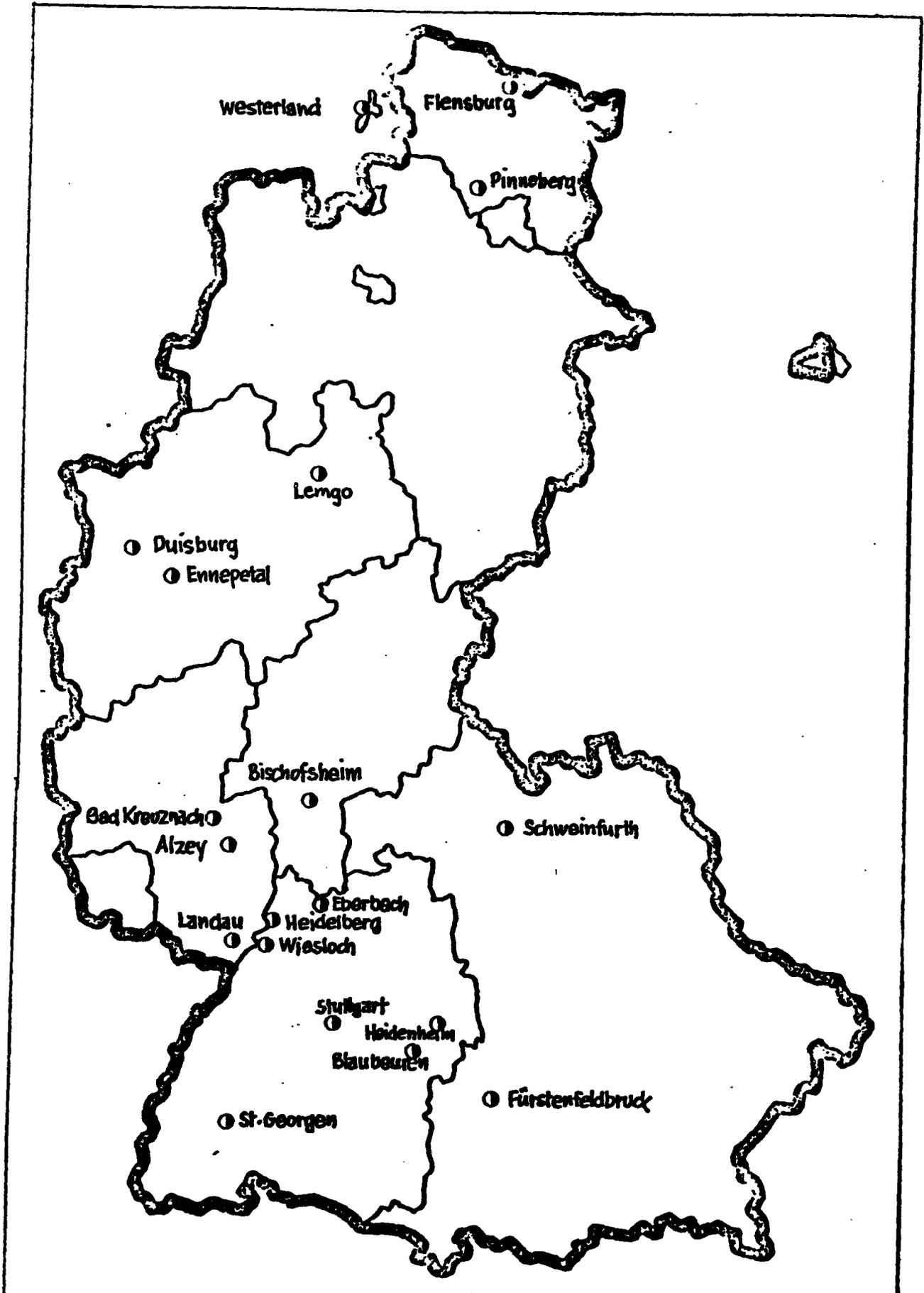


Figure 1. Locations of refuse/sewage sludge composting plants in the Federal Republic of Germany; status October 1976.

Table 3. Composting plants in the Federal Republic of Germany (22)

| Nr. | Location      | Year of Operation | Population served<br>x 1000 | System      | Manufacturer of System |
|-----|---------------|-------------------|-----------------------------|-------------|------------------------|
| 1.  | Alzey         | 1970              | 100                         | Mühle       | Hazemag                |
| 2.  | Blaubeuren    | 1953              | 22                          | Beatmung    | Eigenbau               |
| 3.  | Duisburg      | 1957              | 88                          | Dano        | Dano/VKW               |
| 4.  | Eberback      | 1967              | -                           | Herbold     | -                      |
| 5.  | Ennepetal     | 1974              | 50                          | Prat        | BMA                    |
| 6.  | Flensburg     | 1973              | 180                         | Rheinstahl  | Rheinst./Bühler        |
| 7.  | Geiselbullach | 1971              | 105                         | Freiland    | Voith/Müllex           |
| 8.  | Heidelberg    | 1973              | 170                         | Multibacto  | Hazemag                |
| 9.  | Heidenheim    | 1970              | 85                          | Halde       | Voith/Müllex           |
| 10. | Kreuznach     | 1958              | 45                          | Dano        | Dano                   |
| 11. | Landau        | 1966              | 110                         | Miete       | Diefenbacher           |
| 12. | Pinneberg     | 1974              | 300                         | Büttner     | Dano/VKW               |
| 13. | Schweinfurt   | 1965              | 90                          | Brikollare  | Oliver/Dorr            |
| 14. | St. Georgen   | 1963              | 17                          | Freiland    | Voith/Müllex           |
| 15. | Stuttgart     | 1961              | 40                          | Oliver/Dorr | Oliver/Dorr            |
| 16. | Sylt          | 1973              | 65                          | Rheinstahl  | Rheinst./Bühler        |
| 17. | Wiesloch      | 1971              | 100                         | Halde       | Voith/Müllex           |
| 18. | Gross-Gerau   | 1975              | 300                         | Tafelmiete  | Hazemag                |
| 19. | Lemgo         | 1976              | 220                         | Tafelmiete  | Hazemag                |

### Sewage sludge

There are currently about 20 sludge composting plants (frequently described as "bioreactors") in the Federal Republic of Germany. These plants were erected in the last 3-4 years. In spite of this, the proportion of total arisings of sludge which is composted is very small. This stems firstly from the small number of sludge composting plants in relation to a total of 7000 sewage treatment works. Apart from this, sludge composting plants have until now been erected almost exclusively in small sewage treatment works (under 50,000 population equivalent). With regard to the last three years, development in this sector has been proceeding relatively rapidly so that it was expected that an increasing number of operators of sewage treatment works would choose this form of sludge handling. However, because of technical difficulties in operation at some recently built plants, some Federal States have shown a reserved attitude to these plants so that the new construction of further plants is experiencing a certain delay. The difficulties which have arisen appear, however, to be solvable. In the long term, development in the area of sludge composting is viewed much more positively than in the area of refuse composting. The increasing number of firms which are active in this area also suggests this. The following enumeration, which, because of this rapid development, cannot be guaranteed as complete, only lists the firms with information:

Table 4: Firms manufacturing sludge composting plants

| <u>Name</u>  | <u>System</u>                        | <u>Comments</u>   |
|--|--------------------------------------|---|
| 1) Biologische Abfallverwertungs GmbH & Co (BAV),<br>6369 Schöneck/Hessen  | Decaying tower<br>"Kneer" bioreactor | Numerous - about 15 in the Federal Republic of Germany - numerous abroad              |
| 2) Fa. IBP<br>Internationales Büro für Projektentwicklung,<br>5100, Aachen | Trommel                              | Currently, pilot plant in Aachen  |
| 3) Fa.<br>Wilhelm Roediger<br>6450, Hanau                                  |                                      | Pilot plant in Switzerland under construction<br>(ARA Ergolz I/Sissach)               |
| 4) Fa.<br>Gebrüder Weiss KG,<br>6343 Frohnhausen                           | Decaying tower<br>"Kneer" bioreactor | - 2 BAV - plants under construction<br>- 1 plant under construction in Bad-Liebenzell |
| 5) Fa. Dambach<br>Industrie-Anlagen GmbH,<br>7560 Gaggenau                 |                                      | - Rastatt<br>- further plants under construction in the Stuttgart area.               |

2.2.3. Status of Research and Development

2.2.3.1. Organic residues from agriculture

The material and structural pre-conditions for sound decaying as well as process control for composting agricultural residues and the conversion of components of interest for their fertiliser value, are well known. Research work on these subjects is not currently being conducted in the Federal Republic of Germany.

A process for composting liquid manure enriched with solid matter has been developed at the Institute for Agricultural Machinery Research of the Agricultural Research Establishment, Braunschweig-Völkenrode. Here, either liquid manure is mixed with composted and dried manure so that a moist, crumbly mixture with a moisture content of about 50% emerges, or low-moisture excrement (e.g. pre-dried poultry excrement) is processed. This moist material is composted in a crumbly or compacted form in digestors or windrows. Part of the composted material is dried and is used in the process as a drying-agent for binding the liquid manure. This system has so far found no practical application.

### Liquid substrates

Liquid manure exhibits concentrations of dry mass, BOD<sub>5</sub>, COD, etc, which lie in order of magnitude at approximately twice those of sewage, so that the results of sewage purification techniques are transferable only to a limited extent. Also with respect to substrates in industrial fermentation processes, substantial differences exist. Above all, the economically justifiable marginal costs for treatment of liquid manure lie far below those values usually found in industry.

### Process data

Investigations into the particular microflora in manure have been running for some time at the Bacteriological Institute of the University of Munich, Weihestephan, with the aim of determining the microbiological variables of various treatment methods.

The biologically reducible organic matter in liquid manure is present for the most part in an undissolved form. If aeration is interrupted, the organic material goes into solution through anaerobic transformation processes. With heavily diluted pig and cattle manure, the same or occasionally even greater COD reduction was measured after a 5 to 7 day period of intermittent aeration, as with continuous aeration.

Numerous tests in technical and semi-technical plants have given an insight into the complex interrelationships of liquid manure fermentation under various operating conditions. The results to date do not permit any clear statement on the relationship between decomposition and temperature.

The nitrogen content of liquid manure is influenced by aerobic fermentation. The urea in excrement is rapidly converted to ammonia. Ammonia is also formed from other nitrogen compounds; however, without aeration, only to a limited extent. With aeration, undissociated ammoniac is given off, which leads to nitrogen loss and troublesome odour emissions. Ammoniac-desorption increases with rising pH value and temperature. This can be stopped by timely preliminary nitrification. Its optimum lies at pH = 6.8 - 7.2 and 25 - 35°C, and is sensitive to temperatures over 40°C. In a fermenter in which nitrification

takes place, injected ammoniac-gas is also nitrified. Recent investigations show that the nitrification process can also be influenced by the introduction of protozoa.

With discontinuous or locally insufficient aeration, the oxidised nitrogen is denitrified and escapes into the air. In the case of insufficient fertiliser-producing surface areas, this can be advantageous. Unrequired denitrification can only be avoided by constant and intensive aeration. In contrast to the composting of solid matter, hardly any nitrogen is built into the high molecular fraction of the humic matter with fermentation of liquid manure.

The nitrogen loss by ammoniac desorption was determined at about 40% in tests with hot fermentation; with intensive, continuous aeration this can be considerably higher, and with intermittent aeration, substantially lower.

If the C/N ratio of the liquid manure is raised through addition of readily available carbon, for example from molasses or decayed saw dust, with continuous aeration, a part of the ammoniac becomes fixed in the mass of micro-organisms, and thereby does not form waste gas. After introducing aeration, the biomass goes rapidly over to autolysis and releases the ammoniac again.

In order to hold the pH-value, which normally rises to 8 - 9, below the neutral point through admixing of acid, and thereby to stop the emission of ammoniac, so much acid must be added to the heavily swollen liquid manure, that the costs then become too high.

Apart from ammoniac, other odorous substances can escape through poor or intermittent aeration. This happens particularly when stored manure is aerated. Very little is known about possible remedies.

A similar insufficiently researched area is the odour stability of the treated material and its relationship to the nature of the initial material and its treatment. Very little can be said about this, or which performance parameters are important

for odour stability, and what degree of reduction is needed. Heavily diluted liquid manure could be stored for some months without odour emissions after intensive aeration, whereas concentrated liquid manure only has limited storability. In certain circumstances, emissions can arise from manure, where aeration has been interrupted too early, which are even more unpleasant than those of untreated manure. Observations confirm that the presence of nitrate or nitrite retards the commencement of the fermentation process.

Pathogenic agents can be rendered harmless by hot fermentation. Whereas for untreated liquid manure, 90-300 days are required (depending on type of organism) for the mortification of Salmonella, fermentation at a temperature of 45°C and a pH-value of  $\geq 8.7$  can destroy Salmonella after 2 days aeration. In contrast, Salmonella were still virulent after 5 days aeration at temperatures around 35°C.

Investigations gave similar results with parasites. The most important forms were killed after 3 days at 45°C, whereas, at 35-40°C, 10-14 days and more were necessary. In unaerated liquid manure, parasite eggs remain alive for 60-90 days. Work on the influence of fermentation on viruses is also being undertaken in the Federal Republic of Germany.

#### Process Technology

The aeration arrangements should provide the liquid manure with the necessary oxygen and ensure thorough mixing so that the particles and flock are held in suspension. For the most part, all of these functions are observed in one apparatus.

In aeration containers under stall floors, where excessive foaming cannot be permitted, and in processes where denitrification should not occur, sedimentation must be avoided. For this purpose, a certain minimum overall velocity is maintained by agitation in proximity to the bottom, so that, for mixtures with 2% - dry weight content, 0.4 m/s is specified. Besides this, turbulence is necessary for thorough mixing and supply of oxygen. The most important variables are known from investig-

ations with other materials. While data on the influence of some constructive parameters can be drawn from this, further investigations are needed into the rheological behaviour of liquid manure. The introduction of air in the vicinity of the agitator, which is needed with regard to a good supply of oxygen, reduces the effectiveness of the agitation. Hydraulic efficiency factors of 4% for an air intake of only 0.6% have been calculated, for the flow velocity in the oxidation trench.

The supply of oxygen and the associated oxygen yield, i.e. the oxygen mass dissolved in the liquid per unit of energy, has been until now predominantly measured in water. As a result, there appeared, for surface aeration, efficiency values of between 1 and 2 kg/kWH, which are derived from sewage purification techniques, while other values for aeration of liquid manure have not been obtained. In general, a reduction in oxygen supply is measured when transferred to liquid manure. In contradiction to this, considerably higher oxygen supply values can be calculated with suction aeration in warm production, as was shown by the instruments in the water supply. The reasons for this can be indicated in that the salinity, the ability of the gas bubbles to coalesce, and the high viscosity of the liquid manure impede the acceleration of the bubbles. These relationships require further clarification as does the influence of temperature on the oxygen supply.

The oxidation of the dry matter in the liquid manure yields about 24 kJ/g organic mass, from which about half is biologically released in hot fermentation. As a rule, this energy exceeds considerably the energy required for agitation. In the thermophilic process, 2/3 or more of the heat delivery can escape through evaporation. The equipment design plays a considerable role here. Evaporation is also significant because of the associated reduction in volume. This can be considerable in summer with cold fermentation.

The development of foaming is dependant to a great extent on factors which are still unknown, and can be very intensive where the process is disturbed. In hot fermentation, the foam forms a certain layer of insulation. The foam increases the container volume needed. Excessive foam development can cause damage. The use of de-foaming agents is too expensive; apart from this, the micro-organisms can adjust to de-foaming agents, so that this becomes ineffective. Mechanical foam-preventers such as rotating blades frequently fail with excessive foam development. Aerators with foam intakes have until now proved to be the best.

The stabilising of liquid manure in a short period of months has only succeeded until now with a low dry mass content. Therefore the separation of the liquid manure prior to fermentation into a liquid phase, with a low dry mass content, and a solid phase, promises a substantial improvement. The fermentation of the liquid phase is also made easier.

Objectives of any future research project can be described as follows:

#### Microbiological Research

- Optimising the reduction efficiency;
- Removal of odour emissions, also in energy saving process variations;
- Improving the biochemical stability of the fermentation product;
- Minimising nitrogen losses;

#### Development

- Reducing the energy demand;
- Reducing construction costs;
- Facilitating and improving fermentation by combination with other processes, e.g. by separation;
- Simplifying process control;

#### Hygiene

- Clarifying the hygienic effects of various processes;

Commercialisation

- Clarifying the value of treated liquid fertiliser under various circumstances and uses;
- Economic recovery of nutrient-rich, hygienically perfect feed additives;
- Using the heat released by fermentation.

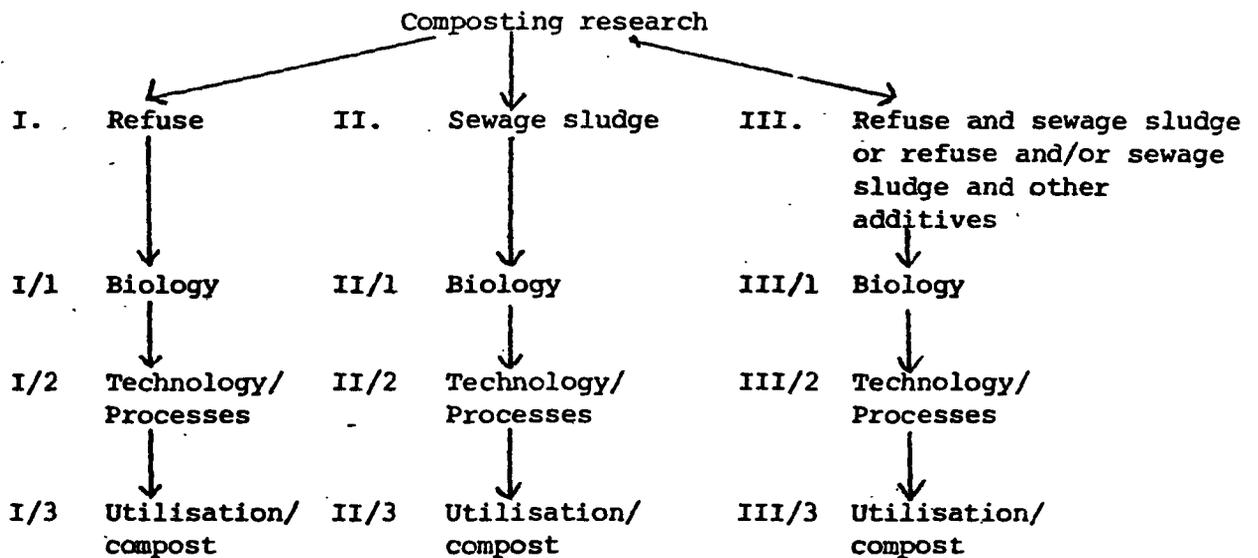
### 2.2.3.2. Urban wastes

Composting on urban wastes has been conducted for about 20 years in the Federal Republic of Germany. The first large-scale plant went into operation in 1954 at Baden-Baden. Since then, intensive basic research as well as process and technology development work has been carried out. In spite of this, the complexity of the biological processes has meant that many questions still remain unanswered and at the present time, new problems have arisen because of changing circumstances.

The activities can be divided into:

- a) Investigations of the biological processes
- b) Improving the technology to aid the decay processes and to recover high value products
- c) Searching for opportunities for using the products
- d) Investigation and analysis of the raw materials and products.

Table 5: Areas of research into composting



The intensity with which the individual areas shown in table 5 are being pursued is nevertheless very variable. The following information refers to those research activities which are being undertaken through ministries of the Federal Republic of Germany.

I/1 Refuse - Biology:

In this area, numerous questions remain open of which currently the most topical is the question of odour emissions and their control. One project is being promoted in this area and a second is proposed. Further projects of the Federal Ministry of the Interior/Federal Environment Agency are examining to what extent:

- highly cellulosic urban wastes can be rapidly reduced by controlled treatment and
- antibiotic materials are formed in composting, which have an influence on the decontamination of the compost.

I/2 Refuse - Technology/Processes:

Currently, no necessity is seen for intensive research activities in this area. The Federal Ministry of the Interior/Federal Environment Agency are promoting a project which concerns the further development of the composting process in Blaubeuren. A project to compile and evaluate operating experience (costs) of existing composting plants is envisaged.

I/3 Refuse - Utilisation/Compost:

In contrast to the other forms of waste treatment (landfill, incineration), the area of compost utilisation (sale of compost) and related questions plays a decisive role in the Federal Republic of Germany. For this reason, numerous projects are being promoted which deal with the following questions:

- improving compost sales with regard to sales organisation and marketing,
- improving the quality characteristics of the products,
- improving production techniques,
- hygienic questions in connection with the end product,
- toxic materials in the product and their effects.

The issue of toxic substances has acquired a particular emphasis in the last few years. Extensive research work has already demonstrated that the dangers of toxic substances in compost have been overrated and can be avoided by appropriate application of this material. These findings have already been presented in various leaflets and information publications. One can specify here in particular the "Leaflet for Quality Criteria and Recommendations for the Use of Compost from Refuse and Sewage Sludge". Nevertheless, further in-depth investigations are required in order to confer greater weight on this leaflet. Investigations are envisaged on:

- standardisation of hygiene tests,
- compilation of standard methods for determining the degree of decay,
- establishment of limit values for heavy metals in soil contingent on soil characteristics,
- development of criteria for the concept of "storage stability".

#### II/1 Sludge - Biology

No projects are currently being promoted in this area. Results from refuse composting can in part be transferred, so that promotional measures do not appear to be required.

#### II/2 Sludge - Technology/Processes

Currently, only limited promotion of research is taking place in this area, as development is being rapidly advanced here by industry and therefore State promotion does not appear to be necessary at the present time.

#### II/3 Sludge - Utilisation/Compost

Because of the comparatively small quantities of compost which currently arise in sludge composting plants, no problems are known to exist with the sale of the compost until now. On the subject of hygiene, a project is being promoted by the Federal Ministry for Research and Technology in which various sludge decaying systems are being examined for safety from the hygiene point of view. The present problems in connection with toxic substances are comparable to those dealt with in paragraph I/3 and are predominantly covered by the corresponding projects.

III. Composting of Mixtures of Refuse and Sewage Sludge and other Additives

Public funding is not currently being applied in this area as the issues arising are included in the projects being carried out on refuse or sludge composting.

The following list gives an overview of the currently running and planned research projects which are being promoted by both of the responsible Federal Ministries, the Federal Ministry of the Interior and the Federal Ministry for Research and Technology.

Table 6: Research Projects in the Area of Composting, Supported by the Federal Ministry of the Interior, Federal Environment Agency and the Federal Ministry for Research and Technology

| Theme   | Researching Body   | Duration  | Status  |
|---|--|-----------|---------|
| I/1   |  |           |         |
| - Formation of antibiotic substances in the composting of urban wastes                          | Institute for Agricultural Microbiology/Giessen                                  | 1975-78   | Current |
| - Flexible intensive composting of solid waste materials  | Institute for Water Development/Stuttgart  | 1975-77   | Current |
| - Metabolic products of composting  | Institute for Organic Chemistry/Heidelberg                                       | 1975-77   | Current |
| - Comparative measurements of odour emissions from waste treatment plants (composting/landfill) | ?  | from 1978 | Planned |
| I/2   |  |           |         |
| - Silosystem with controllable aeration   | Institute for Soil Hygiene/Blaubeuren  | 1977      | Current |
| - Compilation and evaluation of operating experience from existing composting plants            | Siedlungsverband Ruhrkohlenbezirk/Essen (Urban Authority for the Ruhr Coal Area) | 1977-(?)  | Planned |
| - Federal Ministry for Research & Technology/ Investigations of cascade mills & tunnel windrows | Engineering Bureau for Health Technology/Mannheim                                | from 1977 | Planned |

| Theme  | Researching Body   | Duration   | Status   |
|--|--|--|--|
| <p>I/3</p> <ul style="list-style-type: none"> <li>- Compost production on a steep incline</li> <li>- Compost marketing</li> <li>- Federal Ministry for Research and Technology/ demonstration project for compost utilisation</li> <li>- Assessment of various compost qualities/decay grade</li> <li>- Examination of "the Preliminary Leaflet for Compost Quality" in practice in selected composting plants</li> <li>- Investigations of hygiene in refuse composting processes</li> <li>- Standardisation of hygiene - investigations for composting plants</li> <li>- Soil loading with organic materials</li> <li>- Concentration of toxic substances in soil</li> <li>- Heavy metals displacement in soils</li> <li>- Toxic substances in vini-culture</li> </ul> | <p>Institute for Advanced Technology/Karlsruhe</p> <p>Dr. Döspohl</p> <p>Association of Agricultural Commerce/Stuttgart</p> <p>Institute for Water Development/Stuttgart</p> <p>?</p> <p>Institute for Animal Hygiene/Stuttgart</p> <p>Institute for Environmental Hygiene/ Marburg</p> <p>Institute for Phytogeny/Braunschweig</p> <p>Institute for Soil Science/Giessen</p> <p>Institute for Phytogeny/Braunschweig</p> <p>Institute for Vine Diseases/Bernkastel-Kues</p> | <p>1977</p> <p>1976-77</p> <p>from 1977</p> <p>1977-79</p> <p>from 1978</p> <p>1975-79</p> <p>1977-78</p> <p>1975-78</p> <p>1975-78</p> <p>1975-78</p> <p>1975-78</p> <p>1975-79</p> | <p>Current</p> <p>Current</p> <p>Planned</p> <p>Planned</p> <p>Planned</p> <p>Current</p> <p>Planned</p> <p>Current</p> <p>Current</p> <p>Current</p> <p>Current</p> |
| <p>II/1 Not applicable</p>   |  |  |  |
| <p>II/2</p> <ul style="list-style-type: none"> <li>- Composting of granulated sewage sludge</li> <li>- Further development and optimisation of mechanical sludge composting with the HKS-process</li> </ul>  | <p>Institute for Soil Technology/Braunschweig</p> <p>Institute for Construction Technology/ Aachen</p>   | <p>from 1977</p> <p>from 1978</p>  | <p>Planned</p> <p>Planned</p>  |

| Theme   | Researching Body                          | Duration | Status  |
|---|---|----------|---------|
| II/3<br>- Federal Ministry for<br>Research and Technology<br>/Sterilisation of Sewage<br>Sludge in composting<br>plants | Institute for Animal<br>Hygiene/Stuttgart | 1976-78  | Current |

2.2.4. Economic Evaluation

2.2.4.1. Organic residues from agriculture

No generally valid statement can be made about the economic viability of aerobic treatment processes because of the variety of currently employed technical methods and the large differences in operating conditions under which the individual plants function. Comparative investigations currently running and scheduled for termination at the end of this year (BML/KTBL - model project "Liquid manure treatment") will furnish operating data which can be drawn upon for a process evaluation.

2.2.4.2. Urban wastes.

Despite over 20 years experience, composting has not yet unequivocally succeeded in the Federal Republic. One reason for this certainly lies in the fact that only a relatively small portion of the components of refuse are specifically suitable for composting, namely ca. 30 - 50%. The separation of the inert, troublesome and noxious matter, so far as this is at all technically possible, is expensive, and leads to a low compost yield and high arisings of residual matter.

A solution to this problem emanates from its combination with sorting processes, where further raw materials are recovered either before or after composting.

Composting has particular significance as a process for using sewage sludge.

The problem of odorous emissions from compost windrows is not yet completely resolved.

Costs of compost production and revenues.

The costs for composting are customarily expressed in DM/t waste, that is they are described as disposal costs and not as costs of compost production.

For the following four briefly characterised, short examples, approximate estimates of costs are set against production data in table 7.

- a) Simple composting of pulverised wastes in aerated or ventilated windrows.
- |                |  |
|----------------|--|
| Advantages:    | low costs  |
| Disadvantages: | large site requirements,<br>odour formation cannot always be avoided with certainty,<br>insensitive location needed,<br>composting site drainage problematic |

- b) Simple composting with linked pre-retting in closed cells or reactors, and subsequent retting in windrows.  
Advantages: smaller site requirements,  
no emission problems,  
Disadvantages: higher costs compared with a)
- c) Composting as in b), however with additional expense for technology and construction to improve the quality of the compost.  
e.g. fine sieving, air classification for the separation of heavy matter and avoidance of emissions, e.g. subsequent retting in a ventilated chamber.  
Advantages: good compost quality,  
no emission problems,  
Disadvantages: high costs, low compost yield,  
higher residue portion.
- d) Composting of specific, suitable, homogeneous types of wastes such as e.g. "the organic residual fraction" from a household refuse sorting plant, production-specific industrial organic waste (e.g. bark), de-watered sludges.  
Advantages: good compost quality (standardisable by controlling the raw material mix),  
high compost yield and low residue portion.  
Disadvantages: only possible in combination with a sorting plant.

Table 7.

Costs for Compost Production and Possible Revenues in 4 Typical Examples

|   | a                           | b  | c                           | d   |
|---|-----------------------------|--|-----------------------------|---|
| Process   | Windrow Composting          | Composting with linked pre-retting in closed cells, etc. |                             | Composting in connection with sorting plant |
|   |                             | simple operation   | more expensive operation    |   |
| Costs, DM/t waste   | 35.--                       | 50.--  | 80.--                       | 50.--                                       |
| Compost yield as % of crude refuse throughput   | 50%                         | 50%  | 30%                         | 70%   |
| Production Costs, DM/t compost  | 70.--                       | 100.--   | 270.--                      | 70.--                                       |
| -----   |                             |  |                             |   |
| Deductible offset for landfill costs and volume saving DM/t compost<br>(alternative DM/t compost) | - 30.--<br><u>(- 50.--)</u> | - 30.--<br><u>(- 50.--)</u>                              | - 30.--<br><u>(- 50.--)</u> | - 30.--<br><u>(- 50.--)</u>                 |
| Remaining production costs DM/t compost<br>(alternative production costs DM/t compost)            | 40.--<br>(20.--)            | 70.--<br>(50.--)   | 240.--<br>(220.--)          | 40.--<br>(20.--)                            |
| Compost quality   | low                         | medium   | good                        | good (standard-<br>isable?)                 |
| Possible sale price   | 0 to 5.--                   | 5.-- to 15.--  | 15.-- to 30.--              | 20.-- to 40.--                              |

Table 8.

Investment Costs for Composting Plants, by Performance  
and Operating Type in Millions DM.

| Operating<br>Type<br><br>Performance | Plant with:  |  |                             |  |
|--------------------------------------|--|--|-----------------------------|--|
|                                      | Sewage sludge<br>processing,<br>Residue inciner-<br>ation,<br>Bulky waste<br>reduction | Sewage sludge<br>processing,<br>Residue inciner-<br>ations | Sewage sludge<br>processing | Residue inciner-<br>ation,<br>Bulky waste<br>reduction |
| 2 x 40 t/d                           | 24.081   | 23.009   | 13.023                      | 23.835   |
| 2 x 100 t/d                          | 36.830   | 32.235   | 20.566                      | 36.463   |
| 4 x 100 t/d                          | 60.965   | 58.954   | 33.086                      | 60.459   |

Whereas in examples a), b), and c), the disposal stands in the foreground, in case d) it is a matter of applying composting as a recovery process in connection with other processes for the recovery of raw materials from wastes, as is envisaged in the "Federal Waste Recovery Model".

In the first cases, where conventional composting is employed, the raw material to be processed is a waste mixture with an indeterminate composition; an increase in the quality can only be bought by a lower compost yield.

In the last case, raw material components with a relatively homogeneous composition are available, and by controlling the circumstances of the mixing of these components, the quality of the end-product can be influenced.

The comparison of costs for compost production and compost revenues in table 7 must lead to the conclusion that, from an economic point of view, either the simplest technique of windrow composting or the conceivable future combination with sorting plants, should be aimed for.

With existing plants of type b) or c), the subsequent addition of a sorting plant would be a future method of improving the cost situation.

Nevertheless, precipitous deductions must be warned against as the economic benefits of composting presented in table 7 are very much simplified and incomplete.

#### Marketing problems

The newer, large-scale composting works suffer above all from marketing problems.

The reasons for this are, inter alia:

- lack of information on and consideration of potential customers,
- shortcomings in quality standards and inspection in the composting works,
- exaggerated "noxious odours",

- lack of support for compost use from the side of the responsible specialised authorities and institutes,
- the sensitivity of composting to transport costs.

These problems are predominantly organisational in nature. The operators of composting plants, as a rule the regional authorities responsible for disposal, are overburdened when confronted with problems that require solving. It would therefore be desirable to aim for supra-regional, private-sector organisations, e.g. active at the state level or nationally.

## 2.3. Carbohydrate Hydrolysis

### 2.3.1. Raw materials and products

There are essentially four classes of polymeric compounds which are individually or entirely found in biogenic residues and which cannot be readily exploited microbiologically. These are starches, celluloses, hemi-celluloses and lignins. Before fermentation, these must be split into their monomers. In this regard, the polysaccharides starch and cellulose create fewer difficulties than the hemi-celluloses and lignins. In general, the splitting is carried out by acid, basic or enzymatic hydrolysis. The addition of an acid or alkali follows stoichiometric principles, and enzymatic fission must observe all the conditions specific to such a reaction.

#### Starch (1,7,8,13)

Starch is stored in the form of so-called starch granules as reserve material in grain and potatoes (amongst others). It is found there in two forms; amylose, which amounts to about 20 - 30% natural starch, and amylopectin (ca. 80%). Both forms are composed of glucose units. They differ in the type of bonding of the units and, in this respect, amylopectin still contains groups of phosphates. With heating in water, amylose goes into colloidal solution, whereas the amylopectin swells up (starch paste). The fission to D-glucose occurs by the controlled admixing of diluted acids or bases. The starch can be reduced enzymatically with the assistance of amylases ( $\alpha$  and  $\beta$  amylases) to maltose units which are split via maltase into glucose.

#### Cellulose

Cellulose is the most common naturally-occurring poly-saccharide. It is likewise based on glucose. Its molecular weight lies between 600,000 and 1,500,000. In natural matter, cellulose occurs in two forms, amorphous and crystalline. Amorphous cellulose is easily fissionable, the crystalline form difficult to split (1, 24,25). Treatment through acid, basic or enzymatic hydrolysis also leads to splitting into the monomeric elements, the glucose units. The output of sugar depends essentially on whether the cellulose is made available for the hydrolysis reagents by suitable treatment of the vegetable material.

### Hemi-celluloses

Hemi-celluloses are polysaccharides of low molecular weight which are found and in the cell walls of plants, in plant mucilage and reserve organs and are closely combined there with pectin substances, lipids, proteins, pigments, mineral substances (silicic acid) and particularly with cellulose and lignin (24). From a chemical viewpoint, hemi-celluloses are made up of a limited number of sugars, especially from D-Xylose, D-Mannose, D-Glucose, D-Galactose, L-Arabinose, glucuronic acid, galacturonic acid and small quantities of L-Rhamnose, L-Fucose as well as various sugar derivatives.

The most abundant hemi-celluloses are the xylans which occur in large quantities in the lignified tissue of plants (bran 20 to 30%, Grain straw 20%, bast 20%, coniferous wood 7 to 12%, deciduous wood 20 to 25%). They are made up primarily of xylose and small quantities of arabinose which means they are, in contrast to starch and cellulose, built to a large extent from pentoses (1).

With the hydrolysis of vegetable residues, one can always reckon on a considerable proportion of pentoses by virtue of the content of hemi-celluloses. These can be used by many micro-organisms as a carbon source. The important industrial yeasts are, however, almost all specialised in exploiting hexoses. In order to be able to fermentatively utilise such materials, one is directed to employing mixed cultures of yeasts, bacteria or specialised fungi in multi-stage processes.

In contrast, the pentoses can be processed into furfural by a chemical process with dilute acids, a method employed for the processing of straw. Furfural is used as a selective solvent in oil refining or as a primary material for chemical syntheses (8).

### Lignin

Lignin is the incrustated escort material of cellulose and is combined with this in a reactive form. The content varies in different ligneous tissues between 18 and 30% (8,13). It involves a very complex bond, the structure of which has not yet been completely established for it has not until now been successfully

isolated in unaltered form. The techniques for separation always lead to powerful transformations. Fragments result which contain phenyl-propane derivatives, as basic elements; in woods predominantly coniferyl and sinapinal alcohol and in grasses cymaryl alcohol (1).

Two types of lignin digestion are generally used, alkali melting and separation with strong acids (72%  $H_2SO_4$ , supersaturated HCL). With alkali decomposition, the chemical attack results from the acidity of the phenolic-OH group while with acid decomposition, it arises from the ether links.

A microbial process for the exploitation of lignin fragments is not available at present. The means that non-decomposable residuals remain after the recovery of cellulosic residues.

Tests conducted with various types of pre-treatment of the vegetable material, aimed at achieving more rapid and better reduction, have partially succeeded and are being continued.

The tests involve:

1. purely photochemical processes
  - microwave radiation
  - UV-radiation
  - electron radiation
  - laser radiation
2. photochemical processes in combination with chemical processes
  - UV-radiation (355 and 253 nm) in the presence of  $NaNO_2$
3. purely chemical processes.

All processes produce significant changes in the polymer structure. Technical application does not however appear to be possible at the present time.

2.3.2. Technical Processes

Technical processes for the hydrolysis of the carbohydrate compounds described here are known both for starch-containing and cellulosic materials. Starch hydrolysis (saccharification processes) plays a role in industrial practice for obtaining special foodstuffs e.g. biomalt from barley starch with the aid of diastase (maltase) or pure sugar products, primarily starch sugar, dextrose and starch syrup. Acid hydrolysis is becoming increasingly superseded by enzymatic processes in industrial practice, as these can be better controlled. The following diagram (figure 2) gives an overview of the practically significant saccharification processes.

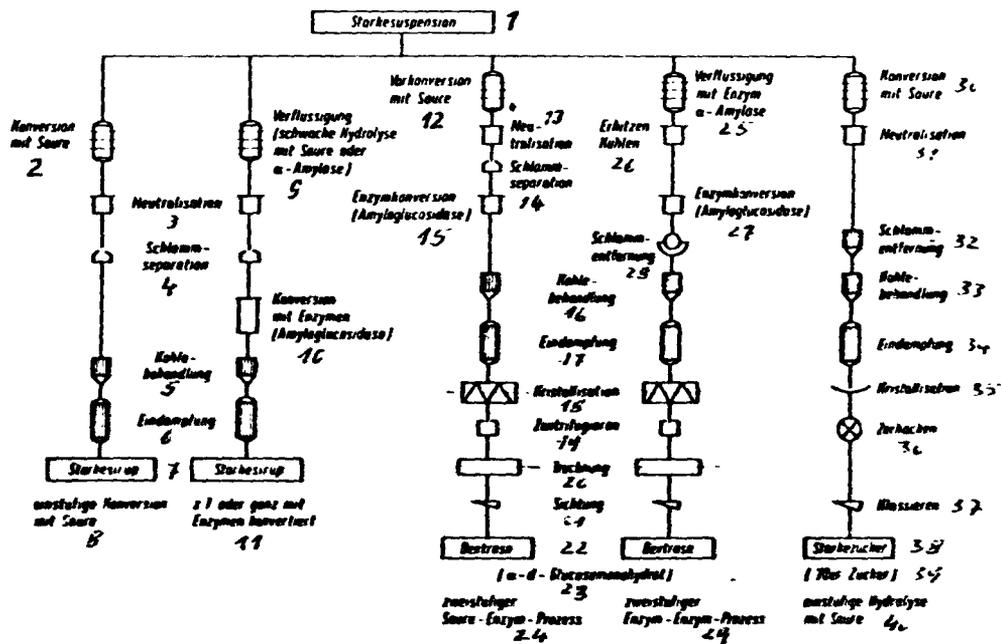


Figure 2

Figure 2. Saccharification Processes (overview) (8)

1. Starch suspension
2. Acid conversion
3. Neutralisation
4. Sludge separation
5. Carbon treatment
6. Evaporation
7. Starch syrup
8. One stage conversion with acid
9. Liquefaction (weak hydrolysis with acid or  $\alpha$ -amylase)
10. Conversion with enzymes (amyloglucosidase)
11. Partially or totally converted with enzymes
12. Pre-conversion with acid
13. Neutralisation
14. Sludge separation
15. Enzymatic conversion (amyloglucosidase)
16. Carbon treatment
17. Evaporation
18. Crystallisation
19. Centrifuging
20. Drying
21. Screening
22. Dextrose
24. Two stage acid-enzyme process
25. Liquefaction with enzyme  $\alpha$ -amylase.
26. Heating/cooling
27. Enzymatic conversion (amyloglucosidase)
28. Sludge removal
29. Two stage enzyme - enzyme process
30. Conversion with acid
31. Neutralisation
32. Sludge removal
33. Carbon treatment
34. Evaporation
35. Crystallisation
36. Chopping
37. Classification
38. Starch sugar
39. (70% sugar)
40. One stage hydrolysis with acid.

The other carbohydrates always occur together with cellulose so that one can confine the discussion to processes for utilising cellulose.

Cellulose is a base material for such products as:

- paper,
- chemical pulp,
- cellulose derivatives:   - ester  
                                  - ether  
                                  - acetate  
                                  - oxicellulose
- xylose, and others.

A large percentage of cellulose requirements are met by the processing of wood which, in the course of time, has led to a range of large-scale digestion processes that are also capable of being applied to the processing of cellulosic residues.

Sugar recovery by digestion of wood

The classic processes for the extraction of sugar from wood (under current conditions, these have become uneconomic in the Federal Republic of Germany) were the Bergius and Scholler processes.

**Bergius:** Hydrolysis of wood chips with ca. 40% HCL at room temperature to a polymeric, tasteless, still non-fermentable but digestible dry sugar, which can already be employed as cattle feed. Further hydrolysis of the dry sugar with 10% HCL from which a mixture of glucose, mannose, galactose, fructose and xylose emerges, that, with fermentation, gives yeast (foodstuff) and wood spirit.

Disadvantages of the process:

- acid-resisting reactors,
- vacuum evaporation for removing the acid,
- lignin and hemi-cellulose accumulation,
- danger of secondary decomposition of the hexoses.

By recovering further products such as lignin-plastics, sorbit, glycerin, xylose, lignite, efforts have been made to increase productivity, which have clearly not proved successful.

Yield: 100 kg of pinewood result in 60 kg of wood sugar,  
2 kg of acetic acid, 3 kg of resin, 33 kg of lignin.  
66 kg of wood sugar result in 25 kg of yeast or  
34 l. of 94% ethanol, 15 kg sugar slops (8,13).

Scholler:

(Percolation)

Hydrolysis of sawdust with dilute sulphuric acid (0.2-0.6%)  
at ca. 170°C and 8 atm, to fermentable sugar. Result: 4%  
sugar solution with low acid content. Neutralisation with  
lime and phosphate; after addition of malt culms, fermentation  
to ethanol and CO<sub>2</sub>.

Yield: 100 kg of woodpulp result in 50-55 kg of sugar and  
22-24 l. of 98% ethanol (8).

Disadvantages of the process:

- high energy consumption,
- acid-resisting reactors,
- vacuum evaporation for removing the acid,
- lignin and hemi-cellulose accumulation,
- danger of secondary decomposition of the hexoses.

#### Wood digestion processes for producing woodpulp:

1. Alkaline process (alkaline or sulphate pulp). Digestion is carried out with a solution of 25% Na<sub>2</sub>S and 75% NaOH at 6-7 atm. and 170°C for 5-6 hours. Pine is mainly used. By-products can be recovered such as turpentine (10 kg. turpentine oil per tonne of wood), colophonium, woodpulp, as well as tall oil. Straw chaff can also be treated with this process (8,13).
2. Acid process (sulphite pulp). The cooking liquor comprises calcium bisulphite Ca (HSO<sub>3</sub>)<sub>2</sub>. Depending on the type of cooking, the temperatures lie between 115°C and 150°C, the pressure required between 2.5 - 6 atm., the duration of cooking amounts to 20 -35 hours with indirect cooking (Mitscherlich) and 7 - 15 hours with direct cooking (Ritter-Kellner). These values change according to the type of wood used and the desired form of pulp.

The sulphite liquors were previously used as a common substrate for yeast production (8,13).

3. Nitric acid digestion (N-cellulose).

Beechwood is particularly used in this process and, in Japan, also the straw from annual plants. It is characterised by low digestion temperatures (less than 100°C), and short cooking times (7 hours). The pulp yield is relatively high and the material contains only small quantities of quality-impairing hemi-celluloses. The cellulose is particularly suitable for manufacturing cellulose derivatives (8,13).

### 2.3.3. Status of Research and Development

No information exists on concrete projects for the hydrolysis of starch-containing residues. Starch-containing effluents arise, for example, from the processing of potatoes, with, however, considerable proportions of protein, and treatment takes place primarily with the objective of recovering protein. The methods used here are purely precipitative and extractive methods without any fermentation.

The following institutes and firms in the Federal Republic of Germany are involved in possibilities for the utilisation of cellulosic residues:

- University of Hohenheim
- GBF Stöckheim
- the firm of Böhlinger
- the firm of Haas
- the nuclear research plant at Jülich
- land research Weihenstephan (University of Munich)
- FAL - Braunschweig
- the firm of Bayer, Leverkusen
- the firm of Ikato Dortmund.

The investigations are primarily directed towards achieving better enzymatic digestion processes. In addition, work is going on to develop more efficient reactors.

Currently, the following R and D projects are being supported by public funds (17):

| <u>Research Project</u>   | <u>Institution</u>   |
|---|--|
| Cellulose and cellulosic raw materials as unconventional biotechnical substrates. | Institute for Food Technology, University of Hohenheim                 |
| Conversion of cellulosic wastes from agriculture into SCP.                        | Institute for Biology, Federal Food Research Establishment, Karlsruhe. |

| <u>Research Project</u>   | <u>Institution</u>  |
|---|---|
| Manufacture, storage and utilisation of inoculants of productive micro-organisms for the fermenting of liquid manure. | FAL - Braunschweig  |
| Microbial processing of straw to fodder.  | FAL - Braunschweig  |
| Preservation of microbial fodder from processed straw.  | FAL - Braunschweig  |
| Basic investigations into the microbial reduction of bark wastes.   | Federal Research Establishment for Forest and Wood Management, Hamburg  |
| Biochemical utilisation of wood   | Federal Research Establishment for Forest and Wood Management, Hamburg. |

The aim of these research projects is the biological or enzymatic reduction of problematic materials into metabolisable units to enable secondary production to be subsequently carried out.

For the reduction of cellulosic residues, various aerobic and anaerobic micro-organisms are being tested. While activities in the Federal Republic of Germany are still at the initial testing stage, there already exist reports from the USA containing the results from semi-scale pilot plants (9). There, the best reduction results were obtained with highly cultivated strains of the fungi-type *Trichoderma Viride*.

A limiting factor in development is that the mechanism of the cellulose-function is not completely understood at present. It appears that the combined action of an entire enzyme complex is necessary for splitting cellulose (25,26).

However, as cellulose is always combined with other components in vegetable residues, many more details investigations are required before maximum yields can be achieved. Aside from research into hydrolytic reduction, greater attention must be paid to the comminution and preparation of the material.

2.3.4. Economic Evaluation

Digestion processes by themselves cannot be easily subjected to economic analysis. They must be seen in relation to associated process stages, as they themselves represent only the preliminary section of a process. Nevertheless, something can be said, if only a few generally worded statements.

With respect to the hydrolysis of cellulosic materials, there is a high likelihood of a surplus emerging for enzymatic digestion, as opposed to the physical/chemical treatment of the materials. If one examines the digestion processes mentioned, there are at least two cost-intensive variables to consider:

1. They must be operated at a high temperature and under pressure, which is related to energy costs.
2. Technically, with these processes, the danger exists that the glucose formed is further reduced. This means that controlling the process is relatively expensive.

Without doubt, an advantage lies in the fact that, with reference to plant size, they can be operated at high throughputs.

Enzymatic reduction processes have the advantage of high selectivity and specificity. In using pure enzymes, there is no danger of uncontrolled reduction of the end products, though the theoretically possible yields are substantially determined by the condition of the substrate. This must be prepared in such a way that the enzymes can act and should not contain any disturbing structural elements. The recovery of active proteins and their optimal use will be a substantial contributory determinant of economic viability. The performance of known enzyme systems can certainly be improved with further development.

In the case of physical/chemical processes, comparable throughputs are achievable only with increased expenditure on process engineering. In particular, one can proceed on the assumption that the enzymes must be introduced in excess, as a part of the protein is rendered inactive by indefinable substances in the culture medium. The product, e.g. glucose, must be extracted at a defined concentration in the system, as there exists the danger of product constraint (27).

2.4. Protein Recovery

The annual protein demand for fodder purposes in the EEC region amounts to ca. 10 million tonnes. The provision of such quantities is expensive and can only be achieved by importing soya and fishmeal. In recent years, dependence on imports has proved to be disadvantageous.

Microbial protein is of equivalent nutrient value to conventional protein sources. It is therefore suitable as a substitute product and can, apart from this, be manufactured domestically.

Many organic residues can, either directly or after suitable reduction, be used as a substrate for the production of microbial protein. Prior to the second world war, a large part of the demand for feed protein was met by the fermentation of sulphite liquors (28). With the exception of a few small-scale processes (baking yeast, diet yeast, brewing yeast), this originally widely-used technology was unable to withstand economic progress. In the last few years, however, it has experienced something of a revival, stimulated by the recent development of processes for the recovery of microbial protein products from components of petroleum. Two reasons can be identified for this motivation:

- a) the imbalance between the rapidly increasing world population and the less flexible production of food;
- b) Growing environmental awareness, particularly in industrialised countries where, instead of just disposing of wastes, the efficient utilisation of biogenic residues is aimed for.

The processes which are currently being tested with varying degrees of success, are directed towards the mass cultivation of yeasts, fungi, bacteria and algae, and are all distinguished by a short growth period and a high protein content. The application of these products for human alimentation is at present uncertain, but is regarded as fundamentally feasible.

2.4.1. Raw Materials and Products

Nutrient substrates and initial materials for the production of yeasts and bacteria are:

- starch-containing raw materials,
- sugar-containing raw materials,
- hydrolysate from wood and annual plants.

These substrates have been commonly used for a long time. One can regard them as conventional substrates. The fermentable/assimilable materials contained therein are primarily sugars (28):

- maltose
- saccharose
- glucose
- galactose, etc.

Unconventional substrates are:

- alkanes (ca.  $C_{10} - C_{23}$ )
- alcohols (methanol, ethanol, isopropanol)
- aldehydes
- organic acids
- whey and other discharges from the food industry
- wastes from livestock production after processing
- municipal sewage
- $H_2$  and  $CO_2$

The synopsis shows that particularly the discharges from food production as well as solid residues must be subjected to specific pre-treatment, with the objective of transforming these into a usable condition for micro-organisms. But as the range of substrates and organisms is very broad, there inevitably emerges an abundance of process possibilities.

For biosynthesis, one should obviously select, for technical reasons, fermentative reduction for the processing of the material to be treated. However, complications are not thereby excluded.

The combination of enzymatic reduction and microbial growth can result in undesired enzyme reactions, for instance, the reduction of amylases or cellulases by proteinases, whereby the hydrolysis of the polysaccharides is disturbed.

The inorganic raw materials for biosynthesis are essentially potassium, magnesium, phosphate and, in particular, ammonium salts as a nitrogen source. In addition, most micro-organisms require vitamins for their growth.

The end products are various biomasses (depending on the type of organisms) which are usable after undergoing a separation process. The following yeasts have proved successful for the recovery of microbial protein (for many of the yeasts listed, there exist synonyms which are not explicitly mentioned) (29):

Table 9: Fermentation and Assimilation Characteristics of Important Yeasts (29)

| Type                            | Growth-<br>Temperature | Lactose-<br>assimilation     | Lactate-<br>assimilation | Maltose-<br>assimilation | Glucose-<br>assimilation | Citric acid<br>assimilation |
|---------------------------------|------------------------|------------------------------|--------------------------|--------------------------|--------------------------|-----------------------------|
| <i>Saccheromyces fragilis</i>   | 37°C                   | +                            | +                        | -                        | +                        | +                           |
| <i>Torula cremoris</i>          | 44 - 47°C              | +                            | +                        | +                        | +                        | weak                        |
| <i>Torula (Candida) lactosa</i> | 37 - 42°C              | +                            | +                        | -                        | +                        | -                           |
| <i>Torula utilis</i>            | 39 - 43°C              | -<br>(+ after<br>adjustment) | +                        | +                        | +                        | +                           |
| <i>Saccheromyces cerevisiae</i> | 28 - 40°C              | -                            | +                        | +                        | +                        | -                           |

The protein content of the yeasts varies according to the process. The highest protein yields are obtained with *Pseudomonas methylum* i.e. on a methanol-basis (over 80% raw protein). *Saccheromyces cerevisiae* produces ca. 60% raw protein based on whey (without process modifications, but these are detailed in the section on "Status of Research and Development"), *Candida utilis* based on sulphite waste liquor lies under 60%; from information from Dietrich (Waldhof yeast) (28) the values even lie under 50%, thus about the order of magnitude of soya meal.

Further end products depend on the process itself and on the initial material. That is, with the processing of sulphite discharges by the Waldhof process, in addition to a high  $BOD_5$  from the centrifuge outlet, considerable quantities of lignin still occur.

The combination of special fermentation processes for the recovery of secondary materials and biomasses is also possible; an example of this was already shown with alcohol fermentation. Other examples are:

Fermentation with bacteria to:

- acetic acid
- gluconic acid
- dihydroxyacetone

Fermentation with fungi to:

- citric acid
- oxalic, fumaric and gluconic acids
- antibiotics

(30, 31)

#### 2.4.2. Technical Processes

Two processes are of particular significance:

- manufacture of baking yeast with *Saccheromyces cerevisiae* based on molasses, and,
- production of yeast from spent sulphite liquors with *Candida utilis*.

##### 2.4.2.1. Production of yeast from molasses

By the term molasses, is meant exclusively beet molasses.

The primary component of molasses is saccharose; other sugars are raffinose, ketose and galactose. The following table gives average values for the most important components (32):

|                   |          |
|-------------------|----------|
| H <sub>2</sub> O  | 20%      |
| Saccharose        | 48 - 50% |
| Raffinose         | 0.8%     |
| Invert Sugar      | 0.3%     |
| K <sub>2</sub> O  | 4.4%     |
| Na <sub>2</sub> O | 0.9%     |
| CaO               | 0.3%     |
| Amino compounds   | 1.0%     |
| Betaine           | 0.6%     |

The production of yeast from molasses is generally coupled with alcohol fermentation. Only in certain cases is yeast exclusively produced. Control takes place by the introduction of atmospheric oxygen.

In relation to saccharose, six Mol (gram-molecules) of oxygen are required per Mol, that is five times this value of air must be introduced. Hence, the resulting quantities of air increase in relation to the OTR (oxygen transfer rate) of the reactor under multifarious circumstances.

The molasses are normally diluted by a factor of 6-8. Modern, efficient loop-reactors aim to process more concentrated solutions.

Forms of aeration are diverse; there are yeast factories which operate concurrently with turbine-, submersed jet- and jet pipe - aeration. The size of the fermenter lies between 50 and 200 m<sup>3</sup> inside volume.

Clarification and sterilisation of the molasses occurs mostly by hot-clarification with sulphuric acid at a temperature of 85 - 90°C.

For yeast propagation, the addition of nitrogen and phosphate sources is necessary. Through the dosage, the protein content and yield can be varied within certain limits.

The pH-value must be regulated, depending on the type of yeast (commercial- or pitching- yeast), at between 4.2 and 5.0. The fermentation temperature rises from 26° to ca. 30°C. If one wishes to extensively suppress the production of alcohol, which is necessary in particular for fermenters with a poor OTR, one operates on the running-in process. Thus, only just sufficient sugar is added to the nutrient solution as the growing yeast cells are able to consume in a short period, so that the sugar is utilised as a priority for the construction of cell matter.

After a growth phase of 10 - 12 hours, the yeast cells and the fermented nutrient solution ("wort" with a high betain content) are separated. The wort constitutes a large residue problem because of its high oxygen demand. It is measured at 1600 pollutant units on the basis of 1 tonne of molasses.

Yeast manufactured in this way contains 40 - 60% raw protein in the dry mass, depending on process conditions.

A variation of interest for developing countries is witnessed in biochemical fat synthesis with molasses (raw molasses, after Lundin and Törnquist). In working with sugar-rich but nitrogen-poor nutrient solutions, lipid formation is favoured instead of protein formulation (32).

|                         |                   |
|-------------------------|-------------------|
|                         | Preference for:   |
| C : N ca. 50 to 100 : 1 | Fat formation     |
| C : N ca. 20 : 1        | Protein formation |

The following micro-organisms are particularly suited for fat formation:

- *Torulasporea Delbrückii*
- *Rhodotorula gracilis*
- *Endomyces vernalis*
- *Oidium lactis*
- *Candida arborea*
- as well as some types of *Mucor*, *Aspergillus* and *Penicillium*

With *Rhodotorula gracilis*, Lundin and Törnquist (32) have obtained fat yields from 40% up to a maximum of 60%. The fat produced contains vitamin-B in particular, and thereby is just as well suited for human alimentation. In principle, this method of fat manufacture can also be carried over onto other substrates. So, for example, from 100 g lactose, one can recover ca. 13g raw fat in 5 days. The yield is better with surface aeration than submersed aeration. Worthy of further note is that dried fungus mycelium can contain more than 15% raw fat.

|  |        |
|--|--------|
| <i>Penicillinium bialowiezense</i>       | 17%    |
| <i>Penicillium flavocinerium</i> Biourge | 28.5%  |
| <i>Penicillium piscarum</i> Westling     | 26-28% |

#### 2.4.2.2. Production of yeast from sulphite liquors

In contrast to molasses which, as already mentioned, are traded at ca. DM15 per 100 kg, sulphite liquors constitute a very serious residue problem. The spent sulphite liquors contain the soluble constituents of the wood. The dried residue consists of up to ca. 65% lignin sulphonic acid, 20% reduced sugar, ca. 8% sugar - SO<sub>2</sub> - derivatives and ca. 7% calcium. The composition of these main components alters radically according to the type of wood and reduction process. The dry weight content of the digester discharge can also fluctuate by over 100% (between 6 and 16%). 8,000 - 15,000 litres of waste liquor result per tonne of pulp.

Spent liquors are not currently used in the Federal Republic of Germany for yeast production. The reasons for this are:

- (a) obsolete plant,
- (b) uncontrollable contamination by various bacteria,
- (c) high effluent loading

The production of yeast from spent sulphite liquors probably only has a future if, through further systematic development of the process, it can contribute to a reduction in the effluent loading. Currently, for the waste producer, there exists only a small incentive to recycle, when further problems in the form of yeast effluents arise.

Ca. 55 - 70% of the sugars contained in spent sulphite liquor are fermentable. Fermentation to alcohol is conceivable instead of yeast production. 10 - 12 litres ethanol can be recovered per m<sup>3</sup> of pine spent liquor. Fermentation to butanol is also possible. Alcohol fermentation reduces the BOD<sub>5</sub> (ca. 40,000 mg O<sub>2</sub>/l), but only by about 50%, which gives another meaning to yeast production with which one can achieve a higher standard of clean-up.

The lignin sulphonic acid salts are biologically difficult to reduce and cannot currently be utilised by fermentation methods. Where yeast is produced from sulphite liquor after removal of the sulphurous acid and clarification without alcohol fermentation, this results in 10 to 12 kg of yeast provender and 20 to 30 kg of baking yeast with 25% dry solids, from 1m<sup>3</sup> of undiluted waste liquor. Baking yeast manufacture can thus be undertaken on a thoroughly economic basis, but is only employed to a limited extent.

An average analysis of sulphite yeast gives the following values (28):

|                                       | %    |
|---------------------------------------|------|
| Moisture after 2 hrs. drying at 105°C | 5.9  |
| Ash                                   | 9.1  |
| Phosphorous (P)                       | 1.9  |
| Calcium (Ca)                          | 0.9  |
| Raw protein (N x 6.25)                | 47.4 |
| Raw fat                               |      |
| Standard method                       | 1.0  |
| With pre-hydrolysis                   | 4.8  |
| Raw fibre                             | 0.8  |

Amino-acid composition of sulphite yeast in % of dry mass (values in brackets are for whey yeast):

|               |           |            |           |
|---------------|-----------|------------|-----------|
| Arginine      | 3.6 (5.2) | Isoleucine | 3.8 (3.6) |
| Glycine       | 0.2       | Lysine     | 4.1 (6.3) |
| Phe-alanine   | 2.4 (2.8) | Tryptophan | 0.7       |
| Glutamic acid | 6.9       | Cystine    | 0.7       |
| Histidine     | 1.3 (1.2) | Leucine    | 3.6 (5.2) |
| Methionine    | 0.8 (1.0) | Threonine  | 2.9 (4.1) |
| Valine        | 3.0 (3.5) |            |           |

The values for sulphite yeast are less favourable when compared with those of whey-yeast (see section "Status of Research and Development"), in terms not only of total protein content but also of essential amino acids content.

### 2.4.3. Status of Research and Development

The processes commonly used today for the production of yeast from molasses have only been slightly altered in the past few years. The sugar content primarily serves as the nutrient for the yeasts. The separation of the yeast biomass after termination of the growth period is incomplete, so that yeast recovery is normally associated with the formation of highly loaded effluents.

From 100 l. molasses, ca. 400 l. of effluent arise. This corresponds to about 8 - 12 litres per kilo of yeast. The  $BOD_5$  lies at over 10,000 mg.  $O_2/l$  with a dry solids content of ca. 1 - 3%. Quantities of effluent from more recently introduced yeast production lie even further above these values. The gain from the production of high value yeast protein is thus impaired by the resulting effluent loads. An improvement in the situation can only be achieved through fundamental changes in the process technology.

An R and D project nearing completion on the production of yeast from whey, carried out by the Federal Milk Research Establishment in Kiel, approaches a 'model character' because of the positive results that have been forthcoming until now.

Whey consists of the fluids remaining after removal of the products in the manufacture of cheese, curd and casein.

Total arisings of whey in the Federal Republic of Germany amount to ca. 4.3 million tonnes per year, of which ca. 2 million tonnes are not processed. This corresponds to a potential production of yeast protein of ca. 100,000 tonnes per annum and a value of ca. 100 million DM/year.

Depending on process and origin, whey has the following approximate composition (11):

|                    |              |
|--------------------|--------------|
| Water              | 93 - 95      |
| Dry mass           | 7 - 8        |
| Fat                | traces - 0.8 |
| Nitrogen compounds | 0.8 - 1.1    |
| Lactose            | 3.8 - 5.0    |
| Lactic acid        | traces - 0.8 |
| Citric acid        | 0.1          |
| Ash                | 0.5 - 0.8    |

Conventional whey recovery is directed particularly at the separation of protein in the form of milk powder and the extraction of lactose. . However, these forms of recovery contribute only slightly to improving the effluent situation as the discharges are still highly loaded with lower molecular components.

With the production of yeast from whey, almost all the contents can be taken up by the yeast cells as nutrients, depending on the conditions selected. In particular, the application of optimally-functioning aeration systems which enable maximum growth, permits the amount of dissolved matter in the discharges to be reduced to a minimum.

The trials conducted in Kiel are based on well-known yeast production processes (11):

| <u>Process</u>      | <u>Ash<br/>Content</u><br>% | <u>Yield</u>                            | <u>Operating<br/>form</u> | <u>Remarks</u>         |
|---------------------|-----------------------------|---|---------------------------|------------------------|
| Linzer<br>process   |                             | 15-18 kg yeast                          | Batch                     | Centrifuge<br>effluent |
| Waldhof<br>process  | 10                          | 13-15 kg yeast<br>and 5-7 kg<br>protein | Continuous                | Centrifuge<br>effluent |
| Wheast-<br>process  | 8-10                        | 22-23 kg yeast<br>and protein           | Batch                     | Centrifuge<br>effluent |
| Polyvit-<br>process | 18                          | 36 kg in total                          | Batch                     | No effluent            |
| SAV-<br>process     | 16                          | 43-45 kg in<br>total                    | Continuous                | No effluent            |

The tests were carried out with the following objectives:

- optimal reduction of  $BOD_5$  during the growth phase,
- low effluent processes for harvesting and drying,
- high yield of protein,
- submersed cultivation,
- lower salt-content in the dry solids,
- lower ribonucleic acid content in the nitrogen compound,
- application of nutritive-physiologically harmless micro-organisms.

The essential innovation in contrast with traditional methods lies in the utilisation of mixed cultures of lactic acid bacteria (*Lactobacillus Bulgaricus*) and yeasts (*Candida Krusei*). The lactose is fermented to lactic acid by the bacteria and used in a subsequent process by lactic acid-assimilating yeasts as an energy source.

The whey protein found in solution is likewise processed to assimilable components by applying enzymatic fission.

In addition, a defined quantity of pre-cultivated yeast is autolysed with ammonium sulphate (in a continuous process, the yeast is taken directly from the fermenter) and the saline mixture is added to the whey. Besides the desired protein-splitting enzymes, the autolyte contains a range of substances which influence positively the growth of the living cells, except that one cannot exactly determine to what this influence is attributable. The timing of the harvesting is determined primarily by the complete consumption of the lactic acid.

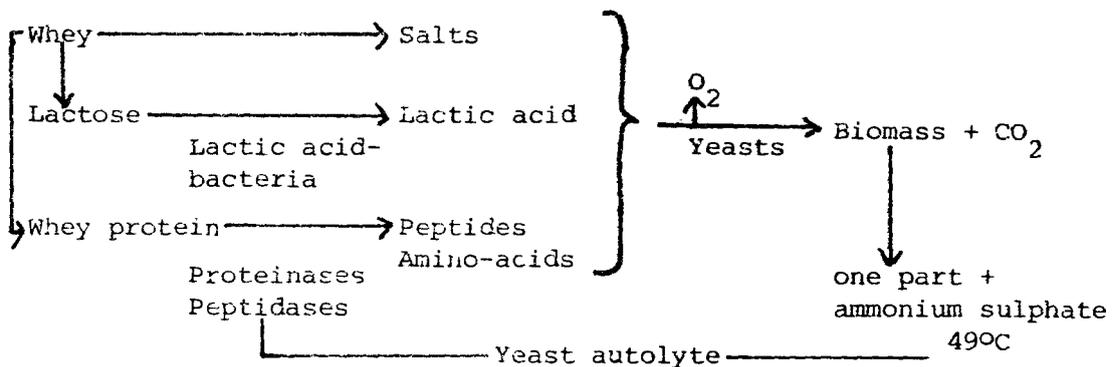


Figure 3: One stage production of yeast from whey with enzymatic protein reduction (11).

The  $BOD_5$  in the centrifuge discharge could, depending on the whey value, be lowered to 2000 mg  $O_2/l$ , which amounts to a 95% reduction and represents the attainable limit if thickened with centrifuges.

The alternative of drying the entire contents of the fermenter, results in a product in which the salinity of the dry mass, conditional on the autolysis, at 10% lies too high. This means that the process cannot be readily operated without an effluent. The compatibility of the yeast is diminished by too high a salinity. Besides, simple evaporation of the contents of the fermenter would greatly increase costs as the dry mass amounts to only ca. 3%.

A critical factor in applying the yeast for human nourishment is its high nucleic acid content. As the enzyme uricase is lacking in human beings, the purine compounds are only reduced to uric acid and not (e.g. as with pigs) to soluble allantoin. With this there exists the danger of forming an increased uric acid concentration in the blood, which can lead to accumulation and metabolic disturbances.

The current status of the work at Kiel is as follows:

- the production of yeast from whey is proven at the semi-technical scale (20 m<sup>3</sup> fermenter);
- the whey- $BOD_5$  is reduced by ca. 90%;
- the yeast can be marketed as a fodder additive. The revenue will cover the process costs (ca. DM80 per 100 kg of dried product);
- the protein yields lie, at 50-60%, close to the theoretically possible yields of biomass. In comparing the biological values of other single cell proteins, yeast from whey lies at the top;
- the high RNA-content does not permit any human application.

Further work is aimed at finding an effluent-free process.

Initial tests have shown that the admixing of additional carbohydrate sources, such as residual meal, maize swelling-water etc., does not disturb the yeast fermentation process. The dry mass is increased by the content of non-convertible escort substances while the proportions of salt and nucleic acid are diminished. On the other hand, the OTR (Oxygen Transfer Rate) falls with an increasing proportion of solids. Thus it would be necessary to convert the plant

to a loop-reactor. The higher solids content eventually makes direct drying in a drum-drier possible, leading to an improvement in the economics of the process. The high additional drying costs are swallowed and exceeded by gains from better yields and savings in effluent charges. In fermentation with *S. Cerevisiae*, a protein content of over 70% was achieved in preliminary tests. On this basis, such a product can be suitable as a substitute for fishmeal. If this should succeed, an economically attractive form of whey recovery would have been found. An extensive R and D project with similar objectives is currently being formulated by the Nuclear Research Plant at Jülich. There, important cellulosic residues will be examined with regard to their recovery potential. In the preliminary programme, it states:

"The research and development work of the Institute will deal with the microbial conversion and purification of defined waste products. Such substances are, for example, cellulose, hemi-cellulose and lignin, which will be introduced as microbial substrates for biotechnological processes, e.g. for protein recovery as food or fodder, or for the production of pharmacologically or technically interesting metabolites." (33)

Other projects (17)

Institution

Conversion of cellulosic waste materials from agriculture into SCP.

Federal Food Research Establishment, Karlsruhe.

Preliminary work for the recovery of SCP from silage percolate.

Institute for Soil Biology of FAL - Braunschweig

Preparation of base substrates for the cultivation of higher fungi from residues, for agricultural production.

Institute for Soil Biology of FAL - Braunschweig

Microbial treatment of straw for fodder purposes.

Institute for Soil Biology of FAL - Braunschweig

2.4.4. Economic Evaluation

The production of yeast from spent sulphite liquors on the basis of the technology commonly used up until 1975 has proved to be uneconomic and was discontinued. The production of yeast from molasses represents the classic form of yeast manufacture. This is, however, only a form of molasses exploitation. Its economic viability is thereby guaranteed in that it is operated in strict conformity with the respective demand and, with market fluctuations, is restrained in favour of other manufacturing processes. The current price for molasses stands at 15 DM/kg.

For the production of yeast from whey in the form described, preliminary studies of economic viability were conducted and published (11). These can be summarised as follows:

"In the Federal Republic of Germany, whey can be processed into numerous alternative products. The production of yeast from whey, as a new form of utilisation, must therefore be compared with the already proven and quantitatively significant processes. The potential for using the yeast will be decisive in this respect. Given present market price relationships, in particular the low prices for protein feedstuffs and high prices for lactose, the production of yeast from whey, where the yeast is employed as protein feed, is not competitive compared with other forms of whey utilisation.

The significance of yeast production from whey, as an additionally available form of protein feedstuff, arises from its substantial but nevertheless limited potential as a substitute for the highly import-dependant soya protein, with increasing soya prices. It thus is faced with two-fold competition:

- a) from the other forms of whey utilisation,
- b) from a number of other possible substitutes for soya protein which, with increasing prices for protein feedstuffs, can likewise be brought into play at a lower price level.

However, if it were to prove successful to also employ whey-yeast for human alimentation (e.g. as a baking agent) and thereby to obtain more than 1 DM per kg of dried yeast, then this would be an immediately competitive form of whey utilisation." (11).

3. COMPETITIVE PROCESSES

The term competitive processes refers to those processes which do not positively comprise fermentation/hydrolysis processes or use wastes as raw material, but which nevertheless deliver comparable end products.

The following processes are worthy of mention:

- Leaching
- Cell-culture processes
- Biological N<sub>2</sub> - fixation
- SCP from n-alkanes and methanol
- Other processes

### 3.1. Leaching

Leaching processes are employed for recovering metals from lean ore bearing rock. The bacteria-containing ores are mixed with pyrites (FeS) and steeped in water. Sulphuric acid emerges through biological oxidation processes. The acid leaches the available metal out in the form of readily-soluble salts. In this manner, for example, some 40% of world copper production is operated. Uranium and copper are microbially leached in particular in South Africa, Canada, the USA and the east-block countries.

The best-known bacteria are species of the type Thiobacillus. Currently, leaching occurs through percolation processes; higher yields (close to 100%) would be attainable by submersion, but the necessary technology is still lacking and besides this, the material must be very fine-grained (1).

In the Federal Republic of Germany, the Federal Geological Science and Raw Materials Establishment in Hannover is engaged on several leaching projects. The work is directed particularly at the lean ores available domestically, the yield of which is currently not very rewarding with conventional technology (34). Whatever is valid for lean ores is also true in the long-term for the leaching of dumps and land-fill sites, particularly those with a high proportion of slags, electrolytic sludges, etc (35). The work is significant not only from the point of view of the environment but also in terms of raw material conservation. The preparatory technical and laboratory work has been completed; a pilot plant will be constructed.

The yield from deposits of uranium in the Black Forest is the theme of investigations by the Uranerzbergbau - Bonn and the Saarbergbau Interplan. The associated laboratory tests have been completed.

Another project is concerned with lead-zinc leaching in Rheinland-Pfalz (Manbacher Bleiberg). The laboratory investigations for this are likewise completed.

3.2. Cell-Culture Processes

Vegetable as well as animal somatic cells and tissues can be maintained in cultures in the laboratory, inter alia, also those which are capable of special metabolic performance (15). There thus exists the possibility of manufacturing biosynthetically certain important substances, in particular for the pharmaceutical-medical area, which until now could only be obtained by extraction from plants and animals. This means that for individual natural vegetable and animal materials, domestic production can become independent of imports. Furthermore, through careful selection of the tissue material and observance of uniform culture conditions, production becomes independent of fluctuations in quality and crop failures, which otherwise play a significant role, particularly for tropical products.

Apart from this, cell-culture processes have the advantage that intermediate products can be isolated, which is not, for example, possible with plants, so that much greater product variability can be attained.

The scale-up of this presumably environmentally-safe and, in particular, resource-conserving process, is not yet ensured; currently, practical knowledge of several products is available at a semi-technical scale.

3.3. Biological N<sub>2</sub>-fixation

Nitrogen belongs to those elements which are indispensable for growth processes and the production of foodstuffs. Atmospheric nitrogen is of course available in abundance; however, only some micro-organisms are able to utilise it. For all higher living organisms, nitrogen is only usable in compound form. It can be transformed to this state by numerous methods and then used as a fertiliser for boosting yields. Since by intensive research one comes to be better conversant with the nitrogen cycle, one works upon biological processes for the recovery of compound nitrogen (1).

By gene manipulation, the transfer of nitrogen-fixation (nif) from Klebsiella, Rhizobium and Azotobacter to other micro-organisms (bacteria) has been successful, by which these were capable of N<sub>2</sub>-fixation and, as a metabolic product, ammonium ions (NH<sub>4</sub><sup>+</sup>) were produced. With this, the biological or fermentative recovery of ammonium salts draws closer to being achieved. The substitutable quantities of Haber-Bosch ammonia are high; this amounts to ca. 40 million tonnes per annum which for the most part goes to agriculture.

#### 3.4. SCP from Methanol

The methylomonas species is a necessarily methylotrophic bacterium, which can utilise methanol as a carbon source. The other nutrient materials are the same as for other SCP-processes (36).

In the Federal Republic of Germany, this was first fermented on an n-alkane basis but, for practical reasons, this was succeeded some two years ago (1975) by methanol conversion. The firms of Hoechst and Uhde are working jointly on the current development. The project is being supported by the Federal Ministry for Research and Technology (BMFT). The changeover from n-alkane to methanol has at least two grounds:

1. n-alkanes i.e. paraffins are comparatively expensive as, for this purpose, they can only be used in a highly refined form.
2. It cannot be excluded that a part of the hydrocarbons becomes accumulated in the cells. They are therefore suitable only to a limited extent for the feeding of long-lived domestic animals or humans as they can evoke metabolic disturbances and carcinogenic afflictions.

Apart from nutritive-physiological advantages, the change-over also has technological advantages as it can be operated with a single phase system.

The work at Hoechst-Uhde is at an advanced stage of development. Currently, at Hoechst, a pilot plant is being built directly alongside the technical school, in order to ensure good feedback to the other phases of the development work.

The technology applied is of the currently most modern status. The reactor is a loop fermenter, of the jet nozzle type. The performance of this reactor is significantly better compared with the conventional agitator-boiler:

The energy costs are low at  $2 \text{ kW/m}^3$ , and the OTR is very good (over 40% oxygen yield).

With continuous operation, the operating costs are significantly lower than with batch operation.

The protein content of methanol-SCP is over 80%, and clearly exceeds those SCP-types known to date. The RNA-content and the pattern of distribution of essential amino-acids is comparable with other SCP products. Nutritive-physiological investigations are being carried out.

With acceptable results in respect of digestibility, fishmeal could eventually be replaced by this product in the fodder market.. Nothing is known about the economics of the process. But as it operates without any residue (methanol is returned again from the centrifuge outlet to the fermenter), and as a pilot plant is currently being constructed by the interested firms at their own cost, one can assume that the economic viability of the process is assured.

3.5. Other Competitive Processes

There is an abundance of biotechnical processes which can either already, or after subsequent development, be applied for the recovery of residues or for improving the present environmental situation. Two of these will be mentioned:

Enzyme Technology:

The practical significance of enzymes in technology has increased greatly, particularly as one can command more and more the manufacture of active immobilised enzymes. This involves enzymes which are combined with durable carriers and which, with appropriate manipulation, can be summoned and employed for controlled reactions and syntheses. Immobilised enzymes are already used for the large-scale recovery of glucose concentrates (Isomerase ca. 1 million tonnes p.a.). The possibilities for these catalysts are until now by no means exhausted and as active research in this sector can be cited, one can reckon on substantial progress soon.

Algae Propagation:

The production of algae is a branch of biotechnology which, in the Federal Republic of Germany, is gaining a footing only with some difficulty. This is due primarily to climatic conditions which do not permit continuous production (also in colder periods of the year). Processes for effluent purification with algae are being tested in a few research institutes. However, the results until now are not very hopeful.

4. PROPOSALS FOR FUTURE RESEARCH AND DEVELOPMENT WORK

4.1. Anaerobic Reduction of Organic Residues

Optimising of sludge fermentation in order to achieve maximum yields of methane:

- investigation of the biological and biochemical processes during fermentation:
- investigation of the selection of sub-processes necessary for methane formation and the influence of other fermentation processes on this;
- investigations of the special contributions of groups of organisms involved in the whole process;
- development of reactors for optimising methane production;
- development of economically viable, small-scale fermenters for use in agriculture.

4.2. Aerobic Reduction of Organic Residues

Optimising process technology for the recovery of high value composts:

- optimising the technology with regard to the materials balance (reduction of the nitrogen loss);
- optimising the technology with regard to the energy balance (pulverising, sorting, aeration).

4.3. Hydrolysis of Carbohydrates

- microbial and enzymatic reduction of cellulosic residues for the recovery of sugar;
- microbial and enzymatic reduction of lignin to usable primary materials.

4.4. Protein Recovery

- development of processes for effluent clarification through the application of phototrophic bacteria; examination of the potential uses of the biomass;
- development of processes for the recovery of Single Cell Protein from discharges of the food processing industry;
- preparation of a demand analysis for microbial protein.

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COMMISSION OF THE EUROPEAN COMMUNITIES  
(Directorate General XII - Research, Science, Education)

FERMENTATION - HYDROLYSIS PROCESSES  
FOR THE  
UTILISATION OF ORGANIC WASTE MATERIALS

Report by

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## S U M M A R Y

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COMPOSTING

The importance which composting of solid refuse has for Italy derives from the fact that the content of organic substances in many agricultural areas is below 2%, limit value by which soil is defined fertile.

The quantity of organic substances which can be supplied from other available sources (animal manure, agricultural-forestry residues, slaughtering refuse) is not sufficient to re-establish a balance of organic substances such as to maintain the fertility of the soil in equilibrium (§ 1.1).

Numerous types of refuse are suitable to be sent to composting processes:

- a) town refuse
- b) sludge from urban, zootechnic and some industrial wastewater treatment plants
- c) agricultural-forestry refuse
- d) waste from determinate industries (sugar, paper, wood, food, tanning, alcoholic beverages, etc.)

Of these, the most significative is formed by solid town refuse about 14,000,000 t/y (§ 1.2).

The composting plants of town refuse are 35 in Italy, with an overall capacity of about 4,400 t/d; 5.6% of town refuse produced is treated in these (§ 1.2 and 1.3).

Only three plants at industrial level exist for industrial and agricultural-forestry refuse, one of which treats town and industrial refuse at the same time.

None of the commercial processes treats the sludge (§ 1.3).

However, for the town refuse, in spite of there being numerous composting systems, very few are satisfactory both from the

engineering point of view and the quality of the product point of view (§ 1.3.1).

In the field of the R & D projects on laboratory or pilot plant scale (6 projects), attention is paid to the composting of mixtures of town refuse and sludge or of refuse different from domestic ones (§ 1.4).

The costs of treatment by composting town refuse vary from 5,500 to 8,000 Lit. per ton of refuse treated, according to the type of plant used (§ 1.5): the yield of the transformation is about 50%. 30% of the compost produced from the town refuse is dumped, the 70% is sold at prices which go from 200 to 7,000 Lit/q.

This is because of the fact that compost up until now is not a product in conformity with standards and therefore products of various qualities are sold under the label of "compost".

Referring to reliable technology, the proceeds from the sale of the compost produced, could reduce by 20 - 30% the costs of running the plant (§ 1.5.1).

The two plants which produce organic fertilizer from agricultural-industrial refuse have assets (§ 1.5.1).

Products which substitute compost do not exist in sufficient quantities (§ 1.6).

On the contrary, there exist systems for treating solid town refuse as alternatives to composting, such as recycling which can in certain situations detract the refuse from this form of re-use (§ 1.6.1).

Two proposals for research are forwarded:

- experiment on pilot scale of the laboratory research for the composting of town refuse and all the town sludge produced by

the same municipality (§ 1.7.1), with the aim of obtaining a product with defined characteristics, using a reliable and economic technology.

- Improvement of technology of composting of some agricultural and zootechnic (§ 1.7.2) waste.

### ANAEROBIC DIGESTION

The import of petroleum in Italy represents the first item of deficit of the trade balance ( $94.4 \times 10^6$  tons in 1975 for a value of 5,355 miliard Lit.) (§ 2.1).

A policy of recovering energy and/or reducing the energy requirements is of vital interest.

The processes of anaerobic digestion allow the production, apart from residue organic fertilizers, but also of a biological gas with a content of  $\text{CH}_4$  around 60 - 70% (§ 2.2).

The quantities of gas which could probably be produced from town and pig-sty sludge is an overall  $5.5 - 6.0 \times 10^8$  NCuM/y equal to 3.2 - 3.5% of the Italian consumption of natural gas.

However, in the best of hypothesis, the processes of anaerobic digestion could allow to realize plants or systems self-sufficient from the energy point of view, but would not let energy be available for external use (§ 2.1, 2.3).

Twenty-eight plants for the treatment of town wastewater exist which use the anaerobic digestion of the sludge, while others are under construction (§ 2.3).

Commercial plants for other types of waste do not exist until this present moment.

The R & D projects (§ 2.4) revealed are 3 and concern zootechnic or industrial waste.

The production of energy (electrical and/or thermic) from biological gas allows a remarkable reduction of the running costs of a plant for the treatment of wastewater (§ 2.5).

Where the alternative processes are concerned, given the energy situation in Italy, these do not exist in sufficient manner (§ 2.6).

Three research proposals are made:

- one which would give significant results in a short period is the development on prototype scale of reliable and high yield technology for pig-sty waste (§ 2.7.1).
- The second is anaerobic digestion of urban refuse and sludge (§ 2.7.2).
- The third is concerning anaerobic digestion of liquid waste with high BOD content (§ 1.7.3).

## CARBOHYDRATE HYDROLYSIS

There is an enormous quantity of waste of cellulose nature (several millions of t/y). The principal sources are agricultural-forestry residues and solid town refuse (p. 3.2). The hydrolysis of such cellulosic material represents the first stage of the fermentation processes which produce yeasts, enzymes, SCP, alcohols, acetone, polyols, organic acids (p. 3.2).

In spite of the great quantities of refuse in question, no commercial processes for enzymatic hydrolysis exist in Italy at this present moment (p. 3.3).

Processes of acidic hydrolysis are applied (p. 3.6). Actually, fermentation processes exist but their original material cannot be considered refuse (molasses).

Only two research programmes (projects) (p. 3.4) were founded. The first is at laboratory level and starts off from straw, paper, residues from wood working with the aim of producing biomasses and/or ethanol.

Instead, the second is at feasibility study level and aims, amongst other things, to evaluate economically the possibility of producing ethanol from town refuse and agricultural waste.

Experiments have not yet been foreseen.

The existence of alternative processes of the treatment or recovery of refuse of cellulosic nature seems to be prevalent and so it is quite difficult to find the secondary material necessary at low cost (p. 3.5, 3.6).

Only the development of economically competitive processes with existing uses could arouse interest for this type of research.

A proposal of research is presented which aims at producing ethyl alcohol from town refuse (p. 3.7). It should go as far as the carrying out of a demonstration plant.

PROTEIN RECOVERY

Italy is largely dependent on Foreign Countries to satisfy its proteic requirements, both for meat (716,500 t in 1975 as import-export deficit) and for fodder and agricultural products.

The food deficit of the trade balance is the most substantial item after petroleum (§ 4.1).

Various organic waste is suitable as substratum for the production of SCP: agricultural-forestry refuse, household refuse, sludge animal waste, waste from the paper industry, residue from food industries (p. 4.2).

Four plants of the same type exist in Italy which produce fodder from solid town refuse on industrial scale (§ 4.3.1) without using fermentation processes.

The use of animal manure to produce protein has been studied in 3 projects (§ 4.4.1, 4.4.2, 4.6.2) respectively from pig, cattle and poultry manure.

However, in spite of the positive results obtained from the finished research programmes, these processes cannot be applied in Italy because of the Regulations and Laws (in general made after the research) which forbid the use of animal refuse (manure) in animal feeding.

Two other research programmes were carried out at laboratory level with the aim of recovering protein from the treatment of waste-water from oil mills and distilleries. The results obtained are considered promising (§ 4.4.1, 4.4.2).

A plant exists which operates at industrial levels and carries out the recovery of protein from tanning scraps, slaughtering residue, etc. by acid and basic hydrolysis processes (§ 4.6.1).

Numerous schemes using a variety of substrata, fermenters, methods of purification and separation have been proposed (p. 4.5).

Esperiments on industrial scale are lacking and so therefore is a reliable evaluation of costs. However, on the basis of feasibility studies carried out in other Countries, it seems that there is economical convenience in producing proteins at marketable prices only in large sized plants.

And this, if it is verified, represents an obstacle to the spreading of the use of such recovery systems (p. 4.7).

Three research proposals are forwarded:

- 1) Proteins recovery from cellulosic waste material in farms and breedings organized on an intensive and industrial basis.
- 2) Production of proteins from urban solid waste by fermentation route.
- 3) The study and carrying out of processes which foresee the value of agro food industry wastewater and the excess sludge from biological treatment plants, for the production of protein from protozoa.

## GENERAL INTRODUCTION

This present report forms the final report of the study on the Processes of Fermentation and Hydrolysis for the utilization of organic waste materials, commissioned to TECNECO by the E.E.C. by the study contract no. 305-76-12 ECI I.

It is given for recognized the necessity of treating the refuse examined by the study, which independently from the systems of possible recovery must be carried out. Therefore, particular importance is not given to the aspect relative to the pollution coming from this refuse if it is not treated.

On the contrary, stress was put on the recovery systems, that is, on the possibility of carrying out treatments and recovering substances which have a residue value on the market. These systems were compared from the technical-economical point of view with those where recovery is not carried out and which are alternative.

The informations and data collected by the terms of the contract refer exclusively to Italy.

However, some foreign experiments are briefly mentioned where it has been necessary to complete the study and to be able to put forward research proposals having good grounds.

In fact for some items of the study (Composting, Anaerobic Digestion) experiments and plants have been carried out, while for others (Hydrolysis, Protein Recovery) wide interest has not yet been noticed. The consequent collecting of information is therefore scarce. This then provides the reason for mentioning experiments carried out in other Countries.

## 1. COMPOSTING

### 1. 1. Introduction

The fertility of the soil in its three aspects, chemical, physical and biological, is strictly connected to its content in organic substances, and therefore humus.

Soil is therefore classified according to its humus content:

- humus content between 0 and 1% ,      very poor soil
- humus content between 1 and 2% ,      poor soil
- humus content between 2 and 3% ,      average soil
- humus content between 3 and 4% ,      rich soil
- humus content between 4 and 5% ,      very rich soil.

If, as it happens in agricultural land, the products which the growing crop has furnished are taken away at each vegetative cycle, a negative balance of the organic substances is created as the whole plant does not necessarily return to the earth. In this case, the fertility of the earth decreases from cycle to cycle unless substantial amounts of organic substances are added in such a way as to maintain the fertility of the soil in equilibrium.

In particular, the action of the organic substances is explained by the following actions:

- 1) Physical improvement of the soil, mainly its structure accompanied by an increase of the volume of its pores; the exchange of liquids and gases is improved and ploughing operations are made less hard.

- 2) Increase of the capacity to absorb and retain water. This is particularly important during dry periods.
- 3) Prolonged preservation of the nutritive elements of the plants and the oligo-elements of the soil caused by the ionic exchanges which are created by the humus.
- 4) Prevent the erosion of the soil by the action of the humus which generates a compact glomerular structure.
- 5) Encourage development of the plants by:
  - addition of nutritive elements and micro-elements with lasting effect;
  - activating the microbic fauna and flora of the soil with the consequent formation of organic compounds which can be assimilated and so stimulate growth.

Intense use has notably impoverished the soil in humus and the biological activity of the microbic flora is greatly reduced. This is one of the components which condition the physical and biological fertility. Without this component chemical fertility is not possible.

With this aim in mind, the appeal made by the FAO (Food and Agriculture Organization) to its Member Countries to provide and increase the production of humus in the soil, is rather significant.

In Italy, the agricultural cultivated surface represents the 63% of the national territory (\*). The average content of or-

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(\*) The agricultural surface utilized consists of the part of the total surface engaged and actually used in true agricultural cultivation: sown fields, permanent grass lands and pastures, agricultural wood cultivations, chestnuts and poplars outside woods.

ganic substances is more or less between 1.5 to 2%; that is below the limit by which soil is defined as being fertile.

The addition of organic substances to the soil can be carried out in the following ways:

- solid town refuse and sludge
- animal manure (cattle, pig, poultry manure etc.)
- products with animal organic base (slaughtering waste, etc)
- products with vegetable organic base (sea-weeds, peat, lignites, etc.).

The practice of over-turning and burying crops or part of these at different stages of the vegetative cycle is also used with this same scope in mind.

Concerning the actual need of organic fertilization of soil, some reference data can be established although this need is a consequence of multiple contingent factors which vary from case to case.

It has been estimated that in order to raise by one unit % the organic content of soil, 4,000 q/ha of fresh manure are required (\*).

On the other hand, in order to maintain the fertility characteristics of soil used for cyclic cultivation, 20-40 q/ha/y of organic substances are necessary which are equal to 200-400 q/ha/y of fresh manure (HU. = 90%) or 80-160 q/ha/y of mature manure (HU= 75%) and this in function of the characteristics of the soil and the type of cultivation.

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(\*) M. Matarese "L'importanza della materia organica nei riguardi della fertilità del terreno" - Agrobiological Convention 1, Bologna 1972.

The estimate of animal manure available in Italy is  $110 \times 10^6$  t/y of mature manure. This is theoretical and represents superior limit values. The actual quantity available is less as it is connected with the type of animal raising, the system used to clean the stables, cowhouses, pig-sties, etc., the eventual treatment plant for the wastewater from stables, pig-sties, etc.

In spite of the specific national average of availability being 65 q/ha/y, there exist regions where minimum points of 16-30 q/ha/y are reached. (tab. 2). These quantities are far too small compared to those required to preserve the fertility of the soil.

Therefore, a product which integrates manure is necessary, such as compost produced from town refuse, sludge from wastewater treatment plants or agricultural-industrial refuse.

This is the reason why, in Italy, there is a great interest in this type of recycling of refuse and why much initiative in the compost field has been shown over the last few years.

On one hand, this interest is increased by the necessity of finding a satisfactory solution from the sanitary and technical-economical points of view to the disposal problem of refuse which man continuously produces in notable quantities. On the other hand, the need to exploit, by the correct management of the natural resources, the refuse by finding some useful form of recycling.

A more accurate evaluation on the quantities and types of refuse allows to focalize better the problem.

Most of agricultural waste has possibility to be disposed or reused where are produced. Really the most significative namely animal manure forms a problem only where breedings organized on an intensive and industrial basis are "without land"; which concerns about 20% of total refuse : i. e. 20,000,000 t/y (25% poultry manure, 30% swine slurry, 45% cattle manure: each of them requires different process and treatment technology).

The others are utilized in the sphere of a normal agricultural management; on account of the problems concerning with their collection these refuses are to be considered unavailable.

40% of refuse from industries and mines is reused or sold as raw material for manufacturing; therefore in reality it is not a refuse. The other 21,000,000 t/y are formed by very numerous types which have very different characteristics. Composting process may be developed only for some of them (each present in less significative quantities).

It follows that urban refuse forms one of most significative waste in general, the most significative in order to composting; i. e. where setting up effective process gives the greatest returns on the quantities basis. (tab. 4).

TABLE 1 - TERRITORY UTILIZATION IN ITALY (\*)

|                       | AGRICULTURAL UTILIZED SURFACE |                                    |                                |                 | TOTAL      | WOODS SURFACES | OTHER AGRICULTURAL LANDS | IMPRODUCTIVE LANDS | TOTAL TERRITORY SURFACE |
|-----------------------|-------------------------------|------------------------------------|--------------------------------|-----------------|------------|----------------|--------------------------|--------------------|-------------------------|
|                       | SOWN FIELDS                   | PERMANENT GRASS LANDS AND PASTURES | AGRICULTURAL WOOD CULTIVATIONS | KITCHEN GARDENS |            |                |                          |                    |                         |
| PIEMONTE              | 632,559                       | 541,460                            | 125,572                        | 7,110           | 1,306,701  | 596,634        | 356,143                  | 280,447            | 2,539,925               |
| VALLE D'AOSTA         | 2,758                         | 96,849                             | 1,478                          | 150             | 101,235    | 75,412         | 40,579                   | 109,009            | 326,226                 |
| LOMBARDIA             | 820,782                       | 361,569                            | 44,150                         | 2,972           | 1,229,473  | 477,446        | 232,797                  | 444,500            | 2,384,216               |
| TRENTINO-ALTO ADIGE   | 25,169                        | 389,980                            | 42,395                         | 605             | 458,149    | 596,423        | 103,319                  | 203,418            | 1,361,309               |
| VENETO                | 629,129                       | 209,994                            | 148,306                        | 5,506           | 992,935    | 263,083        | 287,300                  | 293,449            | 1,836,767               |
| FRIULI-VENEZIA GIULIA | 181,854                       | 103,413                            | 25,286                         | 1,964           | 312,517    | 165,334        | 164,658                  | 141,967            | 784,476                 |
| LIGURIA               | 28,163                        | 71,016                             | 42,941                         | 937             | 143,057    | 280,591        | 67,100                   | 50,544             | 541,292                 |
| EMILIA ROMAGNA        | 1,031,662                     | 145,133                            | 189,498                        | 2,697           | 1,368,990  | 368,404        | 244,800                  | 230,083            | 2,212,277               |
| TOSCANA               | 632,207                       | 215,713                            | 210,254                        | 5,519           | 1,063,693  | 855,750        | 211,444                  | 168,261            | 2,299,148               |
| UMBRIA                | 274,012                       | 93,542                             | 59,775                         | 1,465           | 428,794    | 258,968        | 97,197                   | 60,645             | 845,604                 |
| MARCHE                | 493,422                       | 94,764                             | 25,765                         | 2,065           | 616,016    | 151,887        | 144,482                  | 56,942             | 969,347                 |
| LAZIO                 | 525,904                       | 231,875                            | 218,858                        | 3,581           | 980,218    | 365,770        | 209,896                  | 164,376            | 1,720,260               |
| ABRUZZI               | 335,804                       | 193,315                            | 60,965                         | 3,796           | 593,880    | 211,388        | 206,187                  | 67,948             | 1,079,403               |
| MOLISE                | 202,341                       | 55,840                             | 20,778                         | 850             | 279,809    | 68,943         | 68,058                   | 26,963             | 433,773                 |
| CAMPANIA              | 456,614                       | 165,373                            | 179,345                        | 3,916           | 805,240    | 275,017        | 158,840                  | 120,428            | 1,359,533               |
| PUGLIA                | 774,232                       | 226,191                            | 629,950                        | 2,370           | 1,632,743  | 91,290         | 79,977                   | 130,720            | 1,934,730               |
| BASILICATA            | 388,453                       | 233,867                            | 45,304                         | 848             | 688,472    | 171,201        | 94,618                   | 64,936             | 999,227                 |
| CALABRIA              | 363,339                       | 200,625                            | 239,892                        | 2,598           | 806,454    | 422,090        | 176,817                  | 102,669            | 1,508,030               |
| SICILIA               | 1,199,070                     | 300,954                            | 499,532                        | 1,015           | 2,000,571  | 194,600        | 190,863                  | 184,813            | 2,570,947               |
| SARDEGNA              | 343,144                       | 1,271,341                          | 107,489                        | 3,087           | 1,725,061  | 319,890        | 220,882                  | 143,120            | 2,408,953               |
| T O T A L             | 9,340,618                     | 5,202,805                          | 2,917,535                      | 53,051          | 19,239,070 | 6,210,121      | 3,355,957                | 3,045,238          | 30,125,343              |

(\*) Data at June 30, 1972 - surfaces in hectares

TABLE 2 - ESTIMATE OF ANIMAL MANURE AVAILABLE IN ITALY

|                       | SHEEPS AND                        |                                  |                                 |                                   | TOTAL MANURE<br>(10 <sup>3</sup> t/y at 75% Hu) | AVAILABILITY IN per .<br>ha OF AGRICULTURAL<br>UTILIZED SURFACE |
|-----------------------|-----------------------------------|----------------------------------|---------------------------------|-----------------------------------|---|---|
|                       | CATTLE<br>(10 <sup>3</sup> HEADS) | GOATS<br>(10 <sup>3</sup> HEADS) | PIGS<br>(10 <sup>3</sup> HEADS) | HORSES<br>(10 <sup>3</sup> HEADS) |   |   |
| PIEMONTE              | 1,273                             | 118                              | 452                             | 22                                | 14,563  | 11.1  |
| VALLE D'AOSTA         | 39                                | 8                                | 3                               | 1                                 | 464   | 4.6   |
| LOMBARDIA             | 1,874                             | 79                               | 1,608                           | 50                                | 22,460  | 18.3  |
| TRENTINO-ALTO ADIGE   | 177                               | 42                               | 61                              | 9                                 | 2,184   | 4.8   |
| VENETO                | 1,183                             | 30                               | 476                             | 23                                | 13,969  | 14.1  |
| FRIULI-VENEZIA GIULIA | 229                               | 5                                | 89                              | 7                                 | 2,800   | 9.0   |
| LIGURIA               | 43                                | 28                               | 8                               | 4                                 | 595   | 4.2   |
| EMILIA ROMAGNA        | 1,061                             | 113                              | 1,999                           | 14                                | 15,370  | 11.2  |
| TOSCANA               | 298                               | 561                              | 649                             | 20                                | 4,840   | 4.6   |
| UMBRIA                | 200                               | 185                              | 468                             | 9                                 | 3,183   | 7.4   |
| MARCHE                | 373                               | 179                              | 353                             | 5                                 | 4,922   | 8.0   |
| LAZIO                 | 356                               | 670                              | 254                             | 55                                | 4,935   | 5.0   |
| ABRUZZI               | 197                               | 480                              | 133                             | 36                                | 2,798   | 4.7   |
| MOLISE                | 60                                | 107                              | 65                              | 27                                | 867   | 3.1   |
| CAMPANIA              | 431                               | 452                              | 336                             | 42                                | 5,200   | 6.5   |
| PUGLIA                | 179                               | 890                              | 85                              | 44                                | 2,625   | 1.6   |
| BASILICATA            | 74                                | 593                              | 150                             | 58                                | 1,635   | 2.4   |
| CALABRIA              | 180                               | 495                              | 291                             | 31                                | 2,980   | 3.7   |
| SICILIA               | 309                               | 812                              | 262                             | 132                               | 4,980   | 2.5   |
| SARDEGNA              | 282                               | 2,920                            | 248                             | 33                                | 5,030   | 2.9   |
| T O T A L             | 8,818                             | 8,767                            | 7,990                           | 622                               | 114,300   | 6.5   |

Note: Poultry manure is not included.

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∞  
1

## 1.2. Raw materials and end products

In Italy, an estimate of 200 million tons per year of solid refuse has been made. The refuse is divided as follows:

- urban refuse 15 million tons (7.3 %);
- refuse from industries and mines 35 million tons (17.5%);
- agricultural refuse 150 million tons (75.2 %) of which 114 million tons are manure.

The amount of solid urban refuse produced in Italy in 1973 was 14,525,000 t/y (about 96.8% of the solid refuse deriving from civil activities) and the average production pro-capite was 715 g/inhabitant per day.

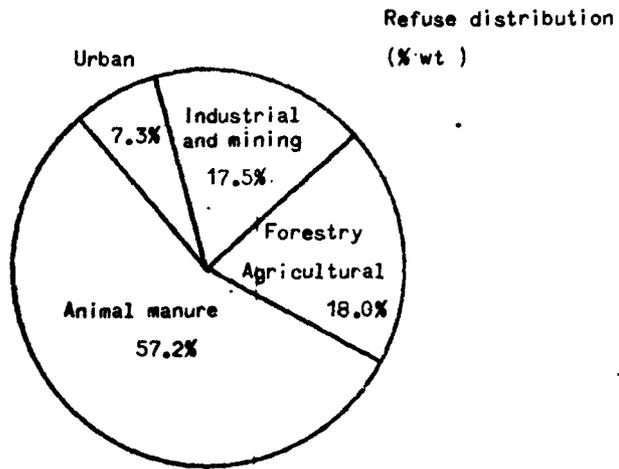
The average density of this refuse is 0.24 Kg/l and therefore the overall volume of the town refuse is equal to about 58,000,000 cu. m/year.

Apart from the metropolitan areas where maximum levels are reached, the regional distribution is not homogeneous. Higher values are found in the coastal regions of the Tyrrhene and the Adriatic due to the existence of commercial centres for agricultural products, fruit and vegetables and fish, the refuse of which is often mixed in with the town refuse.

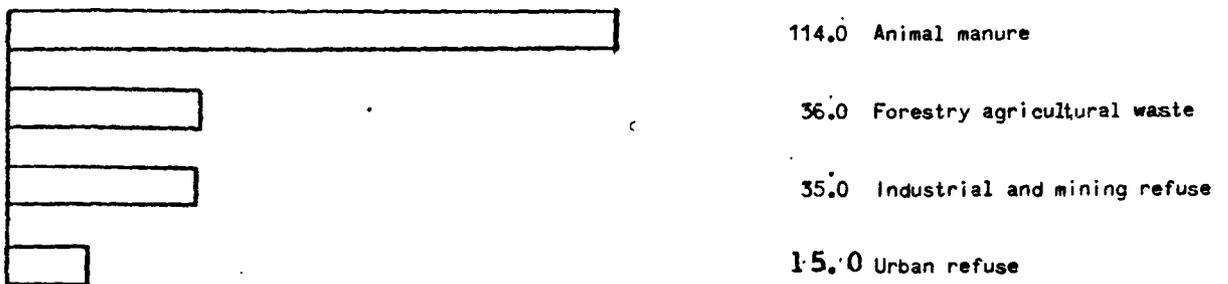
The materials which form the refuse are numerous and present different chemical-physical characteristics according to the season and the geographical area in which it is produced.

The average national composition, based on available data, results as shown in Tab. 3.

Fig. 1 - REFUSE PRODUCED IN ITALY



Refuse produced ( $10^6$  t/y)



Tab. 3

Average composition of solid town refuse produced in Italy

Cellulose materials

(wood, paper, carton, etc.) 31.0 %

Plastic substances

(rubber, polythenes, synthetic  
leathers, etc.) 3.0 %

Putrescible organic substances

(vegetables, kitchen waste, etc.) 37.9 %

Inert

(metals, glass, ceramics, dust, etc.) 28.1 %

The part which could be transformed into compost is formed by the putrescible organic substances and the cellulose materials (in the case of the recovery of paper not being carried out).

Keeping in mind that there is a loss in weight due to evaporation during the biostabilization process, it can be estimated that the quantity of compost which could be theoretically produced is about 60% of the refuse.

As far as the disposal of the solid town refuse is concerned, the quantity treated in plants results as being equal to 3.5 million tons annually which forms only the 23.7% of the total.

The refuse is treated in 161 plants with a total capacity of about 15,000 tons/day. The compost plants are 35 with an overall capacity of about 4,400 t/day (tab. 6); 5.6% of the refuse produced is treated in them.

Over and above the solid town refuse, other refuse is suitable for the compost processes, keeping in mind their chemical content as a fertilizer, their structure, their chemical-physical characteristics as well as their easy of handling and transport.

- Forestry-agricultural : Seaweed, grape-husk, dregs  
refuse : of grapes, manure, straw,  
bark, saw dust, peat;

- Industrial-organic refuse : Generally, the residue from the sugar manufactory, distillery, paper and wood industries, canning, food processing waste, tannery works. More precisely: alcoholic beverages distillation residue, flesh scrap, cellulose pulps, waste pulp from sugar-beet, slaughtering scraps, fish scraps, fruit residues, oil seeds, etc.;
  
- Sludge : Sludge from town wastewater treatment, from treatment of wastewater of the industries which produce the organic refuse mentioned above, sludge from the zootechnical wastewater treatment.

The tabel 4 summarizes the quantities of refuse suitable for composting and the compost which can be obtained from them.

Column 1 indicates the refuse produced in Italy.

Column 2 shows the refuse which is actually used in processes other than composting.

Column 3 shows the refuse which for various reasons are not available for composting plants. This could be due either to their nature or to their distribution which involves collecting systems and costs very expensive.

In column 4 the refuse which can be composted can be found by the difference  $(4) = (1) - [(2) + (3)]$ .

Column 5 shows the compost which can be recovered on the basis of the composition and the consequent transformation yield.

Finally, column 6 shows the actual compost produced. The difference between the contents of columns (5)-(6) is an index of the action margin to be carried out in the composting field in Italy.

TABLE 4 - REFUSE AVAILABLE TO COMPOSTING PROCESSES (T/Y)

|                                | Refuse produced<br>(1) | Refuse utilized<br>in other processes<br>(2) | Refuse not available<br>to composting<br>(3) | Refuse available<br>to composting<br>(4) | Compost which<br>may be produced<br>(5) | Compost actually<br>produced<br>(6) |
|--------------------------------|------------------------|--|--|--|---|-------------------------------------|
| - Urban refuse                 | 14,525,000             | 2,600,000                                    | 2,425,000                                    | 9,500,000                                | 5,700,000                               | 490,000                             |
| - Industrial and mining refuse | 35,000,000             | 14,000,000                                   | 18,000,000                                   | 3,000,000                                | 1,500,000                               |                                     |
| among which:                   |                        |  |  |  |   |                                     |
| papermills waste               | 312,000                | -  | -  | 312,000                                  | 160,000                                 | 10,000                              |
| slaughtering scraps            | 820,000                |  | 210,000                                      | 300,000                                  | 150,000                                 | -                                   |
| pulp from sugar-beet           | 700,000 (c)            | 700,000                                      | -  | -  | -                                       | -                                   |
| oil mills waste                | 600,000                | 600,000                                      | -  | -  | -                                       | -                                   |
| - Forestry agricultural waste  | 36,000,000             |  |  |  |   |                                     |
| among which:                   |                        |  |  |  |   |                                     |
| grape-husk, dregs              | 1,600,000              | 1,430,000                                    | 170,000                                      | -  | -                                       | -                                   |
| straw                          | 13,550,000             | 3,200,000                                    | 10,350,000                                   | -  | -                                       | -                                   |
| rice-husk                      | 180,000                | 125,000                                      | 55,000                                       | -  | -                                       | -                                   |
| - Animal manure                | 114,000,000            | -  | (94,000,000)                                 | (20,000,000)                             | (8,330,000)                             | 50,000                              |
| poultry                        | (5,000,000)            | -  | -  | 5,000,000                                | 5,000,000                               | } 50,000                            |
| cattle manure                  | 97,000,000             | -  | (88,000,000)                                 | (9,000,000)                              | (2,700,000)                             |                                     |
| pig-sties waste                | 12,000,000             | -  | 6,000,000                                    | 6,000,000                                | 630,000 (a)                             |                                     |
| - Sludge                       |                        |  |  |  |   |                                     |
| from urban wastewater          | 1,460,000 <sub>S</sub> | -  | 760,000 <sub>S</sub>                         | 700,000 <sub>S</sub>                     | 1,500,000 (b)                           |                                     |
| from industrial wastewater     | n.k.                   |  |  |  |   |                                     |

Notes: (a) Residue from anaerobic digestion - (b) Water content 50% wt - (c) Water content 6.75% wt  
S = solid matters

### 1. 3. Commercial processes

The situation of the compost processes applied industrial-ly or being studied in Italy is summarized in Tab. 5.

It immediately becomes evident that while for the refuse of town origin numerous applied processes exist which denote a long experience in this area, only three plants at industrial level exist for the industrial and forestry-agri-cultural refuse. One of these three plants treats both town and industrial refuse. None of the commercial processes treats the sludge.

Vice versa, in the field of laboratory research or on pro-totype plants, the attention given to the composting of re-fuse other than town refuse or of mixtures of town refuse and sludge is quite predominant.

However, as it will be shown in the following chapters neither commercial composting technology can be stated satisfactory from the point of view both technic and scien-tific.

#### 1. 3. 1. Composting of solid town refuse

The principle of the method consists in that of stabili-zing the refuse of organic nature using the action of the micro-organisms to be found in the refuse itself. The transformations are fundamentally of aerobic-thermo-phyl type.

Tab. 5

Status of composting processes in Italy

| Processes<br>raw<br>materials      | Commercial<br>processes   | Pilot or pro-<br>tototype plant | R & D   |
|------------------------------------|---|---------------------------------|---|
| - Domestic<br>waste                | DANO<br><br>TECNITAL<br><br>MACCHI-GONDARD<br>" "<br>VOEST-ALPINE<br><br>PUBLIC CONSULT<br><br>FERRERO<br>" "<br>BÜHLER<br><br>ITALCAMPO (1)<br><br>EARP-THOMAS | FIAT (3)                        | TECNECO-<br>BREDA (4)<br><br>PISA UNIVER-<br>SITY (5)<br><br>SIBE |
| - Agricultural-<br>forestry refuse | CARTIERE BUR-<br>GO<br><br>FOMET  | CARTIERE<br>BURGO (2)           | SIBE<br>CRPA  |
| - Industrial re-<br>fuse           | ITALCAMPO (1)   | CARTIERE<br>BURGO (2)           |   |
| - Sludge                           |   | FIAT (3)                        | TECNECO-BRE-<br>DA (4)  |
|                                    |   | CARTIERE<br>BURGO (2)           | PISA UNIVER-<br>SITY (5)  |

Tab. 5

NOTES:

- 1) This process treats waste mixture formed 50% by urban refuse and 50% by industrial refuse (saw dust, waste pulp from sugar-beet, cellulose paste, flesh scrap).
- 2) Raw materials: bark, poultry manure, swine sludge, wood pulp, paper mill thickened sludge.
- 3) Prototype plant to treat solid domestic refuse and/or mixture of domestic refuse and biological sludge.
- 4-5) Laboratory research on composting of domestic refuse and biological sludge.

Following the stabilizing process, the material presents organoleptic characters similar to those of humus. Its content in mineral salts and its physical characters make it utilizable as a fertilizer, especially where the pedological aspects are concerned.

The stabilized material (compost) in fact, carries out various types of action where the soil (structure) is concerned, such as: prevention from erosion due to the action of water and wind, increase of the permeability of the soil, conservation of humidity. The addition of oligo-elements and nutritive elements has yet to be evaluated from the chemical point of view.

At the same time as the stabilizing process, there is a reduction of the initial bacterial charge, both for the transformation of the original "pabulum" and for the action of thermic nature following the heat which is developed in the course of the transformation processes.

The existing plants in Italy and their characteristics are shown in Tab. 6. The location, type of plant, year of construction, construction company, process of composting, plant capacity, inhabitants served are indicated in order.

As far as the type is concerned, most of the plants are "mixed": namely the material which cannot be transformed into compost (about 50% of the weight) is burned in incinerators. In others (indicated as "compost plant") it is dumped.

TAB. 6

SOLID URBAN WASTE - COMPOSTING PLANTS IN ITALY AT 1977

| Locality              | Type of plant | Year of construction | Construction Company (*) | Composting process | Plant capacity (T/d) | Inhabitants served | Comments |
|-----------------------|---------------|----------------------|--------------------------|--------------------|----------------------|--------------------|----------|
| Busto Arsizio (VA)    | Mixed         | 1972                 | Macchi-Officine Saronno  | Macchi-Gon-dard    | 250                  | 310,000            |          |
| Mantova               | Compost       | 1965                 | Public Consult           | Rotoref            | 100                  | 100,000            |          |
| Bolzano               | Mixed         | 1970                 | Macchi-Officine Saronno  | Macchi-Gon-dard    | 125                  | 105,000            |          |
| Merano (BZ)           | Compost       | 1964                 | Public Consult           | Rotoref            | 70                   | 33,000             |          |
| Chioggia (VE)         | Mixed         | 1973                 | Public Consult           | Rotorof            | 120                  | 50,000             |          |
| Iesolo (VE)           | Mixed         | 1965                 | Tevere-Officine Saronno  | -                  | 40                   | 15,000             |          |
| Rovigo                | Mixed         | 1967                 | Tecnital                 | Tecnital           | 35                   | 50,000             |          |
| S. Donà di Piave (VE) | Mixed         | 1972                 | Public Consult           | Rotoref            | 35                   | 23,000             |          |
| Venezia-Mestre        | Mixed         | 1968                 | Public C/Off. Saronno    | Rotoref            | 200                  |                    | Not used |
| Vicenza               | Mixed         | 1970                 | Tecnital                 | Tecnital           | 80                   | 116,000            |          |

| Locality           | Types of plant | Year of construction | Construction Company (*) | Composting process | Plant capacity (T/d) | Inhabitants served | Comments  |
|--------------------|----------------|----------------------|--------------------------|--------------------|----------------------|--------------------|---|
| Gorizia            | Mixed          | 1971                 | Tecnital                 | Tecnital           | 200                  | 40,000             |   |
| Lignano (UD)       | Compost        | 1975                 | Daneco                   | Vöest-Alpine       | 240                  | 5,000              | 180,000 inhabitants in the summer                               |
| Udine              | Mixed          | 1969/75              | Breda-Officine Saronno   | Dano               | 130                  | 90,000             |   |
| S. Agata Bol. (BO) | Compost        | 1974                 | Italcampo                | Italcampo          | 100                  | 100,000            | 50% domestic refuse; 50% agricultural, industrial refuse        |
| Lucca              | Mixed          | 1962                 | Tecnital                 | Tecnital           | 50                   | 80,000             |   |
| Pistoia            | Mixed          | 1969                 | Breda-Shunt              | Dano               | 120                  | 110,000            |   |
| Siena              | Mixed          | 1967                 |                          | Bühler             | 120                  | 50,000             |   |
| Perugia            | Recycling      | 1972                 | Cecchini-Off. Saronno    | Ferrero            | 40<br>(200)          | 180,000            | Composting section capacity. In brackets overall plant capacity |
| Fano               | Compost        | 1968                 | Macchi                   | Macchi-Gondard     | 50                   | 45,000             |   |

| Locality          | Type of plant | Year of construction | Construction Company (*)  | Composting process           | Plant capacity (T/d) | Inhabitants served | Comments   |
|-------------------|---------------|----------------------|---------------------------|------------------------------|----------------------|--------------------|--|
| Roma I            | Mixed         | 1963                 | Slia-Off. Saronno         | Dano                         | 700                  | 780,000            |  |
| Roma II           | Recycling     | 1970                 | Cecchini-Off. Saronno     | 1. Ferrero<br>2. Fernascreen | 150<br>(650)         | 730,000            | Two different systems used.<br>Composting section overall capacity indicated. In brackets overall plant capacity (recycling plant) |
| Roma III          | Recycling     | 1970                 | Saar-Off. Saronno         | 1. Ferrero<br>2. Fernascreen | 120<br>(550)         | 630,000            |  |
| Roma IV           | Recycling     | 1975                 | Sorain-Off. Saronno       | 1. Ferrero<br>2. Fernascreen | 150<br>(650)         | 730,000            |  |
| Terracina (LT)    | Mixed         | 1975                 | Slia-Off. Saronno         | Dano                         | 60                   |                    |  |
| L'Aquila          | Mixed         | 1969                 | Public Consult. Edilforni | Rotoref                      | 50                   | 60,000             | Part of collected refuse dumped in landfill  |
| Salerno           | Mixed         | 1974                 | Tecnital                  | Tecnital                     | 80                   | 155,000            | Part of collected refuse dumped in landfill  |
| Bari              | Mixed         | 1975                 |                           | Dano                         | 250                  |                    |  |
| Brindisi          | Mixed         | 1975                 | Slia-Off. Saronno         | Dano                         | 70                   | 82,000             |  |
| Castellaneta (TA) | Mixed         | 1971                 | Public C-Dal Corno        | Rotoref                      | 30                   | 15,000             |  |
| Foggia            | Mixed         | 1973                 | Public C-Von Roll         | Rotoref                      | 120                  | 140,000            |  |
| Lecco             | Mixed         | 1966                 | Tecnital                  | Tecnital                     | 50                   | 83,000             |  |

| Locality | Type of plant | Year of construction | Construction Company (*) | Composting process | Plant capacity (T/d) | Inhabitants served | Comments   |
|----------|---------------|----------------------|--------------------------|--------------------|----------------------|--------------------|--|
| Palermo  | Mixed         | 1964                 | Biofert                  | Earp-Thomas        | 150                  | 100,000            | The plant serves only a parte of 650,000 inhabitants |
| Stracusa | Mixed         | 1975                 | Public Consult - Shunt   | Rotoref            | 130                  | 108,000            |  |
| Cagliari | Mixed         | 1967                 | Tecnital                 | Tecnital           | 150                  | 223,000            | Part of collected refuse dumped in landfill          |
| Sassari  | Mixed         | 1967                 | Tecnital                 | Tecnital           | 50                   | 56,000             | The plant serves only a parte of 100,000 inhabitants |

(\*) In the case of "mixed" type plant, where two company are indicate, the first indicated has furnished the composting line, the second the incinerating line.

From the technological point of view, the transformation process can be carried out in three ways:

a) By natural way (open windrow composting): in this, the material to be stabilized, after suitable pre-treatment, is placed in mounds in the open-air and turned over and mixed every now and again using a loading shovel.

The TECNITAL, MACCHI-GONDARD, VÖEST ALPINE, BÜHLER, PUBLIC CONSULT (ROTOREF), FERRERO systems are included in this category.

The principal differences between the various processes, concern the trituration phase and that of the refining of the materials. The time of maturation in the open-air should be the same for all the processes. Actually, different times are given which do not appear to be justified.

b) By accelerated way (DANO system): stabilization is reached in two phases: the first phase of homogenization and mixing of the material already graded and freed from ferromagnetic substances. This is carried out in suitable rotating cylinders or digestors (biostabilizers) where the material remains for a certain period (3 days) in controlled temperatures and humidity. After screening, the material is placed in mounds in the open-air on the ground or in specially prepared pits so that the biological process of humification can be completed.

The total time required for transformation is a few months in the case of a) and a few weeks in the second case.

c) by inoculating selected microbic cultures: EARP-THOMAS and ITALCAMPO processes use this system. This method is scientifically very different from the preceding ones independently from how the process is technologically carried out.

Its aim is to accelerate the transformation processes, by the inoculation of determined microbic masses, and to guarantee determinate chemical-physical and biological characteristics of the resulting product.

This allows a consideration. In spite of the necessity of adding organic substances to soil, it is difficult to find a suitable technique which can treat adequately such a changeable product as is refuse. The systems which give a satisfactory product are few.

The compost has not, up until now, been an product in conformity with standards, this has allowed the proliferation of plants which produce a poor quality product which is rejected by the agriculturists and ends up by discrediting such a system of the treatment of solid refuse.

The proof is given by some municipalities who have had such negative experiences in composting plants and who have no intention whatsoever to repeat the experience even with other more valid processes.

The difficulty of commercialization has also contributed to this. In fact, the Municipalities or "Municipalized Un-

dertakings" (\*) who run the composting plants in Italy must also see to the placing of the product on the market and they do not have the suitable commercial structures in order to do so.

From the data available for 21 plants, there is a result that of the compost produced:

- 30% is not sold (it is dumped);

- 70% is sold:

65% at a price of 200 - 400 Lit/q

5% at a price of 2,000-7,000 Lit/q

However, less than half of the plants manage to sell the product. In general, those of a greater capacity.

The prices are intended free on production plant, except, and this is quite significant, in the case when it is sold at 7,000 Lit/q. Of these, 3,000 Lit/q are for commercialization and distribution expenses.

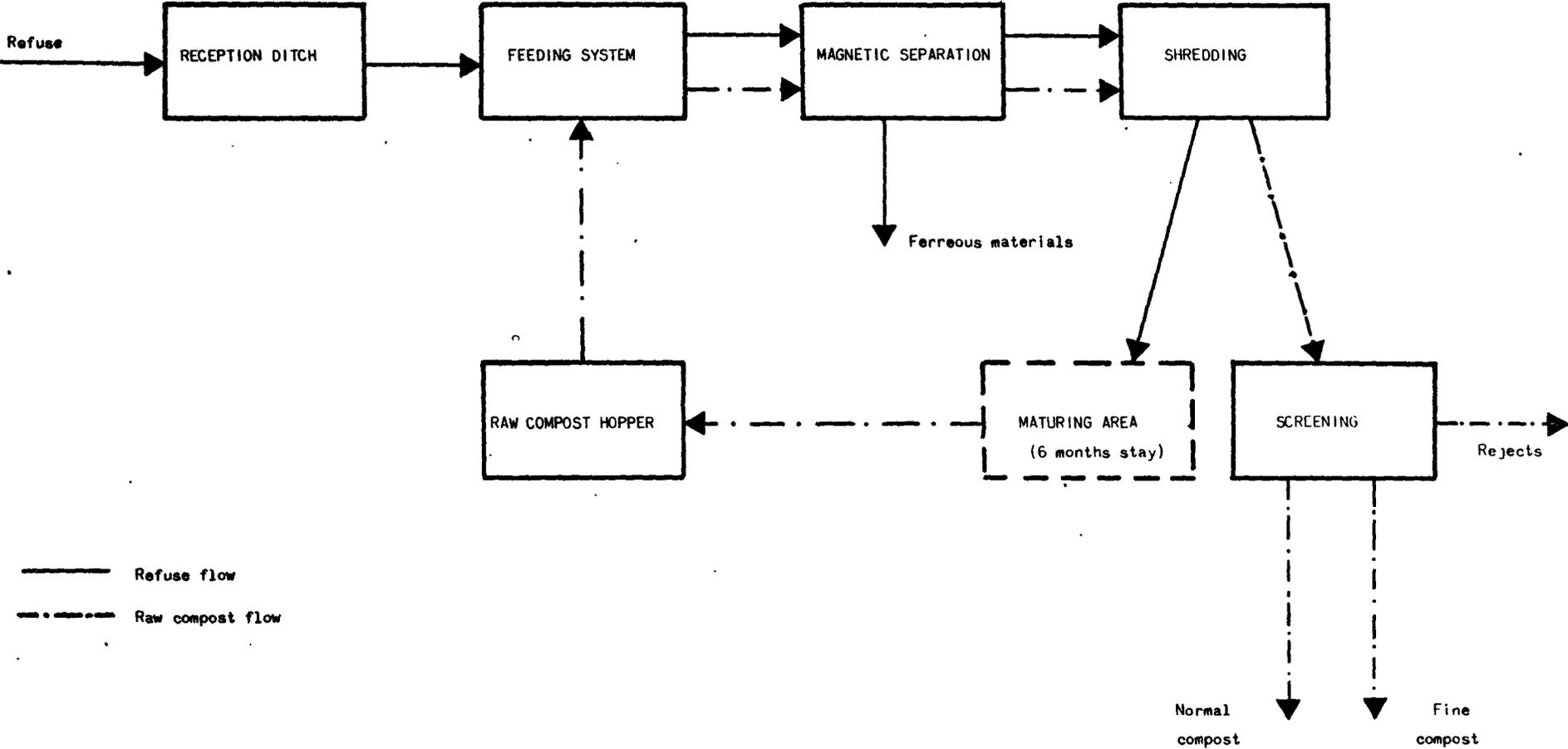
Apart from this, it is clear that the product which is sold at 4,000 Lit/q is not the same as that sold for 400 or 200 Lit/q.

In Fig. 2+8 are summarized block diagrams of the various composting systems adopted in Italy.

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(\*) Special Bodies established according to the Italian law through which Communes may provide for public services by availing themselves of sufficiently autonomous operative tools endowed with their own management and budget.

FIG. 2 - COMPOSTING PROCESS VOËST-ALPINE : BLOCK DIAGRAM



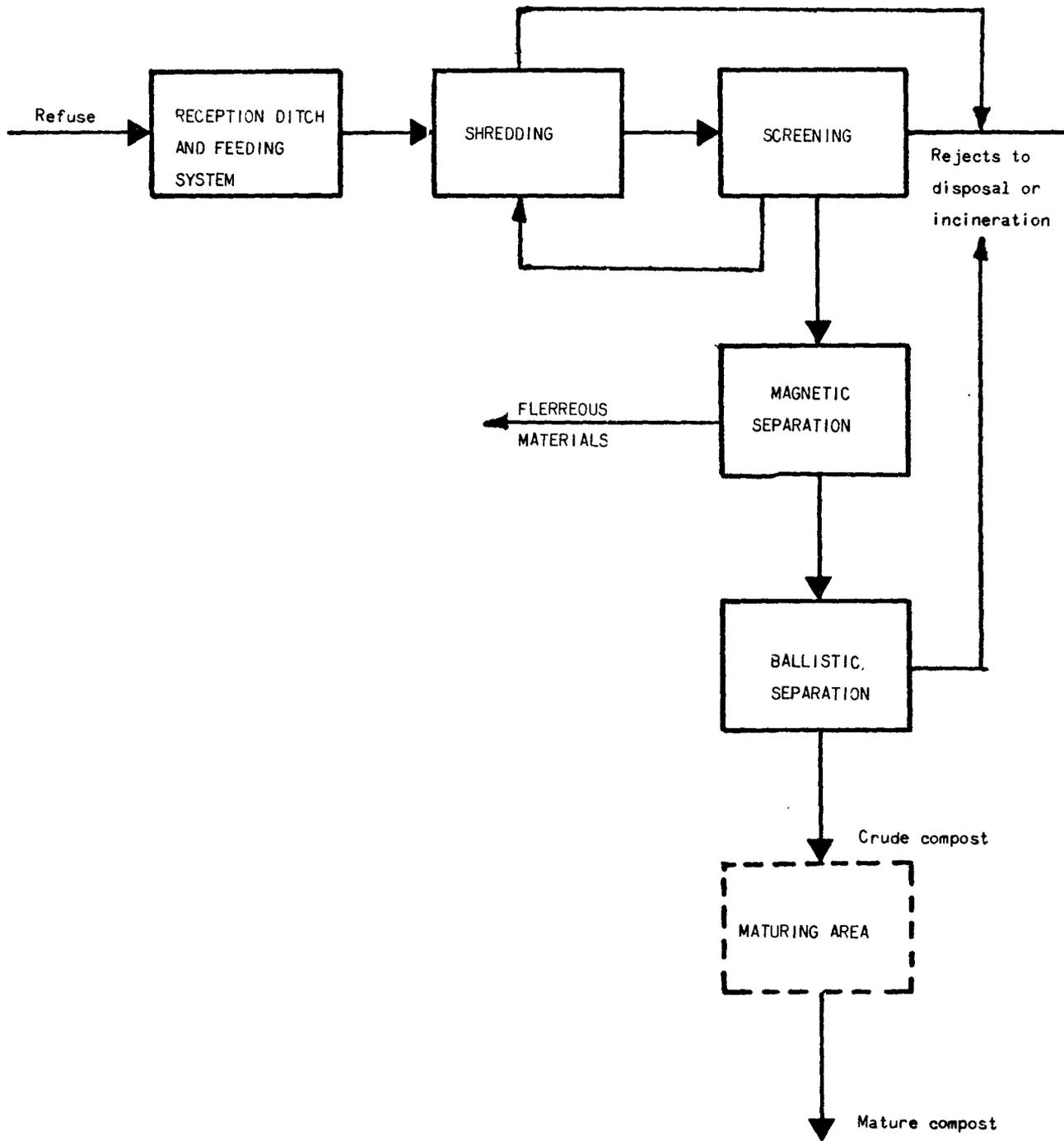


FIG. 3 - COMPOSTING PROCESS MACCHI GONDARD : BLOCK DIAGRAM

FIG. 4 - COMPOSTING PROCESS BÜHLER : BLOCK DIAGRAM

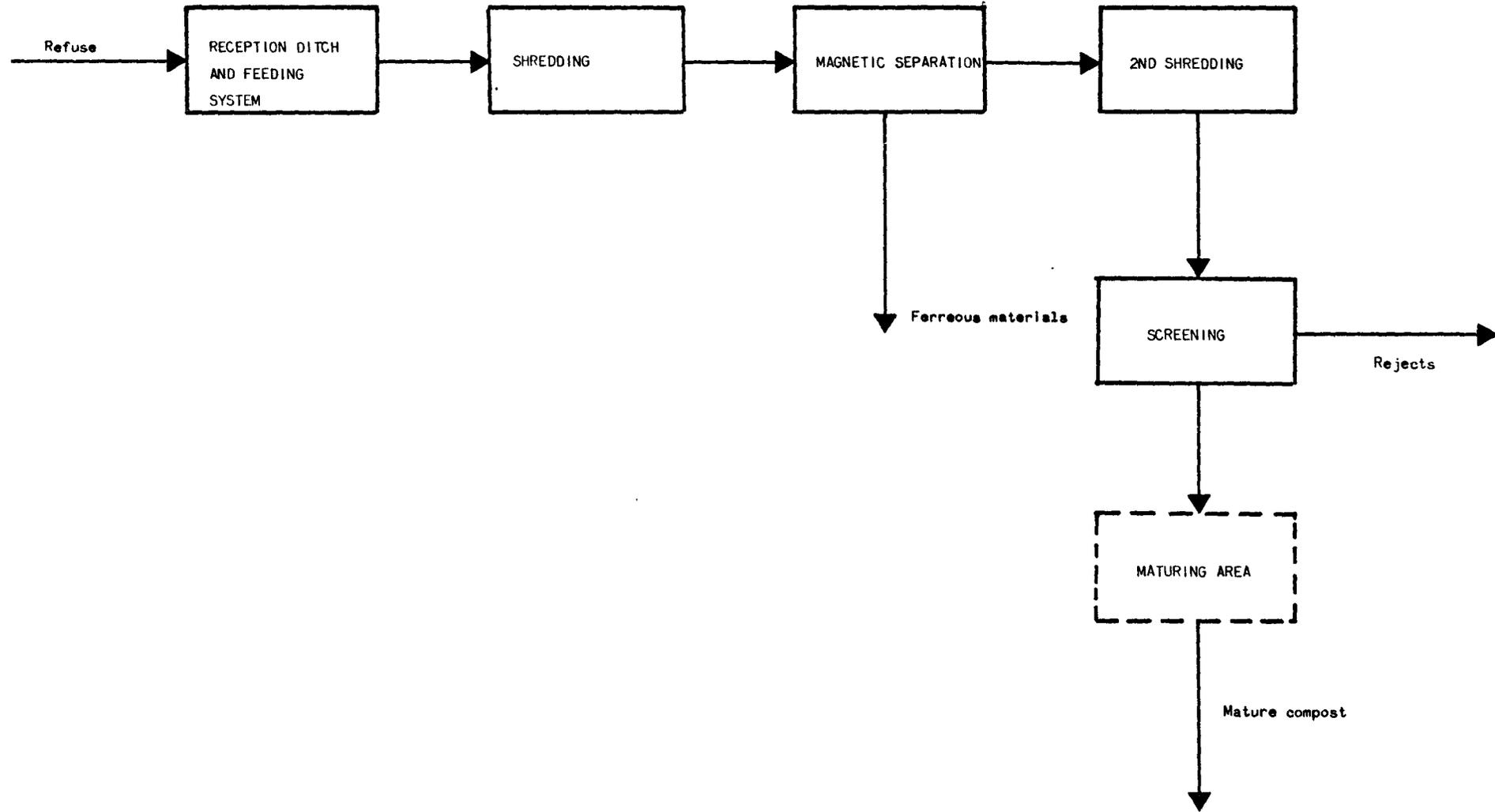


FIG. 5 - COMPOSTING PROCESS PUBLIC CONSULT (ROTOREF) : BLOCK DIAGRAM

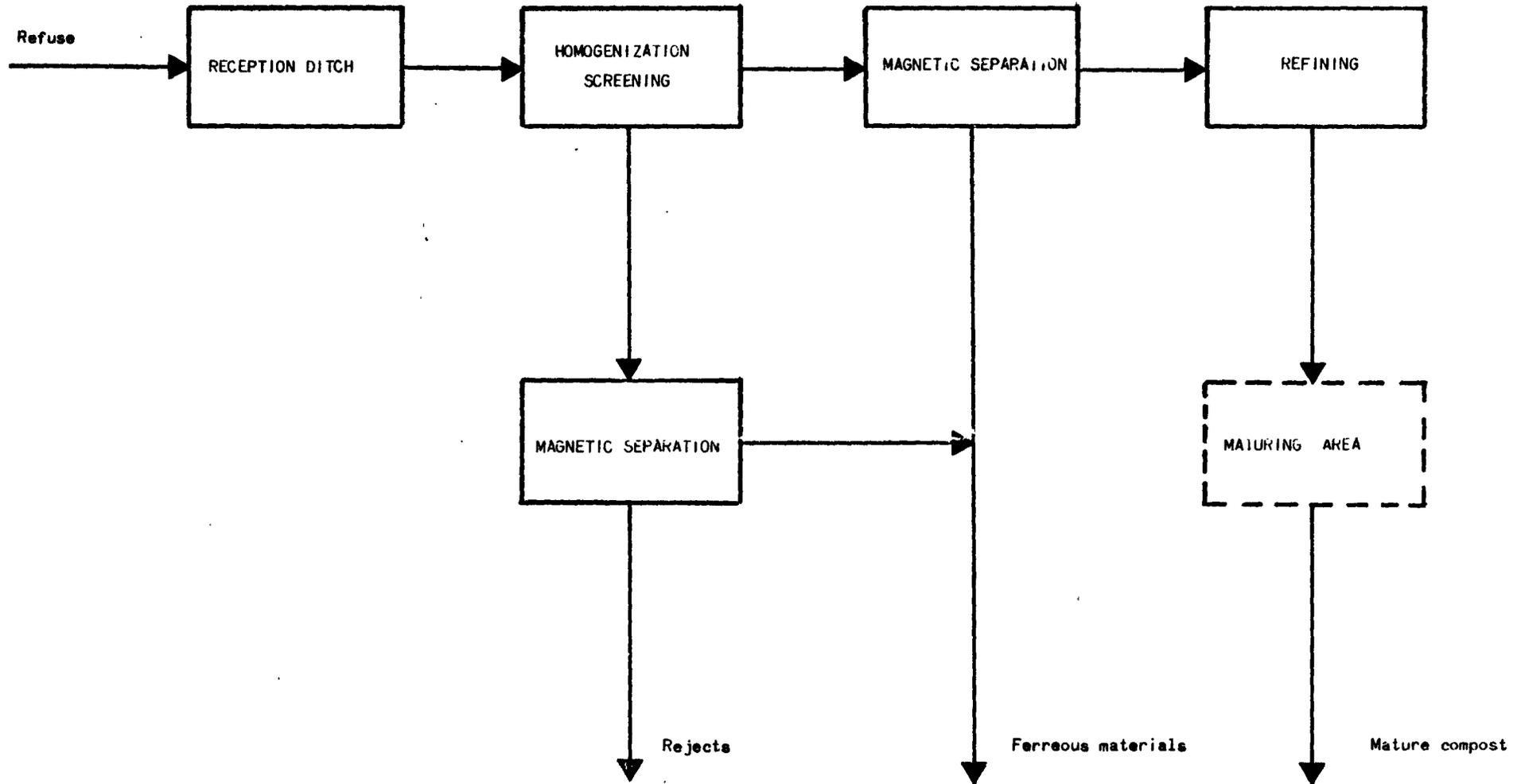


FIG. 6 - COSMPOSTING PROCESS TECNITAL : BLOCK DIAGRAM

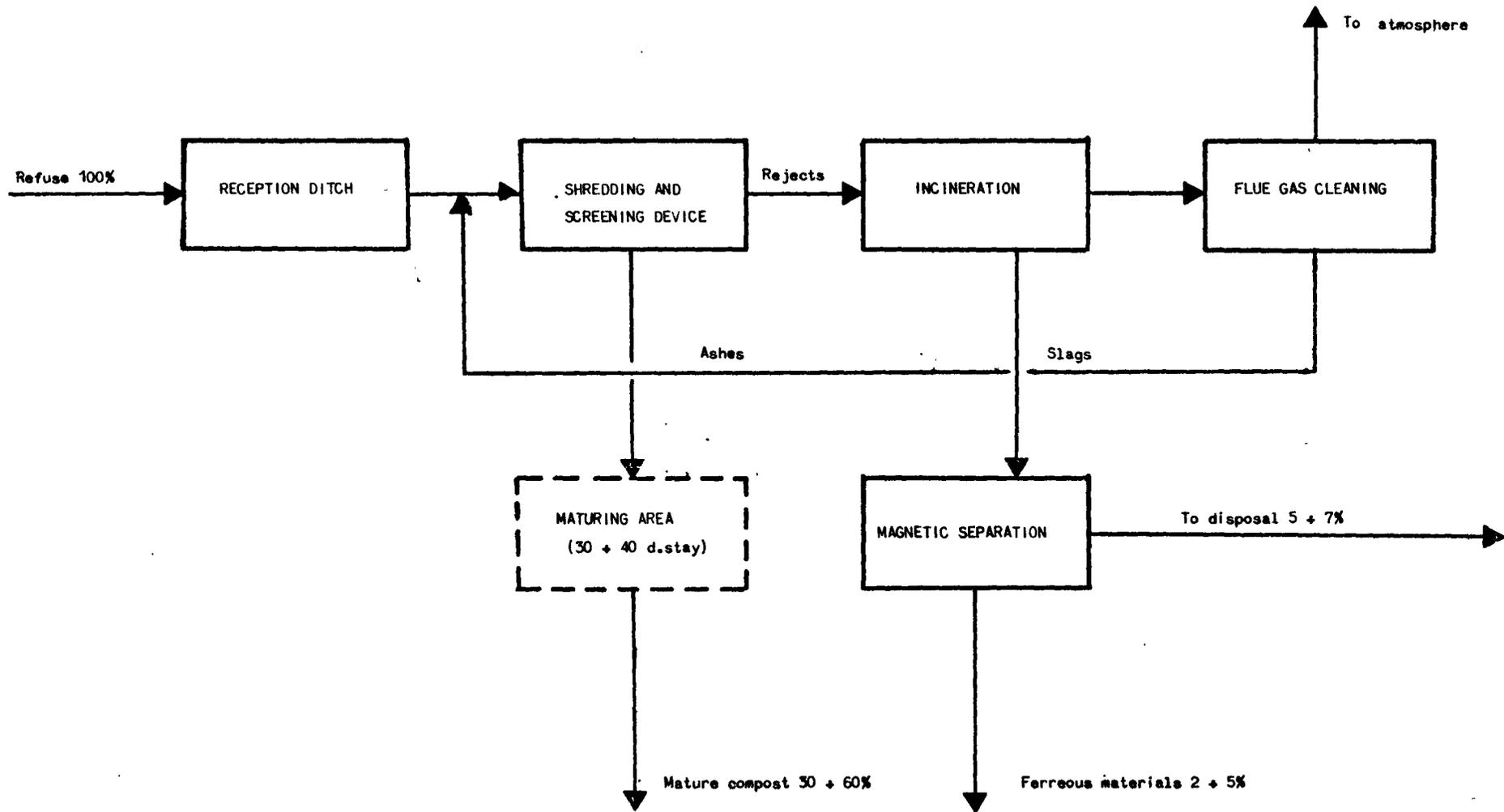
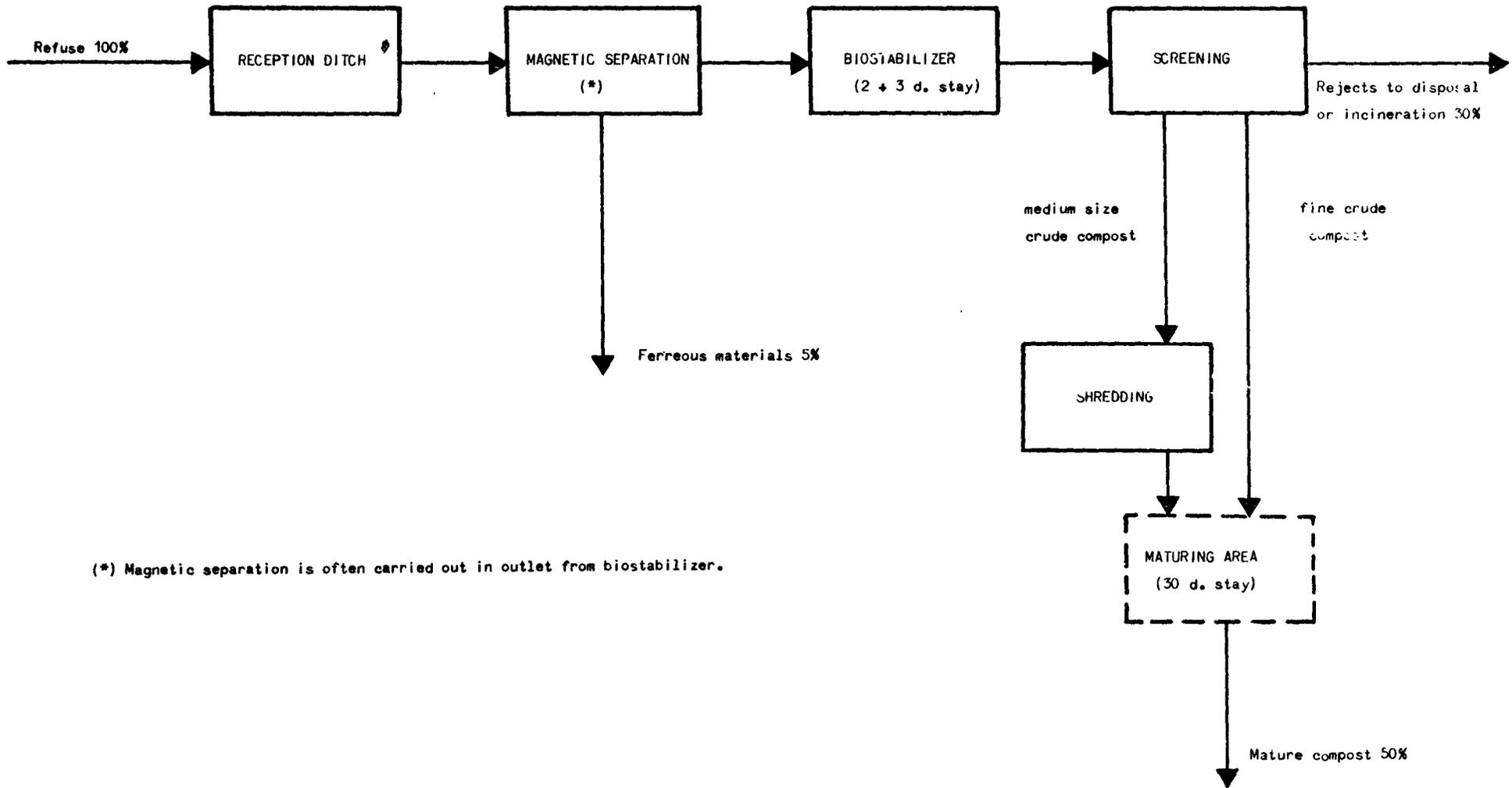
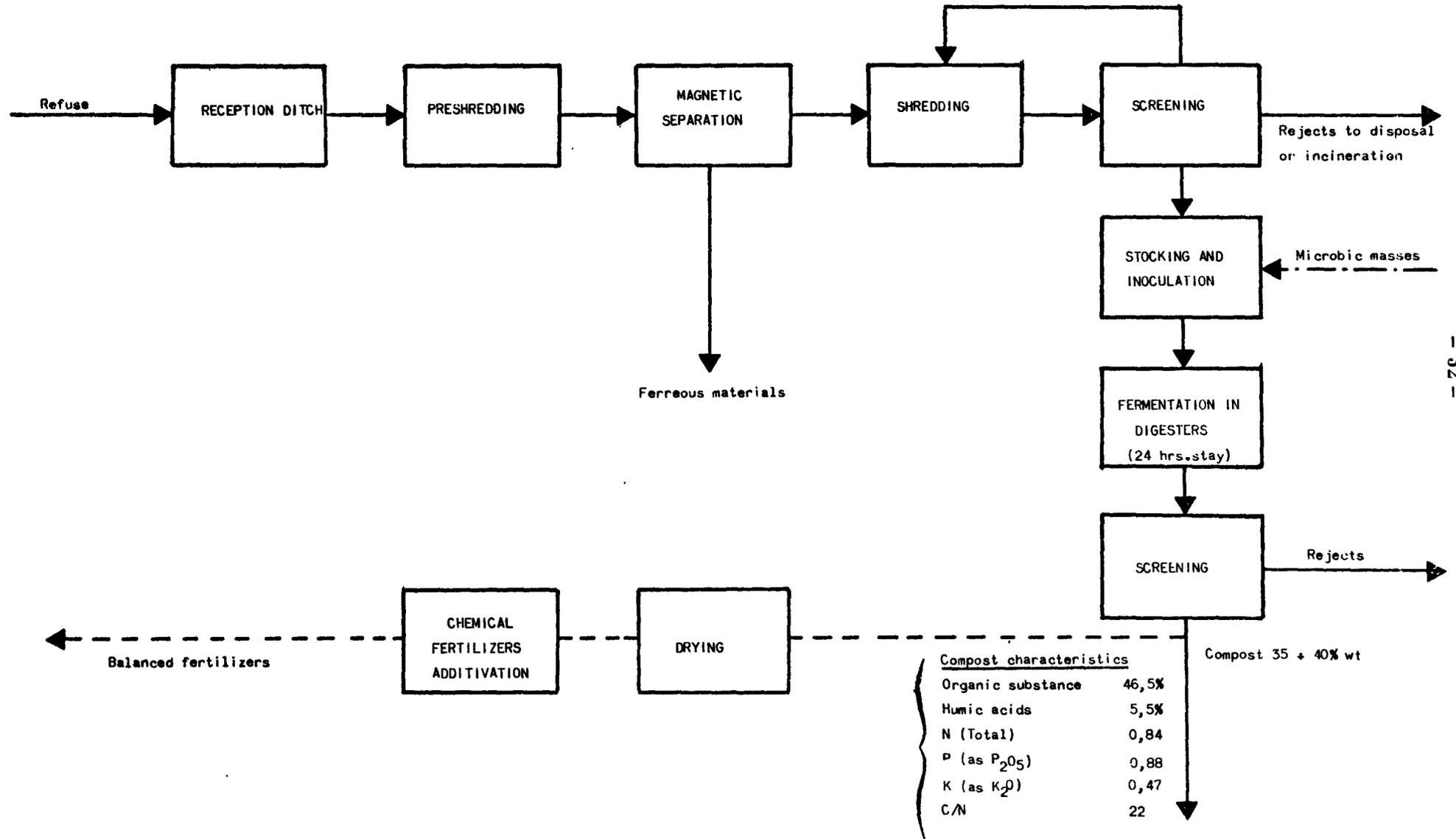


FIG. 7 - COMPOSTING PROCESS DANO : BLOCK DIAGRAM



(\*) Magnetic separation is often carried out in outlet from biostabilizer.

FIG. 8 - COMPOSTING PROCESS EARP-THOMAS : BLOCK DIAGRAM



### 1.3.2 The ITALCAMPO process

This process (Fig. 9) treats solid town and industrial refuse in the following proportions:

- 50% town refuse
- 30% cellulose waste as sawdust, pulp from sugar-beet, paper mill residue
- 20% tanning waste (flesh scraps).

The product recovered is 70% in weight of the raw material.

Due to the inoculation of suitable microbic cultures and the composition of the initial material, the process does not seem to produce residue.

The process uses the materials as they are without pre-treatment.

The time necessary for the bioconversion is of 2-3 months.

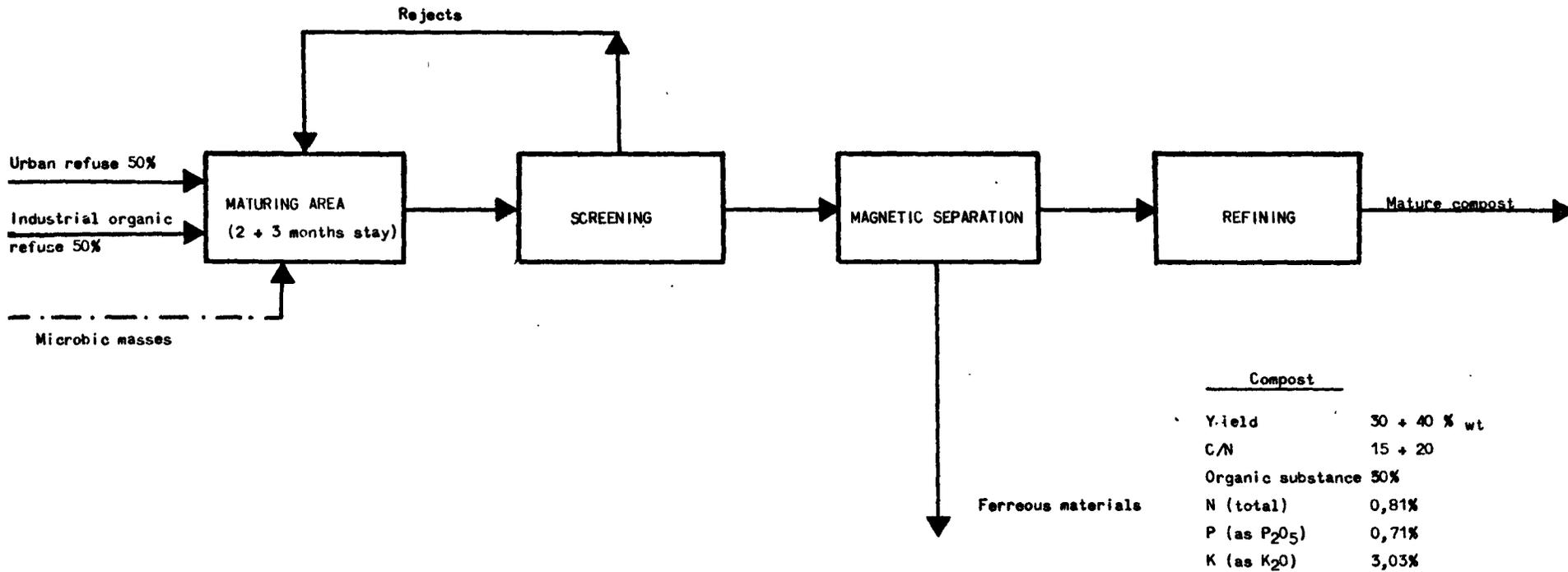
There is a single plant in Italy at S. Agata B. near Bologna with a capacity of 100 t/day. Its management is private.

Some reservations are made on the technological aspects and the operative conditions from the sanitary-health point of view.

The plant is placed in an area of 5 ha, far away from inhabited centres.

The refuse from 15 neighbouring municipalities are sent to this plant. For this service, they pay 5,000 lit/t.

FIG. 9 - COMPOSTING PROCESS ITALCAMPO : BLOCK DIAGRAM



| Compost                               |              |
|---------------------------------------|--------------|
| Yield                                 | 30 + 40 % wt |
| C/N                                   | 15 + 20      |
| Organic substance                     | 50%          |
| N (total)                             | 0,81%        |
| P (as P <sub>2</sub> O <sub>5</sub> ) | 0,71%        |
| K (as K <sub>2</sub> O)               | 3,03%        |

### 1.3.3. The CARTIERE BURGO plant

Of the possible solutions to the problem of eliminating wood residue (\*), the "Istituto Nazionale per le Piante da Legno" of the CARTIERE BURGO Company has studied, over the past years, the possibility of transforming the bark from the paper industry into organic fertilizer. This possibility has been chosen for the following reasons: (oo)

- 1) Humification represents an overall and definite solution to the recycling in nature of refuse;
- 2) There is no pollution problem connected with the transformation process;
- 3) The cost of the operations are recompensed by the commercial value of the product obtained.

The results of this research have brought about the setting up of a humification process of the bark of poplar trees and the building of an industrial plant at the Cartiere Burgo plant at Mantova which is capable of handling up to 200,000 q/year of wood waste.

The productive process is briefly described: the bark of the poplar trees, on exit from the bark-stripping drums, is ground in order to obtain pieces of a maximum size of 5 cm. These are transferred to a special asphalted fermentation area and piled up to average heights of 7 m.

---

(\*) The yearly amount of wood waste in the paper and cellulose industries in Italy is 1,000,000 q bark (mostly poplars); 125,000 fir tree fibres, about 2,000,000 q wood pulp waste and thickened sludge from the wastewaters.

(oo) Other possibilities were examined and discarded as incineration and biomass production in liquid medium.

In this way, fermentation begins in thermophilic phase. The mounds are periodically turned over to air the heap carrying out the control of the chemical-physical parameters (temperature, pH,  $O_2$ ,  $H_2O$  content in the heaps). After 12-14 months of fermentation, the product is transferred to other areas to correct the humidity to an average value of 50% and then it is screened, ground and put into sacks. In the course of the process, no chemical additive is added.

Table 7 summarizes some of the characteristics of the fertilizer obtained after about 14 months of fermentation in mounds.

As can be seen from the Table, the product shows high contents of organic substances (70%) and humic acids (15-18%). This is the result of a quite long fermentation process (mostly because of the nature of the material which is not easily metabolized) which guarantees, however, a stable product with a high content of "lasting humus".

All this product named "humus" is sold at a price of 3,500 Lit/q free on plant.

It is employed at a rate of 5-10 tons/hectare

Different products may be obtained from poplar bark, linked to the period of fermentation process:

- Mulching compost: obtained after 3-4 months of fermentation; employed at a rate of 30-40 t/ha
- \* Compost: obtained after 6-8 months; employed at a rate of 15-20 t/ha

Obviously, the last two products are sold at a lower price (2500-3000 Lit/q).

Tab. 7

Characteristics of the fertilizer obtained from  
poplar bark ("humus")

|                               |          |   |      |     |    |
|-------------------------------|----------|---|------|-----|----|
| pH                            | 7.5      | - | 8.0  |     |    |
| Ash (residue at 550°C)        | 28       | - | 32   | %   | wt |
| Organic substances            | 68       | - | 72   | %   | "  |
| Carbonium                     | 38       | - | 42   | %   | "  |
| Organic nitrogen              | 1.6      | - | 1.8  | %   | "  |
| C/N ratio                     | 21       | - | 27   |     |    |
| Ammoniacal nitrogen           | 50       | - | 250  | ppm |    |
| Nitric nitrogen               | 150      | - | 500  | ppm |    |
| Nitrous nitrogen              | 0        | - | 10   | ppm |    |
| P <sub>2</sub> O <sub>5</sub> | 0.3      | - | 0.6  | %   | wt |
| K <sub>2</sub> O              | 0.5      | - | 2.0  | %   | "  |
| Ratio (mean value) N/P/K      | 1 / 0.26 | / | 0.73 |     | "  |
| CaO                           | 9        | - | 12   | %   | wt |
| MgO                           | 2        | - | 4    | %   | "  |
| Humic acids                   | 15       | - | 18   | %   | "  |
| Fulvic acids                  | 2        | - | 6    | %   | "  |

#### 1.3.4. FOMET process

It is estimated that in Italy (1) there is a residue of about 5,000,000 tons/y of poultry manure. Its chemical composition results (2) as:

|                |       |      |
|----------------|-------|------|
| Dry substances | 85.94 | % wt |
|----------------|-------|------|

Percentage of the composition of the dry substance:

|                            |       |
|----------------------------|-------|
| - organic substance        | 81.60 |
| - raw protein              | 23.38 |
| - pure protein             | 12.97 |
| - digestable protein       | 18.44 |
| - uric acid                | 1.74  |
| - raw fat                  | 1.32  |
| - raw fibres               | 17.45 |
| - cellulose                | 19.45 |
| - non nitrogenous extracts | 39.45 |
| - ash                      | 18.40 |
| - calcium                  | 3.74  |
| - phosphorus               | 1.50  |

One possible way of elimination is represented by the reuse of such organic matter.

The F. O. M. E. T. company at San Pietro di Morubio (Verona) has managed by starting off from poultry manure, to obtain a fertilizer with the following average composition by using bioconversion processes with selected bacteria (tab. 8).

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(1) Siniscalchi, Boschi - Ministry of Agriculture and Forestry  
UNEP/FAO, Seminar on Residue Utilization (Rome 18/20  
January 1977).

(2) Average of the analysis in duplicate.

Tab. 8

Average composition of fertilizer from poultry manure

|   |             |           |
|---|-------------|-----------|
| Humificated organic substances  | 38-40       | % wt      |
| Aerobic bacteria charge   | 483,240,000 | UFC/g (*) |
| Anaerobic bacteria charge   | 410,200,000 | UFC/g     |
| Total nitrogen (organic and ammoniacal)                                       | 2 - 3       | % wt      |
| Potassium oxide (from sulphate)   | 2 - 3       | %         |
| Total phosphoric anhydride (from phosphates and phosphorus organic compounds) | 2 - 3       | % "       |
| Fulvic acids  | 11          | % "       |
| Humic acids   | 9           | % "       |
| <b>MICRO ELEMENTS:</b>  |             |           |
| - magnesium   | 350         | mg/kg     |
| - zinc  | 220         | "         |
| - cobalt  | 5           | "         |
| - boron   | 20          | "         |
| - molybdenum  | 20          | "         |

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(\*) UFC/g = Official per gramme

The material is partly from the various fowl raising farms in the province of Verona and partly from the mushroom-bed (horse manure). It is inoculated with bacteria selected in the laboratory (from a pilot fermenter formed by a static cylinder with plates supplied with air blowers) and then stocked in the open-air in great mounds.

With this phase of orientated pre-fermentation, the production of putrescence can be avoided.

The material then enters the plant through a pre-treatment section which is able to handle 70-120 quintals per hour of refuse (according to the humidity of the material).

The pre-treatment section is composed of a doser where a second inoculation of selected bacteria takes place, (to the extent of a few grammes per quintal of material), provided with a conveyor belt which feeds the screen where separation of the larger pieces of material takes place.

The conveyor belt has an electromagnet to hold back the ferrous metal material.

The screened material is ground in a rolling-mill and then undergoes further screening.

After the pre-treatment phase, the material is placed in mounds under cover of 7,000 to 10,000 quintals each (about 2.5 m in height) where it remains to ferment for 40-50 days. The humidity content, the  $O_2$  and  $CO_2$  content, the eventual variations of the temperature, the potential redox, the enzymatic activity and the microbic charge are controlled.

After this period, the material is aired by mixing with the conveyor belt and, if necessary, it is re-inoculated and crushed.

These operations are repeated other eight or nine times for an overall period of 12 months.

If at the eighth month, the fermentation process is behind, the material is passed through a doser with blades, (70 q/hour) then into an inclined rotating cylinder where the product advances by gravity while a blower blows air in the opposite direction. A screen at the exit of the cylinder separates the larger pieces (larger than 1 cm) from those which will become the finished product.

In exceptional cases, (humidity content about 70% after several months of stabilization) the product is passed through a combustion chamber where the humidity content of the material is reduced to 40%.

Finally, a pelleting machine makes the finished product suitable for sprinkling by mechanical means.

The potentiality of the plant allows the treatment of 500,000 to 600,000 q/year of poultry and horse manure.

The plant covers an area of 1,500 sq. m. covered and 1,500 sq. mt. of uncovered area.

The finished product costs 6,500 lit./q free on the plant and is mostly sold from December to the end of March and from June to November.

The F. O. M. E. T. advises the following applications for

various crops:

|                 |    |         |                    |
|-----------------|----|---------|--------------------|
| Wheat           | q  | 15 - 20 | per hectare        |
| Corn            | q  | 20 - 25 | "                  |
| Rice            | q  | 15 - 20 | "                  |
| Beets           | q  | 20 - 25 | "                  |
| Grass           | q  | 8 - 10  | "                  |
| Strawberries    | q  | 35 - 40 | "                  |
| Vegetable crops | q  | 25 - 30 | "                  |
| Flowers         | q  | 30 - 35 | "                  |
| Fruit trees     | Kg | 1 - 2   | (young trees)      |
| Fruit trees     | Kg | 3 - 4   | (producing plants) |
| Olive trees     | Kg | 1 - 2   | (producing plants) |
| Vines           | Kg | 1       | per plant          |
| Lawns           | g  | 500     | per sq. m          |

The F. O. M. E. T. for the analyses of the initial materials of the finished products used the experience of the C. A. M. Laboratory of Analysis and Agricultural Chemical Research in Milan, while the application on the soil of the finished products was carried out by the technicians of the Experimental Institute of Vine-growers of S. Michele Adige (Trento).

#### 1.4. Status of R and D

##### 1.4.1. The prototype plant of the FIAT at CAMBIANO

At Cambiano (4, 500 inhab. ) in the province of Turin, an experimental plant has been built for the transformation of solid town refuse into compost using a new process.

The experiment was planned, organized and carried out by the FIAT (Direction of Research and Engineering).

The aim is to develop a technology of composting which could be used by average sized and small municipalities, which is cheap and reliable, which gives a product of good quality characteristics.

The plant is actually fed with only the refuse from the Municipality of Cambiano or rather with a part of the refuse.

In fact, a separate collecting service has been devised which allows the collection of two different types of material:

- a) that rich in organic substances containing food scraps, cellulose residue, etc.;
- b) inert and non decomposable substances such as glass, metals, plastic.

Only the organic part is fed into the plant. The forming of the separate collecting system is a conditioning part of the technology of studied composting. When this is not carried out initially, the separation should be carried out at the plant automatically. The principal scope of the experiment would then therefore be useless.

In fact, the apparatus for separating are very costly, not very easy for maintenance and are not suitable for plants of small size.

A few words on the transformation process. Transformation takes in four successive phases:

- 1) Sterilization, shredding and homogenizing of the material in a rotating cylinder. In the course of this phase, there are alternate periods of the same length of the standstill periods in function with the rise in temperature which occurs in the material due to the effect of fermentation. The temperature rises to about 80°C and kills the pathogenic germs. The permanence in the cylinder is 2 days.
- 2) Formation of mounds under cover in special sheds and the natural fermentation of the material. Period: 3 - 5 days.
- 3) Turning over of the mounds and first inoculation of microbic strain which encourages and controls the transformation process. Permanence of material in these mounds: 3 - 5 days.
- 4) Turning over of the mounds and second inoculation of microbic strain which concludes the transformation. Duration of this last phase: 3-5 days. The average overall duration of the process is therefore 15 days.

The material transformed is then screened and packed in sacks for distribution.

The process is under developing: so changes in the scheme as described may be possible.

Future research programmes foresee:

- a) a composting treatment with the sludge from the treatment of the wastewater from the municipality of Cambiano. This plant should be built near the composting plant;
- b) experiments in the application of the compost obtained;
- c) experiments to automatically separate the non decomposable materials.

#### 1.4.2. The CARTIERE BURGO pilot plant

In 1973, in the "Istituto Nazionale per Piante da Legno" (\*) of the Company Cartiere Burgo an experimental programme was developed with the aim of recovery by accelerated humification in mounds of some of the sludge from zootechnical breeding and which is not actually of any use (poultry, sludge from pig-sties), mixed with some wood-pulp residue (poplar bark, wood pulp waste, thickened sludge).

A pilot plant was carried out (unity of 100 q/year) and one on a semi-scale (unity 10,000 q/year) with procedures substantially analagous to those described in

§ 3.3. . .

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(\*) "National Institute for Plants for wood".

The scope of the experiments was that to obtain fertilizer with a high humificated organic substance content and at the same time, verify the contents of the principal fertilizing elements.

The poultry used in the experiments comes from battery-reared fowl. It was collected at the end of the productive cycle in its solid state.

The average humidity results as being 70%. The sludge from the pig-sties comes from screening the waste effluents.

The sludge from the paper industry taken into consideration comes from the centrifugation of the primary sedimentation of the effluents of a newspaper factory. It is formed of fibres of very small size (about 60% is < 100 mesh) with a high cellulose content.

Products deriving from various combinations of initial material have been studied. These are indicate in brackets:

- poplar bark and poultry manure (75-25)
- poplar bark and sludge from pig-sties (75-25)
- wood pulp and poultry manure (80-20)
- thickened sludge from paper industry and poultry manure (70-30)
- poplar bark, thickened sludge from paper industry and poultry manure (55-20-25).

From the experiments, the following points have emerged. From the integration of the wood residue with

sludge from zootechnical breeding, following long fermentation, products are obtained which have a high content of fertilizing elements.

In particular, the presence of zootechnical sludge, compared to the product obtained from only the poplar bark, increases the contents of the fertilizing macro-elements (N, P, K). The fertilizers obtained in this way have more or less the same characteristics as a very mature straw and dung and the analogy is obvious when the fact is considered that the original materials used are similar: the wood pulp residue replaces the straw and the cattle dung is replaced by the industrial sludge from breeding farms.

However, the value of the humic substances decrease, probably due to the slight presence of lignin and other polyphenols in the sludge from breeding farms. The fertilizer obtained only from poplar bark present a content of humic acids double to that obtained in the presence of sludge.

The mineralization of the organic substances is on the other hand intense. This can be seen in a relevant increase of the ash in the material at the end of fermentation.

Within the field of fertilizers obtained, the fact has emerged that the analytic characteristics vary in function of the initial material used in the process. This affirmation which results as being obvious, underlines, however, the necessity in verifying in detail (and therefore select) the materials to be converted. Some indications reflect

on the possibility of application: the fertilizers obtained from poplar bark and poultry manure (or sludge from pigsties) can be used in all agricultural crops requiring elevated nutrition (in horticulture and fruit farming, etc.); the product obtained from poultry manure and wood pulp waste (or thickened sludge from paper industry) cannot very easily be used as an organic fertilizer because of its low humus content as the capacity to retain water is great especially at the intermediary stage of fermentation (3-5 months). This consideration, together with the fact that it has a lower pH (6.3-6.9) is interesting for its possible application in floriculture.

On the basis of the above-mentioned indications, a vast agronomical experiment programme of the products obtained has been started.

As a result of this research, an industrial plant will be set up which treats bark, thickened sludge from the paper industry and poultry manure (in the percentage of 55-20-25) at the Cartiere Burgo plant at Mantova or else the existing plant which treats only poplar bark, will be modified ( § 1.3.3. ).

1.4.3. Researches financed by the CNR on the composting of solid refuse and sludge

The CNR (National Council for Research) financed during the period 1976-77, two experimental research programmes on laboratory scale of the composting of solid town

refuse and sludge.

These form part of the finalized programme "Promotion of the Quality of the Environment", sub-project "Water", theme of the research "Development of treatment processes and elimination of the resulting products".

These research programmes were planned for the period 1976-77 and are therefore still being carried out. They are carried out by specialized Companies or Institutes.

a) The first research carried out by the TECNECO S. p. A. (Fano) and the ISTITUTO RICERCHE BREDA S. p. A. (Milan) has for title "Composting of solid town refuse and sludge".

The research programme is divided as follows:

- research and choice of initial material
- pre-treatment
- chemical, physical and biological characterization of the pre-treated material
- treatments fermentation
- characterization of the obtained product plus pathogenic and germinability research
- selection of the operative conditions for the humification treatment
- characterization of the end products.

Although the experiments are carried out on laboratory scale, they give the operative data of the fundamental parameters of the process.

The results could be applied in pilot plants or suitable existing plants on the basis of indications which emerge from the research.

b) The second has for title "Basic research and application on the microbic colonization of solid town refuse with the aim of elimination by rapid transformation into compost to be used as agricultural fertilizer".

This is carried out by three Institutes of the University of Pisa:

- Institute of Agronomy
- Institute of Chemistry of the Soil
- Institute of the Microbiology of the Soil

This research has the aim of studying the effects of selected inoculation on the transformation processes and the quality of the product.

1.4.4 Production of compost by mixing solid refuse with sludge from various sources and field experiments of the product obtained

The research financed by the CNR within the project called "Energetica" (o) foresees the production of compost in an experimental plant set up by the Swedish Environmental Office with the use above all of animal manure.

The research is carried out by the CRPA (oo) in Reggio Emilia and should last two-years (1977- 78).

This research is seen as an integrant part of the research on composting of the project called "Ambiente" (ooo) (p. 1.4.3).

The reactor or digester is formed by a large tank with air suitably fed in at the bottom and side walls. The material is moved by an archimedean screw.

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(o) i. e. "Energetics"

(oo) Centro Riproduzioni Animali - Animal Reproduction Centre

(ooo) i. e. "Environment"

#### 1.4.5 SIBE Composting processes

The SIBE (Società Impiantistica Bio-Ecologica) of Valenza Po (Alessandria) has developed two processes for the transformation of refuse in compost.

The first treats solid town refuse, the second the sludge from pig - sties.

Before proceeding to their description, it is necessary to point out that these processes have been experimented on laboratory scale.

Application on prototype or industrial scale has not been made and therefore applicative proof is missing. Consequently elements concerning the cost are among the unknown factors.

##### a) Production of organic fertilizers from solid town refuse

The cycle foresees automatic separation of metals, plastic and glass; airing in digestors for 5-7 days; drying of the product so as to be able to stock it, successive selected fermentations.

The experiments were made in digestors. The airing process aims at eliminating determinate pathogenic biomasses and to bring the refuse up to a microbic balancing.

The material obtained in this manner is called "basic precursor".

The SIBE idea is that the material obtained in this way should be dried until 10% humidity is reached so as to allow its stocking for 6-8 months.

It would then be processed according to the requirements of agriculture so as to supply a product which has not only good chemical-physical characteristics but also an useful . microbic charge.

On the other hand, stocking the product for more than 1 month would cause a loss of the microbic symbiotic charge that is, a loss of its biological characteristics (those organic would remain).

However, the processing of stabilized material is possible, without drying (in the case of drying for the successive processing, the dried material must be rehumidified with a content of H<sub>2</sub>O of at least 50%).

On the basic precursor, a first sowing of biomass of the pectinolytic and cellulolytic families is carried out.

Then selected metabolizations, that is, a series of fermentations using suitable inoculation which allow:

- the production of phyto-hormones useful to the growth of vegetables
- the production of specific antibiotics
- the production of stimulators of the growth of root and stalk
- the production of micro-organisms which increase the humic rate of finished fertilizer.

An overall number of 42 strains of bacteria have been isolated and are used in the process. Each of these bacteria has a specific job.

Altogether, the fermentation phases last a few days.

The finished product has a very fine granulometry, a ratio of C/N = 7 ÷ 10, a humus content of 14%.

The yield of the process is 30 ÷ 40% of the initial material.

The experiments for developing the process ended in 1976.

During 1977, experiments on crops will be carried out. The research works is carried out in collaboration with the Institute of Agricultural Chemistry of the University of Piacenza.

A precise estimate of the cost of a plant on industrial scale has not yet been made.

According to the technicians of the SIBE, the investment cost should be inferior to that of an incinerator having the same capacity.

About the cost of running (amortization excluded), the figure of 1,500 Lit/q of finished product has been indicated.

b) Production of organic fertilizers from sludge from pigsties

The process is analagous to the preceding. The residue coming from the first screening of the pigsties waste effluent and that from the neutralization of the wastewater (o) are converted into fertilizers.

The waste products of a farm such as straw, stubs, animal residue from abattoirs, etc. can be added.

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(o) As will be explained in chap. 4, the SIBE recovers fodder from the swine waste effluent.

The cycle foresees airing in digestors for 5-7 days, drying of the product so as to be able to stock it, successive selected fermentation conducted in digestors which give products with different characteristics, mixing of the ferments with different ratios.

In this way, fertilizers which are suitable for all types of crops are obtained.

### 1. 5. Economical evaluation

Some indications of cost have already been given during the preceding chapters.

1. 5. 1. Where the composting process from solid town refuse is concerned, it can be noted that some produce a compost which is dumped and so they work as trituration plants rather than composting plants; others produce a product which is sold at up 4, 000 Lit/q free on plant.

Therefore, the two types of plant cannot be compared from the cost point of view. On the other hand, it must be noted that certain plants which produce excellent compost are not reliable from the technical and health-sanitary points of view.

This makes it difficult to speak about the costs of investment and running of composting plants.

It is therefore necessary to refer to one type of plant which is satisfactory from the technical point of view and from the quality of the product point of view. This narrows the choice to 1 or 2 composting processes in commerce.

With this precision, it is possible to estimate the investment cost:

- |                               |  |
|-------------------------------|--|
| - 15, 000, 000 Lit /t/day (°) | for composting plant with incineration of screen residue |
| - 10, 000, 000 Lit/t/day (°)  | for simple composting plant                              |

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(°) Lit. per ton/day of capacity installed

For the running cost respectively:

- 7,000 ÷ 8,000 Lit/t (o)      including amortization, proceeds excluded
- 5,500 ÷ 6,500 Lit/t (o)      including amortization, proceeds excluded

The presumed proceeds which could come from the sale of the compost (which is about 50% of the material treated) are:

$$(300 \div 400) \times 5 = 1,500 \div 2,000 \text{ Lit/t (o)}$$

This would then cause a reduction of the running costs of about 20 ÷ 25% in the first case  
about 25 ÷ 30% in the second case:

A further reduction of the cost is made by the recovery of ferrous materials which is generally carried out in composting plants.

The selling price of compost mentioned in the preceding calculations is that which is found in the industrial plants to which references are made.

But what is the real value of compost?

Several systems have been proposed in order to estimate a fairly correct selling price.

They are based on the content of organic substances and fertilizing elements (N, P, K) present in the product.

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(o) Lit per ton of treated refuse

- According to a first hypothesis (o), the value of compost would be given by:

$$C = 2(\%N_2 \times C_{N_2} + \%K_2O \times C_{K_2O} + \%P_2O_5 \times C_{P_2O_5})$$

where

$C_{N_2}$ ,  $C_{K_2O}$ ,  $C_{P_2O_5}$  are market prices of  $N_2$ ,  $K_2O$ ,  $P_2O_5$ .

Therefore the effect of the organic substances is assumed to be equivalent to the content of fertilizing element.

- According to another hypothesis (oo), its value is given by:

$$C = \%Organ. Subs. \times C_{SO} + \%N_2 \times C_{N_2} + \%K_2O \times C_{K_2O} + \%P_2O_5 \times C_{P_2O_5}$$

where

$C_{SO}$  is the cost of the organic substance. In order to determine it, the suggestion is made of referring to the cost of one "known substitution" such as stable dung, keeping in mind the relative efficiency of the two products (compost-dung).

It is clear that the value which is obtained largely depends on the value which is given to the organic substance which is the predominating percentage.

From this, the margin in discussion which these criteria leave.

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(o) Marimpietri, Experimental station of the Ministry of Agriculture and Forestry.

(oo) A. Barilli, "Agriculture and organic fertilizers from bio-conversion" Terra e Vita, No. 24, 12 - 19 June 1976

For example, there is a certain perplexity in the fact that the 'type' of organic substance is not taken into account. In fact, the agronomical value of organic substance with high humus content is very much different from that which has none.

However, by applying the second formula, an estimate can be made of 150 - 550 Lit/q for compost from town refuse, with 50% wt humidity.

1. 5. 2 The plant for composting from poplar tree bark carried out by the CARTIERE BURGO has the following costs:

|                   |   |
|-------------------|---|
| - Design capacity | 200,000 q/year  |
| - Investment      | 400,000,000 Lit. referred to year<br>of construction (1974)<br>(700÷ 800,000,000 Lit estimated actual<br>value) |
| - Running cost    | 2,200 Lit/q (amortization excluded)   |
| - Total cost      | 2,400 - 2,500 Lit/q (amortization in-<br>cluded)  |
| - Sale proceeds   | 3,500 Lit/q   |

The plant therefore has assets.

### 1.6. Competitive processes

Due to the lack of organic substances in the soil in Italy, competitive products to compost from refuse do not exist in sufficient quantities, not even if all the refuse were to be transformed into compost.

It was underlined in Chap. 1.1 that the addition of organic substances to the soil can be carried out by:

- town refuse and sludge
- animal refuse
- products with organic vegetable base
- products with organic animal base.

The quantities of animal manure, which are considerable, are not sufficient to cover the need of organic substances.

Where the products with organic vegetable base are concerned, such as peat, Italy already imports this from abroad.

On the other hand, the use of some of the industrial-agricultural refuse (poplar bark, wood pulp, sludge from the paper industry, etc.) to produce compost is not sufficient to solve the problem (\*).

The same can be said for the products with organic animal base (slaughtering scraps, etc.).

On the contrary, alternative treatment processes other than composting exist which can in certain situations divert the refuse from this from of the re-use.

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(\*) For example, the bark, waste wood pulp, sludge from paper industries concern an overall 300,000 t/year while requirements are in the region of millions of tons/year.

1.6.1. Alternative treatments of town refuse to composting

Due to the reasons mentioned above, and since most of the animal manure in some way or another returns to the soil, the analysis will be limited to only the town refuse or those industrial refuse with similar characteristics.

When a municipality finds itself in need to solve the problem of the elimination of refuse, the following technologies are actually possible:

- landfill, sanitary landfill
- trituration with dumping
- pressing with dumping
- incineration with or without heat recovery
- pyrolysis
- recycling
- composting

The characteristics of these methods are well-known and therefore their description is left out. Here, some of their aspects in comparison with composting should be underlined.

Landfill is the most economic system both from the investment cost and running costs point of view.

By this method, the refuse is buried in a place (the dumping ground) with all the value of the substances it contains.

However, the adoptability of this method is tied to the existence of suitable areas at reasonable distances.

to the places of production of the refuse; with becomes increasingly more difficult.

Trituration and pressing are also burial systems of the refuse with all their contests (\*). They require less space than dumping; they have investment and running cost comparable with those of composting.

Incineration, whether it is with the recovery of heat or not, is generally more costly both from the investment and running cost point of view than that of composting.

It has the advantage of being the most tested from the technological point of view and perhaps the most satisfactory from the health-sanitary point of view. The recovery of heat which is carried out in some large plants is really a "low yield" recovery.

For the Pyrolysis more or less the same may be said: however it is a technology up to day non-enough tested.

Recycling, in the policy of recovering refuse, represents a more "advanced" stage than composting. While the latter really only recovers one single species, compost, recycling tends to give a value to various products from the substances contained in the refuse.

The technologies applied on industrial scale in Italy allow the recovery of ferrous materials, paper, fodder for animals, compost.

The fodder is produced from the putrescible organic part of refuse.

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(\*) For pressing actually forms of re-use of the blocks are being proposed which is limited and in any case can help save on construction or filling material.

From one ton of refuse having the following composition:

|                     |      |
|---------------------|------|
| paper, carton, wood | 30 % |
| organic substances  | 45 % |
| metals              | 3 %  |
| glass               | 5 %  |
| plastic             | 5 %  |
| various             | 12 % |

the following can be obtained:

|                        |   |           |   |             |
|------------------------|---|-----------|---|-------------|
| 100 kg paper           | x | 35 lit/kg | = | 3,500 Lit/t |
| 40 kg fodder           | x | 50 lit/kg | = | 2,000 "     |
| 30 kg ferrous material | x | 50 lit/kg | = | 1,500 "     |
| 200 kg compost         | x | 3 lit/kg  | = | 600 "       |
|                        |   |           |   | <hr/>       |
| total                  |   |           |   | 7,600 Lit/t |

From a composting plant, from the same refuse, the following can be obtained:

|                        |   |              |   |                   |
|------------------------|---|--------------|---|-------------------|
| 500 kg compost         | x | 3 - 4 lit/kg | = | 1,500-2000 Lit/t  |
| 30 kg ferrous material | x | 50 lit/kg    | = | 1,500 "           |
|                        |   |              |   | <hr/>             |
| total                  |   |              |   | 3,000 -3500 Lit/t |

The recycling plants have greater investment costs: they are in fact more complex; but they have lower running costs on account of value of recovered substances.

However, they can be adopted for large plant capacities and they have many more problems concerning the commercialization of their products than the compost plants with the difficulties which this implies.

1.6.2. Other processes of production of organic fertilizers

There are numerous companies in Italy which produce organic fertilizers from slaughtering scraps and the tanning industry, such as: hoofs and horns, leather, bones, blood, flesh scraps, hair, hides, wool, etc.

In general, these are small industries and they use thermic treatments or of acid hydrolysis (with  $H_2SO_4$ ).

The product obtained is generally put on the market at a price of 4,000 to 7,000 Lit/q.

The doses for use which are advised are of a few q/ha.

### 1.7 Research proposals

In putting forward proposals for research in this sector it seems useful to indicate the points which emerge during this present study.

- In Italy, the content of organic substances in the soil is often to be found below fertile levels.
- The organic substances available in order to reintegrate the soil (zootechnical refuse, agricultural-forestry refuse, etc) are not sufficient to re-establish the equilibrium nor are they to be found available at reasonable distances from the soil which requires them most.
- In many regions, especially those in the south and islands, the only way of suitably reintegrating the organic substance is tied to the possibility of producing compost from town refuse and sludge.
- In Italy, numerous composting plants exist. However, few of them give an acceptable product. In no case is the quality of the product guaranteed.

The standardization of some parameters should be reached, such as:

- C/N ratio
  - content of organic substance
  - content of humic substance
  - granulometry
  - absence of pathogenic germs or germinable seeds
  - content of toxic metals (heavy metals)
- Plants which treat sludge and town refuse at the same time do not exist in Italy. This possibility would resolve in a single plant, two of the most serious pollution problems which the municipalities are called upon to deal with.

- The few systems which exist abroad for the composting of sludge and refuse either do not treat all the sludge produced by a municipality with the refuse or have such high costs and technological difficulties that widespread use is hindered.
- Some research programmes are being carried out in Italy on the composting of sludge and refuse for which the necessary experimentation on industrial scale has not yet been carried out.

#### 1.7.1 Pilot plant for composting sludge and solid town refuse

This proposal concerns the experiments on prototype scale of the results of research carried out at laboratory scale on the composting of solid refuse and sludge.

It is based on the following:

- starting up of a simple technological process with restricted investment costs also suitable for small and average municipalities for the composting of solid town refuse and sludge.
- In particular, a solution to the problem of wet content of the sludge should be found as well as the joint optimal treatment of two non-homogeneous phases (sludge and refuse) and the use of inoculation and the most suitable forms. All this should be done with the aim of reducing the holding time and size of the plant.
- Should the product result as having good characteristics, agronomical experiment would then be unnecessary.

For this kind of experiment, a two-year time limit would be necessary with the overall use of 8,000 hour/man.

To this expense, the cost of the plant, the modifications or integrations which might be necessary during the course of the experiment would have to be added.

1. 7. 2 Composting of agricultural, industrial, zootechnical sludge and refuse

This research applies to refuse of various characteristics and therefore probably require various forms of treatment.

It can be based on a series of actions and therefore on many small sectoral researches aiming to improve the characteristics of the product.

For example, in the case of poultry manure transformation takes place out in the open, creating unpleasant conditions for those running the plant or those who live near it.

An experiment of fermentation in a reactor could probably bring about substantial improvement in the quality of the plant.

A research programme on the optimal treatment with lower energy consumption to reduce the wet content of the final product or in the raw materials, etc. also appears to be interesting.

1. 7. 3 Commercializing of the final product

In order to have a widespread use of the composting processes, it is thought that the Municipalities should not have to deal with problems concerning the disposal (particularly serious in the case of large plants) of the product.

Action with this aim should be taken and a system developed for the commercializing of the compost once its quality has been ascertained.

## 2. ANAEROBIC DIGESTION

### 2.1. Introduction

The import of petroleum in Italy in the year 1975 has amounted at 94.4 million tons for a value of 5,355 miliard Lit.

It represents the first item of deficit of the Italian trade balance.

This is in spite of the notable decrease of the imported quantity which has been registered as compared to the preceding years. In 1972, the importation was 119.5 million tons for a value of 1,430 miliard Lit. Of this, 87.1 was absorbed by the internal market: the item relative to fuel oil represented about 43% (main item).

These figures show the importance of the development, the diffusion of processes which tend to recovery energy and the adoption of processes with low energy consumption.

Still referring to 1972, the electrical energy produced in Italy was 135 miliard Kwh.

Of this, 70% was produced by ENEL and 30% by self-producers ( "Municipalized Undertakings", large plants, etc. ).

The origins of this energy result for the part produced by ENEL:

|                              | 1972<br>-----                            |
|------------------------------|--|
| - Hydroelectric power plants | 30%                                      |
| - Geothermic power plants    | 2 + 3%                                   |
| - Nuclear power plant        | 3 + 4 %                                  |
| - Thermoelectric power plant | rest of the requirements<br>(63 + 65 %). |

In the same year the energetic deficit (import-export) resulted:

|             |                     |
|-------------|---------------------|
| Petroleum   | 94,443 ( $10^3$ t)  |
| Natural gas | 8,696 ( $10^6$ Nmc) |
| Coal        | 12,421 ( $10^3$ t)  |

Since consumption increases while the energy produced at hydroelectric and geothermic power plants is more or less the same, an increase in the production of energy by nuclear and thermoelectric power plants should be foreseen.

According to the ENEL (o) programmes, in 1990, 40% of the energy produced in Italy will be of nuclear origin.

This programme is thought to be too optimistic by some experts if not unrealistic either because the time needed to build a nuclear power plant is long or because the structures to carry out so vast a programme do not exist.

It is worthwhile pointing out that there is a hold back concerning the nuclear power plant because of the problem of radioactive slags.

This leads to think that thermoelectrical energy will handle for a certain period yet the larger part of energy requirements.

This means a greater consumption of fuel oil, therefore petroleum. Fuel oil is mostly used for combustion in the Italian thermoelectric power plants (the use of natural gas or solid fuels is rarer). However, in Italy, there exists a market situation which does not encourage the production of electrical energy from recovery.

Law, dated 6th December 1962, no. 1643 instituted by ENEL (o) (the State Company which is occupied with the production and distribution of electrical energy) affirms

that: "the companies which produce electrical energy destined to satisfy the inherent requirements and other productive processes carried out by the company itself, are not subject to transfer "(°). This means that in Italy, it is possible to produce energy only for one's own requirements. More exactly the quantity used by the company can not be inferior to 70% of that produced.

In the case of centres with recovery which correspond to the particular technical conditions, outside distribution is not limited to 30% once authorization of the Committee of Ministers has been obtained (DPR 436 dated 4th February 1963).

Therefore, the possibilities of sale are either the transfer to the ENEL network or link to the plant which produces the energy, a new plant run by the same company or contractor which consumes the energy produced besides that autoconsumed. A form which is similar to this type is the case of "Municipalized Undertakings" which produce electrical energy from the incineration of solid town refuse and which make over the energy produced to another 'Municipalized Undertakings". To these two situations, two different sale prices of the electrical energy exist and therefore there are two different degrees of advantages of this form of recovery.

a) Sale to ENEL

As there are no previous articles concerning this, these considerations are only indicative and must undergo

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(°) i. e. nationalization

verification.

The energy available from a plant for the recovery of energy, although it could be a great amount, is really negligible as compared to the annual production by ENEL.

From this, the basic purchase price is fixed by the marginal thermic cost of the actual thermoelectric power plants.

As 200 + 250 g naphta are necessary for Kwh produced, the purchase price is linked with the price of the fuel:

$$P_e = 0.20 + 0.25 P_c$$

where:

$P_c$  = cost of fuel Lit/kg

$P_e$  = presumed price of transferring electrical energy in Lit/kwh.

b) Auto-consumption

In the case of auto-consumption, "the value" of the recovered energy is evaluated equal to the purchase price of electrical energy if it were taken from the public network and therefore 2 + 3 times superior to the probable transfer price in case a).

The anaerobic digestion processes will hardly enable a plant to produce more energy than it can consume.

However, their realization is equally important if this brings about a system of auto-sufficiency from the energy point of view. In this way, their diffusion, if it does not cause a decrease in the requirements of energy, will contribute in limiting the increase in demand and therefore outside supply.

## 2.2. Raw materials and end products

In the wastewater treatment plants, the polluting substances either in dissolved or undissolved form are removed from the wastewater.

These substances produce a sludge, which if left alone, produces a nasty acid decaying phase.

To avoid this, the sludge can undergo a decaying process in the absence of air but controlled and regulated in a slightly alkaline environment.

50% of the organic substances contained in the sludge are destroyed by this anaerobic and alkaline digestion.

The formation of a biological gas results which consists of 2/3 methane ( $\text{CH}_4$ ) and 1/3 carbon dioxide ( $\text{CO}_2$ ) and has a heating value of about 5,500 Kcal/Cu. M.

The sludge residue from the digestion process is not liable any longer to decomposition. The sludge also contains numerous nutritive substances for vegetables and can therefore be used for the formation of "humus" and improvement of the soil.

Table 9 shows the quantity and average value characteristics and composition of the various types of sludge.

Using the biological gas, heat in the boilers and energy in the combustion engines can be produced.

The calories produced or recovered can be used to heat the digester and the control room for the pasturization,

TAB. 9

QUANTITY, AVERAGE VALUE CHARACTERISTICS AND COMPOSITION OF SLUDGES

| CHARACTERISTIC                                  |                  | TYPE OF SLUDGE      |                                  |  |                                |                                |  |
|---|------------------|---------------------|----------------------------------|--|--------------------------------|--------------------------------|--|
| Definition                                      | Unit             | A<br>Primary sludge | B<br>Excess biological<br>sludge | C<br>Mixed sludge<br>(non digested)<br>(A + B) | D<br>Nearly digested<br>sludge | E<br>Well dige-<br>sted sludge | F<br>Sludge from<br>aerobic sta-<br>bilization |
| Dry matters production (TS)<br>(g/inhabitant/d) |                  | 54                  | 31                               | 85   | 55                             | 50                             | 50 ± 60  |
| TS concentration                                | % wt.            | 3 ± 5               | 0.5 ± 1.5                        | 3 ± 6  | 6 ± 10                         | 6 ± 10                         | 2 ± 5 (*)                                      |
| pH  |                  | 5.5 ± 7.0           | 6.0 ± 7.0                        | 6.5 ± 7.0                                      | circa 7.0                      | circa 7.5                      | 6.5 ± 7.0                                      |
| Organic substances                              | % wt<br>on TS    | 65 ± 75             | 65 ± 75                          | 65 ± 75  | 55 ± 60                        | 40 ± 45                        | 50 ± 60  |
| Total carbonium                                 | % wt<br>on TS    | 50 ± 60             | 50 ± 60                          | 50 ± 60  | 25 ± 35                        | 20 ± 30                        | 15 ± 20  |
| Total nitrogen                                  | % wt<br>on TS    | 2 ± 5               | 6 ± 8                            | 4 ± 6  | 2.0 ± 3.5                      | 1 ± 2                          | 2.0 ± 2.5                                      |
| Total phosphorus                                | % wt<br>on TS    | 0.6 ± 1.2           | 1.0 ± 1.4                        | 1 ± 1.2  | 0.5 ± 0.7                      | 0.5 ± 0.8                      | 1.0 ± 3.0                                      |
| Inf. heat value                                 | Kcal/Kg<br>of TS | 3500 ± 4000         | 3500 ± 4000                      | 3500 ± 4000                                    | 2700 ± 3300                    | 1500 ± 2000                    | 1800 ± 2300                                    |

(\*) Greater value for thickened sludge

thermic conditioning, drying of the sludge, or as an auxiliary in the incineration of the sludge. The energy is used for the production of the electric power for the aeration of the biological treatment plant.

In order to be able to spread the sludge as a fertilizer or improvement agent for the soil, it is necessary first of all to destroy the pathogenic bacteria without however destroying the substances which are nutritive to the plants.

This is carried out by the pasturization of the sludge (70°C, less than 0.5 at, 30 min permanence time) usually for which, the biological gas in excess supplied by the anaerobic digestion, is absolutely sufficient.

The characteristics and dehydration capacity of the sludge are improved by the anaerobic digestion in such a remarkable way that the successive treatment which has the aim of reducing the volume to a minimum - for example, the mechanical dewatering with successive drying - can be carried out in the presence of much smaller rates and therefore with smaller apparatus than if the treatment of fresh sludge without digestion had been chosen.

From the technological point of view, the anaerobic digestion can be carried out in one or two stages.

In the process with two stages, the primary digester is heated and the mass agitated by means of blowers of methane gas produced by the digestion of the sludge. The secondary digester is not heated but equipped with a gasometer to collect the methane gas.

The anaerobic digestion system is the only one to stabilize and make the sludge harmless, which produces energy. It also allows the recovery of organic substances such as fertilizers and energy in such quantities which, according to the type of plant, can cover its entire energetic need.

Tab. 10 shows the biological gas and energy which can be recovered.

The specific production of biological gas averages about  $0.85 \text{ Nm}^3$  per Kg of destroyed organic solid.

In reality, from the data given by functioning plants, vast variations of the theoretical values can be noted and a dispersion of data which demonstrate how the yield of the process depends on various specific factors.

In the supposition that anaerobic digestion is used in waste water treatment plants of all the towns in Italy with more than 10,000 inhabitants, a biological gas production of  $2.8 \div 3.6 \times 10^8 \text{ NCum/y}$  at 70%  $\text{CH}_4$  would result; that is 0.8 ÷ 1.0 % yearly Italian consumption of natural gas. (o)

Another source, even more significant, of biological gas, are the pig-sties waste effluents.

With reference to Italian swine bred on an industrial basis (about 50% of total head) an availability of  $3.4 \times 10^8 \text{ NCum/y}$  of  $\text{CH}_4$  by anaerobic digestion routes, is estimated (1.5% of national yearly consumption). (oo)

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(o) 1.6 ÷ 2.0 % of natural gas imported

(oo) 3% of natural gas imported

TAB. 10

BIOLOGICAL GAS PRODUCED AND ENERGY RECOVERED

| T of di-<br>gestion | Gas produced from various type of sludge<br>(l/inhabitant/d) |   |   |  | Energy recovered per day and 1000 inhabitants |                                 |                                 |                                 |
|---------------------|--|---|---|--|---|---------------------------------|---------------------------------|---------------------------------|
|                     | Sludge from<br>primary se-<br>dimentation                    | Sludge from<br>primary se-<br>dimentation<br>and thrick-<br>ling filter | Sludge from<br>primary se-<br>dimentation<br>and thrick-<br>ling filter<br>with recycle | Sludge from<br>primary sedi-<br>dimentation<br>and activated<br>sludge | (Gas as in<br>column 2 )<br>Kwh               | (Gas as in<br>column 3 )<br>Kwh | (Gas as in<br>column 4 )<br>Kwh | (Gas as in<br>column 5 )<br>Kwh |
| C°                  | 2  | 3   | 4   | 5  | 6   | 7                               | 8                               | 9                               |
| 10                  | 1.67   | 1.90  | 2.17  | 2.60   | 2.32  | 2.64                            | 3.02                            | 3.61                            |
| 15                  | 6.48   | 7.40  | 8.40  | 9.60   | 9.01  | 10.29                           | 11.69                           | 13.34                           |
| 20                  | 12.90  | 14.88   | 16.80   | 19.20  | 17.93   | 20.58                           | 23.35                           | 26.69                           |
| 25                  | 18.60  | 21.30   | 24.10   | 27.60  | 25.85   | 29.61                           | 33.50                           | 38.36                           |
| 30                  | 19.40  | 22.30   | 26.20   | 28.80  | 26.97   | 31.00                           | 35.03                           | 40.03                           |

### 2.3. Commercial processes

In the field of treatment of wastewater from town origin, the anaerobic digestion process is diffused and used long since.

With the energy crisis and the consequent increase in price of petroleum and therefore of petroleum products, new attention has been paid to this process.

Tab. 11 shows the plants existing in Italy and their principal characteristics.

Others are in construction : Padova, Teramo, Napoli (Acerra, Cuma, etc.) Cosenza, Lucca, Gorizia, Potenza II, etc.

Tab. 12 on the other hand, shows the use of the resulting product for these same plants.

Pasturization is not carried out in any of the cases where the sludge is used for agricultural purposes. One plant foresees in the future the treatment of sludge with the town refuse in order to produce compost. In this case, the stabilization of the sludge would be carried out in the biostabilizer.

Concerning the recovery of heat or energy, the following cases emerge:

- for some of the plants, the gas produced is not used;
- in most cases, it is used for the heating of the sludge and the keeper's house;
- two large plants (Rome) also produce electrical energy from the biological gas;
- the energy produced covers about 2/3 of that consumed by the entire wastewater treatment plant.

TAB. 11

WASTEWATER TREATMENT PLANTS WITH SLUDGE ANAEROBIC DIGESTION IN ITALY AT 1977

| Location              | Resident<br>inhabitants | inhabitants served<br>by the plant<br>or equivalent<br>inhabitants (*) | Sludge rate<br>(cm/d) (3) | Sludge water<br>content % | Digestors<br>number | Digestors<br>volume<br>(Dm <sup>3</sup> ) | Digestion<br>temp.<br>(°C) | Construction<br>year | Building Firm |
|-----------------------|-------------------------|--|---------------------------|---------------------------|---------------------|---|----------------------------|----------------------|---------------|
| Collegno (TO)         | 41,948                  | 42,000 (*)   | 40                        | 98                        | 1                   | 1500                                      | Ambient                    |                      | Panelli       |
| Rozzano (MI)          | 32,915                  | 30,000   |                           |                           |                     |   |                            |                      | Degremont     |
| Milano                | 1,732,000               |  |                           |                           |                     |   |                            |                      |               |
| Como                  | 97,996                  | 25 + 30,000  | 43                        |                           | 2                   | 500 - 250                                 | 35                         | 1967                 | Panelli       |
| Monza (MI), San Rocco | 114,327                 | 229,990 (1)  | 120                       | 93,6                      | 1                   | 2,800                                     | 33                         | 1966                 |               |
| Venezia Cavallino     | } 363,062               | 45,000 (*)   |                           |                           |                     |   |                            |                      | Panelli       |
| Venezia Carpenedo     |                         | 90,000 (*)   |                           |                           |                     |   |                            |                      | Panelli       |
| Venezia Piccole Ind.  |                         | 11,000 (*)   |                           |                           |                     |   |                            |                      | Panelli       |
| Favaro Veneto (VE)    |                         | 18,000 (*)   |                           |                           |                     |   |                            |                      | Panelli       |
| Zelarino Gazzera (VE) |                         | 18,000 (*)   |                           |                           |                     |   |                            |                      | Panelli       |
| Trento Nord           | } 91,768                | 35,000 (*)   |                           |                           | 1                   |   | 35                         |                      | Dondi         |
| Trento Sud            |                         | 35,000 (*)   |                           |                           | 1                   |   | 35                         |                      | Dondi         |

6666

(1) This plant 16 Municipalities (Partnership "Alto Lambro")

| Location         | Resident inhabitants | Inhabitants served by the plant or equivalent inhabitants (*) | Sludge rate (cm/d) (3) | Sludge water content % | Digestors number | Digestors volume (CuM) | Digestion temp. (°C) | Construction year | Building Firm     |
|------------------|----------------------|---|------------------------|------------------------|------------------|------------------------|----------------------|-------------------|-------------------|
| Trieste          | 271,879              |   |                        |                        |                  |                        |                      |                   |                   |
| Udine            | 100,794              | 65,000  | 25                     | 97                     | 2                | 2800-2200              | 19+38                | 1970              |                   |
| Comacchio (FE)   | 18,779               | 20,000 (1)  |                        |                        | 1                | 1450                   |                      |                   |                   |
| Cervia (RA)      | 23,008               | 40,000 (2)  |                        |                        | 2                |                        |                      |                   | Panelli           |
| Ravenna          | 131,928              |   |                        |                        |                  |                        |                      |                   |                   |
| Reggio Emilia    | 128,788              | 60,000  |                        |                        | 1                | 2,000                  | 35                   | 1977              | Dordi             |
| Bologna          | 490,528              |   |                        |                        |                  |                        |                      |                   | Degremont         |
| Follonica (GR)   | 16,775               | 35,000  | 80                     | 98%                    | 2                |                        | 35                   | 1972              | Panelli           |
| S. Vincenzo (LI) | 7,182                |   |                        |                        |                  |                        |                      |                   |                   |
| Aprilia (LT)     | 28,349               | 72,000 (*)  |                        |                        |                  |                        |                      |                   | Panelli           |
| Roma Nord        | 2,781,993            | 950,000   | 1597                   |                        | 2                | 2 x 7000               | 33+38                | 1976              | Italconsult/Elmco |
| Roma Ostia       |                      | 100-150,000   |                        |                        | 2                | 2 x 3000               | 35                   | 1975              | De Bartolomeis    |

| Location  | Resident<br>Inhabitants | Inhabitants served by the plant or equivalent inhabitants (*) | Sludge rate (cm/d) (3) | Sludge water content % | Digestors number | Digestors volume (CuM) | Digestion temp. (°C) | Construction year | Building Firm |
|-----------|-------------------------|---|------------------------|------------------------|------------------|------------------------|----------------------|-------------------|---------------|
| Salerno   | 155,496                 |   |                        |                        |                  |                        |                      |                   |               |
| Foggia    | 141,711                 |   |                        |                        |                  |                        |                      |                   |               |
| Bari      | 357,274                 |   | 525                    |                        |                  |                        |                      |                   |               |
| Potenza I | 56,597                  | 25,000  |                        |                        |                  |                        |                      |                   |               |

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- (1) Used only in the summer
  - (2) Summer population
  - (3) Inlet charge to digester

TAB. 12

PRODUCTS FROM ANAEROBIC DIGESTION OF WASTEWATER TREATMENT PLANTS

| Location              | Served inh. or equivalent inhabitants (*) | Sludge rate outlet of digester (CuM/d) | Outlet sludge water content % | Digested sludge utilization | Gas rate (NCuM/d) | Gas composition    | Gas utilization                                |
|-----------------------|---|--|-------------------------------|-----------------------------|-------------------|--------------------|--|
| Collegno (TO)         | 42,000 (*)                                | 33                                     | 97                            | Dumped after drying         |                   |                    | Not used                                       |
| Rozzano (MI)          | 30,000                                    |  |                               |                             |                   |                    |  |
| Milano                |   |  |                               |                             |                   |                    |  |
| Como                  | 35,000 (*)                                |  |                               |                             | 700               |                    | Sludge heating                                 |
| Monza (MI), San Rocco | 220,000                                   | 35                                     | 91,2                          | Agricultural use            | 2,000             | 70%CH <sub>4</sub> | Sludge heating and heat recovery for the plant |
| Venezia Cavallino     | 45,000 (*)                                |  |                               |                             |                   |                    |  |
| Venezia Carpenedo     | 90,000 (*)                                |  |                               |                             |                   |                    |  |
| Venezia (Picc. Ind.)  | 11,000 (*)                                |  |                               |                             |                   |                    |  |
| Favaro Veneto (VE)    | 18,000 (*)                                |  |                               |                             |                   |                    |  |
| Zelarino Gazzera (VE) | 18,000 (*)                                |  |                               |                             |                   |                    |  |

| Location         | Served inh. or equivalent inhabitants (*) | Sludge rate outlet of digester (CuM/d) | Outlet sludge water content % | Digested sludge utilization     | Gas rate (NCuM/d) | Gas composition | Gas utilization                          |
|------------------|---|--|-------------------------------|---------------------------------|-------------------|-----------------|--|
| Trento Nord      | 35,000                                    |  |                               | Dumped after drying             |                   |                 | Heating of sludge and the keeper's house |
| Trento Sud       | 35,000                                    |  |                               |                                 |                   |                 |  |
| Trieste          |   |  |                               |                                 |                   |                 |  |
| Udine            | 65,000                                    | 10 + 15                                | 92 + 94                       | Used as fertilizer after drying | 300 + 350         |                 | Heating of sludge and the keeper's house |
| Conacchio        | 20,000 (1)                                |  |                               |                                 |                   |                 | Not used                                 |
| Cervia           | 40,000                                    |  |                               |                                 |                   |                 | Sludge heating                           |
| Reggio Emilia    | 60,000                                    |  |                               | Dumped after drying             |                   |                 | Heating of sludge                        |
| Ravenna          |   |  |                               |                                 |                   |                 |  |
| Bologna          |   |  |                               |                                 |                   |                 |  |
| Follonica (GR)   | 35,000                                    | 8                                      | 50                            | Used as fertilizer after drying |                   |                 | Not used                                 |
| S. Vincenze (LI) |   |  |                               |                                 |                   |                 |  |
| Aprilia          | 72,000 (*)                                |  |                               |                                 |                   |                 |  |

Tab. 12- continued

| Location   | Served inh.<br>or equivalent<br>inhabitants<br>(*) | Sludge rate<br>outlet of di-<br>gestor<br>(CuM/d) | Outlet sludge<br>water content | Digested slud-<br>ge utilization | Gas rate<br>(NCuM/d) | Gas com-<br>position | Gas utili-<br>zation         |
|------------|--|---|--------------------------------|----------------------------------|----------------------|----------------------|------------------------------|
| Roma Nord  | 950,000  | 210   | 75                             | Incinerated                      |                      |                      | Electric power<br>production |
| Roma Ostia | 100, 150,000                                       | 110   | 92                             | Dumped                           | 4350                 |                      | Electric power<br>production |
| Salerno    | 155,496  |   |                                |                                  |                      |                      |                              |
| Foggia     | 141,711  |   |                                |                                  |                      |                      |                              |
| Bari       | 357,274  |   |                                |                                  |                      |                      |                              |
| Potenza 1  | 56,597   |   |                                | Dumped                           |                      |                      | Utilized by the<br>plant     |

1) Used only in the summer

#### 2.4. Status of R & D

The projects of research and development in the field of anaerobic digestion are looking towards the treatment of wastewater from pig-sties and some manufacturing industries.

##### 2.4.1. DONDI pilot plant for the anaerobic digestion of pig-sty wastewater

The DONDI Company of ROVIGC experimented years ago on pilot plant scale, a plant for the anaerobic digestion of pig-sty wastewater.

The plant was carried out near a pig breeding farm of 3 - 4,000 animals.

The economical evaluations following experimentation were not judged to be positive at that time and so for the treatment of wastewater, a conventional wastewater treatment was used without recovery of any kind.

##### 2.4.2. ENI/SNAM PROGETTI research on the anaerobic digestion of pig-sty wastewater

The ENI (\*) to which the Italian State has assigned the task for providing raw materials necessary to cover the energy demand of the Country, started an experimental research in September 1975 for the treatment of wastewater from pig-sties by anaerobic digestion with the production of methane.

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(\*) Ente Nazionale Idrocarburi

The research is being carried out by SNAM PROGETTI, a company forming part of the ENI Group. The research should last five years.

A feasibility study was started to obtain methane and fertilizer from such refuse (excreta and urine) to be used in loco. This demonstrated the possibility of operating with pig-sties with a number of about 10,000 animals.

The laboratory experiments at Monterotondo were begun at the beginning of 1976 and the initial material was taken from a pig breeding farm near Ceprano (Rome). This farm breeds 12,000 pigs of an average weight of 50 kg each. It is also equipped with an adequate plant for the treatment of refuse, an abattoir and a fodder plant.

An experimental pilot plant designed for 10,000 animals will be installed at Ceprano during the course of 1977.

It is estimated that each head will produce 93-94 Ncu/y of biogas with 70-80% CH<sub>4</sub> and 0.14 t/y of fertilizer with ratio N<sub>2</sub>/K<sub>2</sub>O/P<sub>2</sub>O<sub>5</sub> = 6.5/1.4/5.5.

#### 2.4.3. TECNECO research on the treatment of wastewater from distilleries using anaerobic digestion

In 1976, an experimental study was carried out by the TECNECO on the possibilities of treating wastewater coming from the processing of fruit for the distillation of alcohol, on laboratory scale.

The study had the aim of investigating the possibility of biological treatment of the wastewater in question not by using the traditional methods and the possibility of recovery of valuable by-products. The study was based on the following themes:

- a) research on the possibility of recovering yeast biomass;
- b) research on the possibility of treatment using anaerobic digestion (recovery methane).

This chapter deals with the second theme of the study. Information on the first one is given in the chapter dealing with the production of fodder.

The distilleries belong to a group of industries which exploit fermentation processes for the production of ethylic alcohol using organic compound of cellulosic (woody substances, graminaceous plants) or amidaceous (potatoes, hops, wheat, corn) or sugary type (beets, sugar-cane, grapes, apples, figs).

In all three cases, the process which produces ethylic alcohol is fundamentally the same only though when cellulose or amidaceous substances are used, a preliminary hydrolysis is necessary (saccharification).

Fermentation of the sugary must is caused by the action of yeast (generally *Saccharomyces cerevisiae*) which secretes enzymes which transform the sugars into  $\text{CO}_2$  and alcohol in 36 + 40 hours or more and produce a "wine" with an alcohol content of about 4 + 10%.

The extraction of the alcohol from the "wine" is carried out continuously by plate -columns, usually three . The treatment disposal of the residue from the distillation process (non volatile impurities ) was the aim of the research.

The residue then underwent a process of anaerobic digestion.

It is a process which is carried out in the absence of oxygen and concerns three stages of fermentation:

- a) hydrolytic phase in which the organic substances are transformed into more simple and assimilable substances by the effect of the extra-cellular enzymes;
- b) acid fermentation, in which these simple substances are transformed into volatile acids (acetic acid, propionic acid, butirric) by facultative anaerobic bacteria (producers of acid);
- c) methanic fermentation in which these intermediary acid products are transformed into gaseous final products (methane, carbon dioxide...) by a group of very specialized and sensitive obliged anaerobic bacteria which are slow in their reproduction.

All this not only causes the stabilization of the waste but also produces small quantities of sludge and gas with a high calorific potential.

The process is possible within a wide range of temperatures but most of the studies carried out showed that there are two optimal points, one at 35°C and the other at about 55°C.

The experiments were carried out in the range of mesophil digestion (35°C).

The characteristics of the charge used (apple distillation residue) are:

|                                |        |
|--------------------------------|--------|
| - pH                           | 3.6    |
| - COD (mg/l)                   | 47,814 |
| - BOD <sub>5</sub> "           | 15,035 |
| - BOD <sub>20</sub> "          | 26,835 |
| - Sugars (g/l glucose)         | 14.00  |
| - Suspended solids 105°C (g/l) | 18.04  |
| - Suspended solids 600°C "     | 0.38   |
| - Residue 105°C "              | 33.18  |
| - Total N (mg/l)               | 42.80  |

The results obtained relative to the digestion phase can be summarized as follows:

- a) good levels of COD removal were obtained (>94%, better for the BOD) when applying volumetric charges in the order of 1.4 + 1.6 g COD/l x d (\*);
- b) the production of gas was generally rather scarce in the range of 1 + 2 l/d.

The methane was between 54 and 55% vol. of the sum CH<sub>4</sub> + CO<sub>2</sub>, beside small quantities of N<sub>2</sub>, H<sub>2</sub>, O<sub>2</sub>, H<sub>2</sub>S (the inferior calorific power should be about 5,500 + 6,000 Kcal/cu m.). In steady state a volumetric gas/alimentation ratio was normally obtained of about 6 + 7 and, in reference to the elimination of the COD, the specific quantities of gas are around 150 l/kg of removed COD.

The effluent coming from the digestion underwent a treatment of biological degradation with activated sludge because

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(\*) l of fermenter

of its polluting residue.

The results obtained reveal that the treatment with activated sludge which follows the anaerobic one, gives an effluent which can be discharged into surface water according to the Italian law (tabel C of Law Merli no. 319 dated 10/5/1976).

The research has also demonstrated that it is unthinkable to develop a treatment cycle which is valid for all the distilleries because of the enormous variability of the characteristics of the wastewater from industry to industry, from season to season, between industries of different potentiality and the different initial products.

## 2. 5. Economic evaluation

The sludge treatment section of a wastewater treatment plant affects in a remarkable manner the cost of the whole plant (about 40%).

The following treatment are possible for the sludge from primary sedimentation and biological treatment (\*):

- 1) Aerobic Digestion → Mechanical dewatering → Drying
- 2) Anaerobic Digestion → " " → Drying or  
incineration
- 3) Mechanical dewatering → Incineration

Of the possible solutions, only the anaerobic digestion is capable of producing energy: the others only absorb it.

The first method is usually applied for plants which serve less than 100,000 equivalent inhabitants. They have lower investment costs but high consumption of energy.

The anaerobic digestion has higher investment costs. However, the energy recovered covers a good part of the running costs (it causes quite a reduction of the running costs of the plant of up to 50%). Theoretically, it would be able to cover all running costs (except amortization) by supplying the surplus energy outside the plant.

Only the investment cost of the digestion is about 2,800 Lit/inhab. served.

However, it must be pointed out that the recovery of energy increases the complexity of the plant and therefore also the running of it. It requires more qualified personnel.

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(\*) Other techniques, such as lagoons do not seem suitable in Italy because of the lack of suitable areas and the hot climate.



|                                    |                                    |
|------------------------------------|------------------------------------|
| - income:                          |                                    |
| - value of biogas                  | 1,600 Lg/year (10-17p/100,000 BTU) |
| - value of residue<br>(fertilizer) | 2,062 Lg/year (Lit 15/t)           |
| - credit for waste management      | 1,168 Lg/year                      |
| - total (b)                        | 4,862 Lg/year                      |
| - Profit (a-b)                     | 2,700 Lg/year                      |

## 2.6. Competitive processes

With the actual situation of energy in Italy, competitive processes do not exist which produce fuel or energy sufficient enough for the energy requirements.

As an example, it has been reported that if all the town refuse produced in Italy were to be eliminated in incineration plants with the recovery of electrical energy, the quantity which would be produced in this way would not be greater than 2 - 3% of that produced globally (and therefore required).

Therefore, the only worthwhile limit is fixed by the fact that the cost of the products of the recovery process must not be greater than that of the same products on the market.

In the case of anaerobic digestion, the cost of the biological gas produced must not be greater than the cost of methane which is available.

The actual price of methane is between 64 - 67 Lit/ Ncu. m. with a heat value of 9,200 Kcal/Ncu. m.

Therefore, keeping in mind that the heat value of biological gas is 5,500 Kcal/Ncu. m., its cost of production must be less than 38.5 - 40.5 Lit/Ncu. m.

## 2.7 Research proposals

Particularly interesting are the waste treatment systems by means of anaerobic digestion, above all for countries like Italy where there is scarce energy resource.

Furthermore, these systems can permit economising of the running costs as compared to other purification systems.

The case mentioned concerning piggery waste (p. 2.5) is very significant as even a profit has been estimated.

It is not realistic to expect that their widespread use will bring about a reduction of energy requirements, but rather contribute to control the increase as industrial development grows.

The fields, where anaerobic digestion could permit greater turnovers (o) for the quantities being considered, are the waste from piggeries and the sludge from water treatment eventually treated with the solid town refuse.

Intervention in the case of industrial waste with high BOD is more complex and fractional as each requires a special experimental programme and taken singly they are less important quantitatively.

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(o) Turnover here is explained by the following ration:

$$\frac{\text{results obtained (Lit)}}{\text{cost of research (Lit)}}$$

### 2. 7. 1 Anaerobic digestion of piggery waste

Several research programmes and experiments seem to be useful in this sector for the diffusion of the process.

A) Experiments in prototype plants of the results obtained on laboratory scale and in particular for the choice of type of reactor according to the size and type of stock-farm (systems in batches, in continuous, by contact or "film reactor", "plug flow digester" type).

B) Study and application of the products obtained by anaerobic digestion.

In particular, for the intensive breeding farm, it is estimated that once from the biogas has been detracted the biogas absorbed by the methanization plants and that necessary for the energy requirements of the stock farm, there would remain about 40% of methane produced which could be used for:

- the air conditioning and central heating of the stock-farm buildings
- production of electricity and heating of water.

The water could be used in agriculture for hydroponic cultures or for the preparation of mashes for the animals

- for traction (included in cylinders for the use of farm machinery).

C) Modifications and experiments so as to be able to adopt the methanization process on existing plants which use purification processes other than anaerobic digestion.

### 2.7.2 Anaerobic digestion of solid town refuse

Anaerobic digestion should be pointed out as being amongst the new systems for the treatment of solid refuse. There are two products:

- a) a combustible gas
- b) a solid residue which can be used as a fertilizer or fodder after suitable treatment.

Pre-treatment necessary for certain processes increases the number of extractable products (ferreous materials).

By this means, the refuse can be treated with the sludge from purification plants and so the municipalities may resolve both problems in one single plant.

To date, there are no plants or research programmes being carried out dealing with this case in Italy. Experiments are mainly mentioned in North America.

This system of treatment is part of the field of the systems which recover energy and with which it must, however, be compared.

The main problems which should be studied concern:

- the effect of the variability of the composition of the refuse
- the eventual presence of toxic substances in the digested residue.

Since this sector is still little known, it seems reasonable to carry out a research programme in two phases:

**Ist Phase** : Concerns the further research of the state of the technological art and the success of the research; the carrying out of a feasibility study of an industrial plant; the defining of specific

projects or suitable studies (o).

IIInd Phase : Concerns the carrying out of the experimental research projects which have been define and justified by the study carried out in the first phase, on pilot plant scale; determining of the yield, characteristics of the products, costs, ecc.

### 2. 7. 3 Anaerobic digestion of liquid waste with high BOD

This proposal concerns experiments on a prototype plant of the treatment of waste with high BOD with suitable biological filters or contact systems. In them, the methanogenic microbic floia grows on the surface of the solid structures which fill the lume of the reactor and the waste is metabolized by contact during the passage.

The methane produced could cover the running cost of the plant.

However, the system must be experimented for the various types of waste for which optimal conditions will be singled out for the different functions.

There is news from Sweden that an industrial process of this kind has already been started up.

The proposed research then would serve a) to verify how the process can be adopted in different conditions; b) to increase the technological independence from abroad.

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(o) Obviously this phase can be reduced or even eliminated if these elements appear in reports made by other Countries taking part in this present study.

### 3. CARBOHYDRATE HYDROLYSIS

#### 3.1 Introduction

Every day, enormous quantities of potential resources contained in solid town refuse, in industrial and agricultural refuse are wasted.

The cost of extracting and preparing the recovery material for production was, in the past, greater than the cost of the "natural" raw materials.

The situation has, however, changed both because of the increase in the cost of raw materials and because of the perfecting of the technology of recovery and re-use.

Cellulose is the most abundant organic material and world production is estimated as being about  $10^{11}$  tons/year. 40-60% of solid house and town refuse consists of cellulose materials and large quantities of cellulosic waste are produced by industries and agriculture. The transformation of this waste product (apart from helping to solve environmental problems) may contribute to the energy economy (ethanol) and food economy (biomass).

The materials from cellulosic waste represent an important source for the production of SCP.

Hydrolysis to convert the cellulose in sugar represents the first stage and greatly influences the economical factor of the entire process.

In the case hydrolysis is made to SCP recovery, data, experiences, etc. are reported in chapter 4. ("Protein Recovery").

### 3.2 Raw materials and end products

Amongst the organic materials to be found in refuse, substances such as pectines, starches, sugars exist which are readily assimilated by micro-organisms.

There are also resistant substances such as cellulose, emicellulose, lignin which are metabolized by highly specialized microbic forms.

Table 13 has grouped the carbohydrate materials both in the form of vegetable, agricultural or forestry residues and in the form of industrial by-products, but which can be used by micro-organisms as nutrient substratum.

Table 14 shows the final products of these metabolic activities.

Tab. 13 Carbohydrate materials potentially useful for fermentation and hydrolysis processes

Cellulosic refuse

- straw from wheat
- straw from rice
- straw from rye, oats and barley
- rice husks
- corn stalks
- paper selected from town refuse
- residue from wood working
- solid residue from paper industry (bark, wood paste, thickened sludge)
- forestry residue

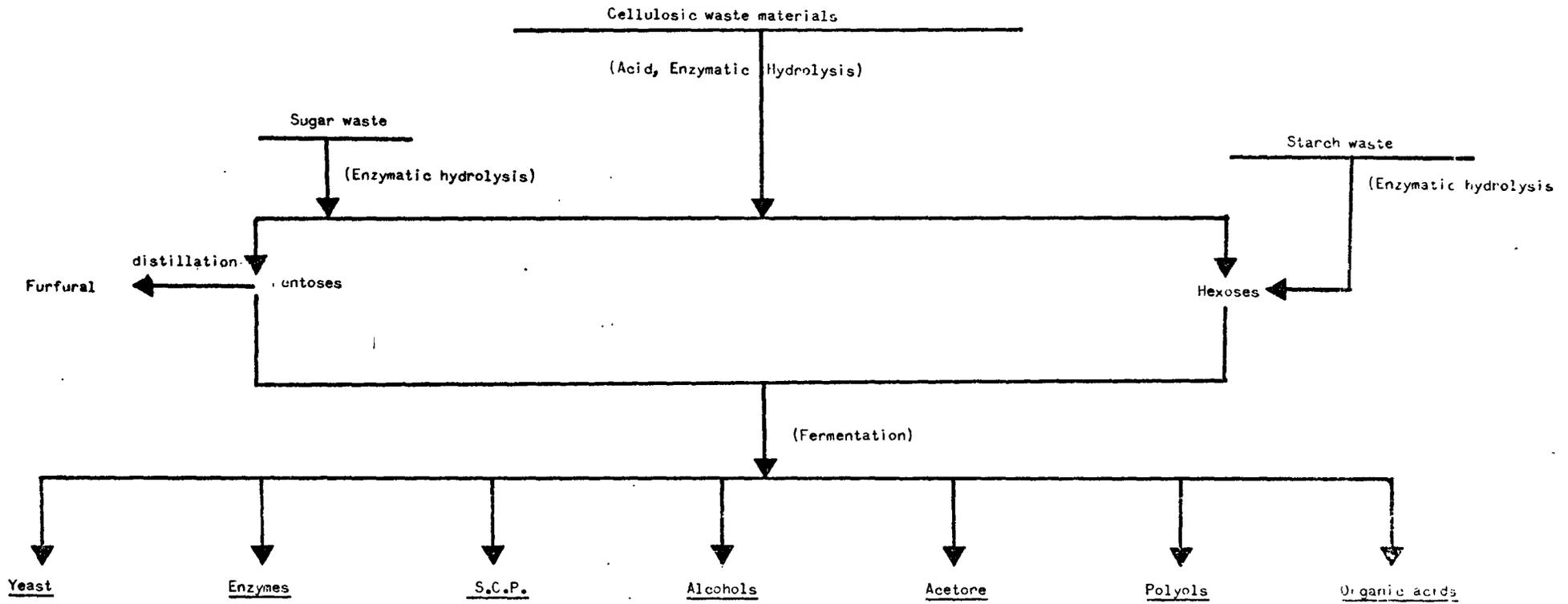
Refuse and sugary by-products

- molasses from sugar-beets
- milk whey
- waste water from the cellulose industry with bi-sulphite process

Starchy waste

- leaves
- seeds
- roots

TABLE 14 - PRODUCTS FROM HYDROLYSIS AND FERMENTATION PROCESSES OF CARBOHYDRATE WASTE MATERIALS



### 3.2.1 Hydrolysis of cellulose, emicellulose, lignin

Cellulose is the most abundant organic material and is formed by polimeric chains of B-D-glucopiranosose re-united by glucosidic links.

The breaking down of cellulose occurs in nature by means of bacteria, actinomycetes, fungi, mixomycetes and protozoa. Within each systematic group, the cellulolysis is limited to a small number of species and strains and such enzymatic activity has never been observed in any yeast origin.

Some kinds of active bacteria are: *Vibrio*, *Cellisibrio*, *Cellulomonas*, *Bacillus* and *Clostridium*.

The actinomycetes install themselves at once in very high numbers on the material which is to be broken down but because of their slow development, they only have very limited activity.

The role of fungi in decomposition of the cellulose is essential because of its high penetration ability and its dense distribution.

Amongst the cellulolytic active fungi are *Chaetomium* sp., *Acremonia* sp., *Monospora* sp., *Myrothecium* sp., *Stachybotrys* sp., *Fusarium* sp., *Aspergillus* sp..

The enzyme which attacks the cellulose is an esoenzyme. Heating at 70°C for 120' and in the presence of substratum, it causes a loss of 40 to 100% of the enzymatic activity.

Recently, it has been observed that the activity of the cellulolytic enzymes increases remarkably by adding small doses of proteic substances to the substratum.

Furthermore, the enzymatic activity registers an optimum at pH between 5 and 7 and at temperature between 55 and 60°C.

The cellulolytic activity also varies in function of the quantitative of substances associated with cellulose such as emicellulose, lignin, pectines.

The decomposition of the emicellulose is a slow phenomenon and is begun by bacteria of the *Azotobacter* genus to be continued by the intervention of Fungus of the *Penicillium*, *Aspergillus*, *Trichoderma*, *Humicola* genus.

These same microbes, capable of hydrolyzing the emicellulose to hexoses (glucose, levulose, mannose, galactose) and pentoses (xilose and arabinose), generally determine the further oxidating decomposition of the sugar produced.

Lignin is a substance having a heavy molecular weight which is contained in the region of 15-20% in straw and up to 30% in wood. It is also very resistant to microbic attack.

*Polyporus versicolor*, *Armillariella mellea*, *Polyporus adustus* are among the lignolytic fungus.

From cellulosic and woody materials suitably triturated and chopped, it is possible to obtain sugary must after warm treatment with acid.

The fermentation of these sugary materials is generally carried out by *Saccharomyces cerevisiae* strains and *Torulopsis* sp.

The fermentation can be increased if sown with fungi of *Fusarium* genus capable of fermenting the pentoses.

3.2.2 Hydrolysis of carbohydrates contained in sugar masts

It is an enzymatic hydrolysis which according to conditions of humidity, aeration and temperature, catalyzes the fermentation reactions to produce yeasts or other SCP, alcohol, organic acids, solvents.

3.2.3 Hydrolysis of starch

Starch is present in leaves especially of the dicotyledons. It accumulates in the storing organs (roots, tubers, rhizomes, seeds and fruits) and is present in the tissues which cover the surface layer of the soil.

The hydrolytic separation of the starch is catalyzed by amylase and gives maltose as end product.

### 3.3 Commercial processes

3.3.1 At this present moment, there are no commercial processes in Italy, which have the aim of recovering cellulosic waste using polysaccharide hydrolysis techniques.

This situation seems to be in contrast with the fact that the raw material necessary is produced in great quantities.

The motives seem to be that for distribution reasons part of the material is unavailable.

On the other hand, it has already found profitable use.

Last but not least, the aspect of pollution should be considered.

The material in question is not excessively polluting as it returns in very short periods of time to the natural cycle; so there is no imposition for treatment due to the law.

3.3.2 There are numerous industrial plants which use molasses from sugar beets as raw material in fermentation processes (o). Molasses is the syrup left over in the industry of the production of sugar and from which saccharose can no longer be crystallized because of the presence of non-sugary substances.

However, molasses cannot be classified as a residue of the sugar industry as it can be immediately placed in the food industry and is used for the production of alcohol, yeasts, citric acid or glutamic acid.

It is really a by-product.

The left over pulp from sugar beets, still in reference to the sugar industry, can also be considered such as it is used as fodder.

---

(o) Amongst the sugar industries in Italy, over and above sugar, four produce alcohol and yeast and two produce yeast.

### 3.4 Status of R & D

#### 3.4.1 SNAM PROGETTI research on enzymatic transformation of cellulose waste materials.

Research has been started at the Microbiological Process Laboratory Centre of the Snamprogetti of Monterotondo (Rome) with the aim of developing a process of enzymatic transformation of cellulose.

#### - Raw materials

##### a) Straw

In Italy, according to data published by ISTAT, the availability and consumption of wheat straw relative to 1975 can be summarized as follows (\*);

|  |                        |
|--|------------------------|
| - production of wheat                          | 9,440,000 tons/year    |
| - corresponding quantity of straw              | 7,552,000 "            |
| - quantity of straw used in zootechny          | 2,633,000 "<br>(34.9%) |
| - quantity of straw used in the paper industry | 596,000 "<br>(7.9%)    |
| - quantity of residue straw                    | 4,323,000 "<br>(57.2%) |

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(\*) Triolo "Straw: availability, consumption, perspectives", IVth Aticelca Assembly, Florence 28-30th October 1976.

It must be kept in mind that 1,000,000 tons of straw from rye, oats and hops, 4,000,000 tons of corn cobs and 1,000,000 tons of straw from rice could be available yearly in Italy.

b) Paper selected from refuse

In Rome, there are actually three plants in function for the recycling of town refuse which are able to recover 50,000 tons yearly of paper. A fourth plant has recently been started-up in Perugia.

c) Residue from the working of wood

These materials are not very available as a use for them has been found in the wood shaving industry and in the specific case of cork, in making insulating panels.

- End products

Biomass (yeasts) and/or ethanol.

- Status of the research

Laboratory experiments were carried out to optimize the production of cellulose enzyme from fungal strains (*Trichoderma* sp.) isolated in the countryside around Rome and cultivated on a natural medium (filter paper) and containing cellulose as an inductor.

To shorten the long lag phase, small quantities of glucose were added.

The influence of various inductors of the enzymatic mechanism was studied (carbon, nitrogen sources, the influences of sugars, organic acids).

A selection of mutants with high enzyme productivity was made.

Studies concerning the influence of pH on the single components of the enzymatic system have to be completed before going on to the development of the pilot plant.

### 3.4.2 Research on "Use of alternative fuels"

Within the Energetics project, sub-project "Traction", the CNR (o) has commissioned research concerning the "use of alternative fuels".

The study group charged with this is composed of experts from the following companies and organizations:

- Snamprogetti (ENI group)
- Agip (ENI group)
- Esso
- Alfa Romeo
- University of Bari
- University of Bologna

The work began in 1976 and should be finished in 1978.

Feasibility studies for the production of ethanol from waste as raw materials form part of this research.

More precisely from:

---

(o) Consiglio Nazionale delle Ricerche

|                       |  |
|-----------------------|--|
| cellulosic substances | { Urban waste<br>Agricultural waste          |
| sugary substances     | { Industrial waste<br>Cultivated sugar-beets |

The work which is to be carried out by 1977 concerns the estimate of the availability of the initial substance and therefore the quantities of ethanol obtainable.

Work in 1978 should, on the other hand, study the technical-economical factors of the adoptable processes with the aim of estimating their economical value.

Experiments have not been foreseen, not even for the study of enzymatic hydrolysis which does not exist either at industrial or at semi-scale levels.

Tab. 15 - SUMMARY OF CARBOHYDRATE HYDROLYSIS R&D PROCESSES

| <u>Institute/company</u> | <u>Type of fermenter</u> | <u>Substratum</u> | <u>Microorganism</u> | <u>Status</u>       |
|--------------------------|--------------------------|-------------------|----------------------|---------------------|
| Snamprogetti             | in vitro                 | cellulose         | Trichoderma s. p.    | Research            |
| CNR                      | ---                      | ---               | ---                  | Feasibility studies |

### 3.5 Economic evaluations

As there are no industrial applications of the fermentation-hydrolysis processes of waste material, it is difficult to form significant economical estimates.

Estimates are made in research programmes which are being carried out or are to be carried out.

However, it is significant to point out the cost of raw material (waste):

- Straw costs about Lit 10,000 per quintal and to this price, the cost of pre-treatment should be added. Furthermore, modest transformation yields are obtained from the product (30-40% of cellulosic substratum in glucose).
- Paper selected from waste is supplied at rather a high cost (Lit 6,000 per quintal) even though, as opposed to straw, no pre-treatment is necessary before the enzymatic attack. Furthermore, this material is not very easily available as it is largely recycled in the preparation of corrugated cardboard.
- The residues from wood-working, over and above the fact that they are not easily available, are also supplied at very high cost (Lit 8,000 per quintal).

Therefore for the materials concerned, a wide market already exist.

Research programmes in this sector should go not so much into the use of materials but more into the finding of a more useful use of those existing; for example, the production of products with higher added values which could eventually be intended for exportation.

The setting up of industrial plants following research in this area seems to be a goal requiring a longer period of time than other recovery processes.

### 3.6 Competitive processes

The chapter on compost thoroughly illustrates the bioconversion processes applied to urban or agricultural refuse with the aim of obtaining more or less humyficated products to use as fertilizers in farming.

The experience of the Cartiere Burgo concerning the use of cellulosic waste from the paper industry to produce compost is very significative (para. 1. 3. 3).

Some agricultural waste (stubble, rice straw, etc.) is turned back into the ground directly as a coadjutor in the fertilization of the soil.

For example, the straw from rice is turned back into the ground in the ratio  $N_2$ /dry straw  $\simeq$  1 kg  $N_2$ /1 q dry straw.

Amongst the competitive processes, the acid hydrolysis of cellulose materials should be pointed out.

In Italy, the acid hydrolysis of rice husks to produce furfural is applied on industrial scale.

The quantities of furfural theoretically obtainable from vegetable matter are:

|                         |           |    |
|-------------------------|-----------|----|
| - husks of oats         | 22-20 %   | wt |
| - corncobs              | 22-19 %   | "  |
| - cotton seed hull bran | 20 %      | "  |
| - corn stalks           | 16.5%     | "  |
| - buckwheat hulls       | 17 %      | "  |
| - bagasse               | 17 %      | "  |
| - rice husks            | 12%       | "  |
| - flax shives           | 14 %      | "  |
| - oak tanbark           | 13%       | "  |
| - beech shavings        | 12%       | "  |
| - fir shavings          | 5.6%      | "  |
| - peanut hulls          | 12 - 11 % | "  |

The rice husks have a very light specific weight (0.1 kg/l) which creates difficulties from the economical point of view during transport.

The annual production of rice husks in Italy is 180,000 t.

This forms the 18-20% of the paddy and has the following average composition:

|           |        |
|-----------|--------|
| silica    | 20% wt |
| cellulose | 50% "  |
| ash       | 15% "  |
| pentosans | 20% "  |

The only plant exists in Lomellina at Valle Lomellina belonging to the Società Italiana Furfurolo.

It treats the husks of rice which come from the rice cultivated in that zone. The quantity treated is about 250,000 q/y.

The furfural obtained is used in the production of plastic materials, as a selective solvent in the refining of mineral oils, in the extraction of butadiene, in the separation of anthracene, phenanthrene, carbazol, as an intermediary in the preparation of nylon.

Furfural and its derivatives can also be applied in pharmaceuticals, insecticides, fungicides, herbicides and such.

Furthermore, it is the initial product for the preparation of furfuryl and tetrahydrofurfuryl alcohol.

Part of the furfural produced is exported.

The conversion of pentosans of the rice husks into furfural consists of two sections: hydrolysis of the pentosans to pentoses in the presence of mineral acids as catalyzers and the successive phase of transformation of the pentoses into furfural by dehydrating.

As catalyzer, it is possible to use any non-oxidizing mineral acid or a strong organic acid.

In this case,  $H_2SO_4$  is used, diluted in proportion of 20% related to the rice husks, in such a way that they are slightly moistened.

With over-heated steam (10 atm.  $270^{\circ}C$ ), hydrolysis is caused and furfural gas is given off.

The ash coming from the incineration of exhaust materials can find use in the refractory industry, in the preparation of prefabricated houses, in foundries.

The investment cost for a plant producing 3,000 t/y of furfural is estimated at Lit  $2.5 \times 10^9$ .

Selling price for furfural is Lit. 280/kg.

The cost of the raw material (husks) has reached Lit 10/kg.

The incidence of the cost of the raw material on the selling price is 30%.

### 3.7 Research proposals

Actually, for many cellulosic wastematerials, there are, in Italy, other forms of re-use which are different from enzymatic hydrolysis route.

Some of the raw materials seem to actually be unavailable (waste paper, straw, wood industry scraps).

For other cellulosic materials (those of agricultural-forestry origin) the question arises as wheter to consider them as refuse or useful elements for the addition of organic substances to the soil.

For this purpose, they have the advantage of being found in their place of production and may be absorbed in the whole quantity they are produced.

#### 3.7.1 Production of ethyl alcohol from town refuse

In Italy, the production of ethyl alcohol in 1973 was 1,790,000 hl anhydrous of which 27,400 for exportation.

The raw materials from which it is produced are numerous, dregs of grapes, molasses, fruit, sugar-beets, etc.

The eventual production of alcohol from refuse must find an outlet by increasing exportation unless new forms of use can be developed such as car fuel.

The production of ethyl alcohol from town refuse has interesting aspects even at the present state of knowledge.

It has been estimated that from a ton of refuse containing 60% cellulosic material, 163-164 l of alcohol can be obtained (o).

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(o) Porteous, A. : "Towards a profitable means of waste disposal"  
ASME Paper 67 WA/PID 2.

The studies being carried out in Italy indicate a production of 86.5 l per ton of treated refuse containing 35% cellulose substances.

This composition is the average one to be found in refuse produced in 33 "metropolitan" areas in Italy. 50% of the population reside in them and 10,000,000 t/y of refuse are produced. The quantity of alcohol which could be obtained would therefore be about 700,000 t/y.

The actual economical data available refer to the U.S.A. They indicate for a capacity of 250 t/d of refuse treated, the investment cost necessary is \$ 2,300,000. In the hypothesis of a selling price of \$ 0.40/US gal, there would be a profit in the running of the plant of \$ 2.50 per ton (= Lit 2,200/t).

It is a known fact that no system for the treatment of town refuse actually in use has a profit.

Probably, the actual costs are considerably higher than those reported in the study mentioned, but on the other hand, the selling price of the alcohol is also higher.

The studies being carried out in Italy do not foresee experimental research which is considered absolutely necessary in order to give a concrete evaluation of the economical factors of the process and verify its feasibility.

Given the promising prospects of the process and the levels of the research done or being carried out, it seems useful to set out a research programme along the following lines:

- definition and experimental research of the separation system of cellulosic materials from refuse to be sent to the following fermentation stage (in particular, a decision should be made as to whether separation is more convenient by wet

means or dry ones finalized to the hydrolysis process);

- experiments still on semiscale plants of the study of enzymatic fission
- definition of the process, type of apparatus and project parameters.

#### 4. PROTEIN RECOVERY

##### 4.1. Introduction

Over the last twenty years, food consumption in industrial countries has undergone remarkable transformation from the qualitative and quantitative points of view in relation to the higher wage levels, the improvement of processes of production of food, the modification of eating habits.

In Italy, there has been a sudden increase in the consumption of meat and dairy products while there has been no significant increase of fish consumption. Only for meat, consumption has gone from 23 kg per head in 1960 to 57.3 kg per head in 1974 (tab. no. 16) (\*).

Tab. 16

#### Meat consumption per head in Italy (Kg/y)

| Year  | Beef  | Pork  | Small<br>species | Total |
|-------|-------|-------|------------------|-------|
| ----- | ----- | ----- | -----            | ----- |
| 1960  | 13.4  | 5.1   | 4.6              | 23.1  |
| 1965  | 15.4  | 7.7   | 8.4              | 31.5  |
| 1970  | 24.8  | 10.7  | 13.7             | 49.2  |
| 1971  | 25.2  | 11.9  | 14.4             | 51.5  |
| 1972  | 24.3  | 12.4  | 15.7             | 52.4  |
| 1973  | 25.9  | 13.4  | 17.3             | 56.5  |
| 1974  | 24.4  | 14.6  | 18.3             | 57.3  |

(\*) Piva G., Santi E., Marica A., "New protein sources of zoo-technical use", Agricultural Faculty - University of Piacenza.

To satisfy the increasing demand on the market of protein of animal origin, remedy was made by importing above all beef with the negative repercussions of the balance of payments. It is only necessary to point out that in 1972, 7,165,000 quintals of meat were imported while the national production was 6,420,000 quintals.

On the other hand, a notable increase in the zootechnical breeding of the so called "rapid production cycle" (pig and chicken sectors) has emphasized and accentuated a dependence on foreign countries (above-all the United States) for the supply of zootechnical fodder .

In the first 10 months of 1974, the importation of flours of soja and fish by Italy was more than 4,000,000 quintals. The consumption of the E. E. C. was nearly 14 million tons, 60% of which was soya flour.

In Italy , the overall consumption of fodder was about 63,000,000 quintals. Table n.17 shows the consumption divided according to the different species.

For the reasons of technical, economical and social character, increasingly larger breeding farms have been developed in Italy which, on one hand have helped improve production but on the other, have raised quite dramatic problems of pollution.

The problems is above all serious for the large pig breeding farms because of the high water consumption and high content of polluting substances.

The treatment plants actually in function are based on bio-

Tab. 17

Consumption of fodder according to data elaborated by the Assalzo in 1974 (thousands of quintals)

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|                     |              |
|---------------------|--------------|
| Chicken sector      | 25,881       |
| Pig sector          | 23,085       |
| Adult cattle sector | 8,650        |
| Calf sector         | 3,180 (°)    |
| Others              | <u>2,729</u> |
| Total               | 63,525       |

---

(°) of which 2,872 as milk replacers.

logical processes which insure more or less the complete destruction of the organic substances contained in pig wastes but they have high running costs which cut into the budget of the zootechnical farms. According to Verrini (\*) at the current prices in 1973, a plant which guarantees the removal of 98% of polluting animal load, cuts into Lit. 21 per Kg of meat produced. Today, it is thought to be more than Lit. 50.

To meet the increasing demand for protein of animal origin, it seems necessary to turn the attention to the possibility of re-using and recycling the organic substances contained in solid town refuse, agricultural and forestry refuse, and in liquid wastewater from zootechnical breeding farms.

However, it must be made clear that in Italy, a Ministry Ordinance dated 10/5/1973 "Sanitary regulations on the Administration of animal refuse and not of whatever origin and of some products of animal origin to animals" forbids the use of animal refuse for animals feeding as it "represents a possible means of propagating infectious diseases of animals" (art.2).

Therefore, interest leans towards the refining of techniques for the production of protein not using the traditional methods but the use of microorganisms which for the biosynthesis of their cellular material, can use as a source of carbon, a series of cheap organic substratum which is normally thrown away as refuse.

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(\*) Piva, Santi, Marica, "New protein sources of zootechnical use" Agricultural Faculty, University of Piacenza.

#### 4.2 Raw materials and end products

Several organic types of waste are suitable as substratum for the production of SCP.

Where Italy is concerned, by the Ministerial Decree 10/5/73, previously mentioned (par . 4.1), the fields of interest for a possible application of recovery processes are limited to agricultural-forestry residues, household refuse, residues from the paper and food industries (both as solid residues and sludge).

Reference to data deduced from experiments in other Countries is made on the yield and characteristics of the finished products since there are no plants for the recovery of proteins by fermentation in Italy.

The yield from 1 t of dry organic substance which could be fermented, is 0.5 t of SCP of which 50% is raw protein.

Considerable variations of these average values must be expected, depending on the type of substratum and the type of process (necessary pre-treatment, type of fermenter and microorganism, separation operations and eventual purification of the finished product).

The significative micro-organisms in the production of SCP can be classified as:

- bacteria
- yeasts
- fungi
- algae

A typical process scheme foresees a section for the pretreatment and preparation of the substratum; a fermentation section; a section for the biomass separation, eventual purification, drying of the final product.

Tab. 18 MATERIALS SUITABLE FOR SCP SUBSTRATUM

| Type                             | Observation  |
|----------------------------------|--|
| Agricultural-forestry residue    |  |
| Household refuse                 | The principal substratum is the paper which it contains  |
| Sludge from wastewater treatment | The sludge of town origin is probably prohibited by law  |
| Cattle manure                    | Prohibited by law for the production of SCP              |
| Poultry manure                   | " "  |
| Pig waste                        | " "  |
| Waste from paper mill            | - Wood, sludge, etc. residues<br>- Sulphite spent liquor |
| Residues from food industry      |  |

### 4.3 Commercial processes

#### 4.3.1 Production of fodder from solid town refuse

Four industrial plants exist in Italy which use recycling as a treatment system for solid town refuse; one of the products is fodder.

There are three in Rome and one in Perugia.

They are run by the private companies which have developed the process (SARR, SORAIN, CECCHINI).

The yields of these plants are shown in Fig.10. The quantity of fodder recovered is 4% of the refuse entering the plant.

The nutritive value is 70% of that of corn. The description which follows is relative to the section on the production of fodder.

The fraction formed by the larger organic parts coming from the automatic selection of the refuse (equal to 23% of refuse, having 45% vegetable and animal organic substances) is first of all washed with water, pressed and stored in an intermediary tank. After this, it is sterilized in autoclaves for two hours at 130°C (Sanitary Regulations require at least 20 minutes at 120°C).

The longer permanence periods and the higher temperatures of sterilization modify the physical characteristics of the materials and create ideal conditions for the successive treatments.

After undergoing a de-watering process at 80+90°C which reduces the water content to 50%, the material

then goes through a hammer mill then a vibrating screen and a pneumatic suction device which liberates it from small splits of glass, and various inert bodies. At the end it is stocked in intermediary silos.

The material now contains 98% of nutritive substances and 2% of inert material.

Under great pressure and with the addition of steam, the material which is already in powder form, is pressed into pellets and stocked or put into sacks for sale.

The proceeds of the sale are around 45 Lit/kg. This type of plant can be economically adopted for large feed rates.

However, the limited number of constructors who practically operate in conditions of technological monopoly forms a negative aspect for a diffused and sure application.

As it is a fact that the above-mentioned companies in Italy do not yet supply plants turn-key, they want to enter into the running of the plant: that is, they offer a service to the Municipalities.

Really the process does not use fermentation way but only chemical-physic operations.

It is likely that fodder produced feels the effects of refuse composition variability therefore it may have non-constant characteristics.

Rather than a fodder this product is to be used as an additive for fodder preparing.

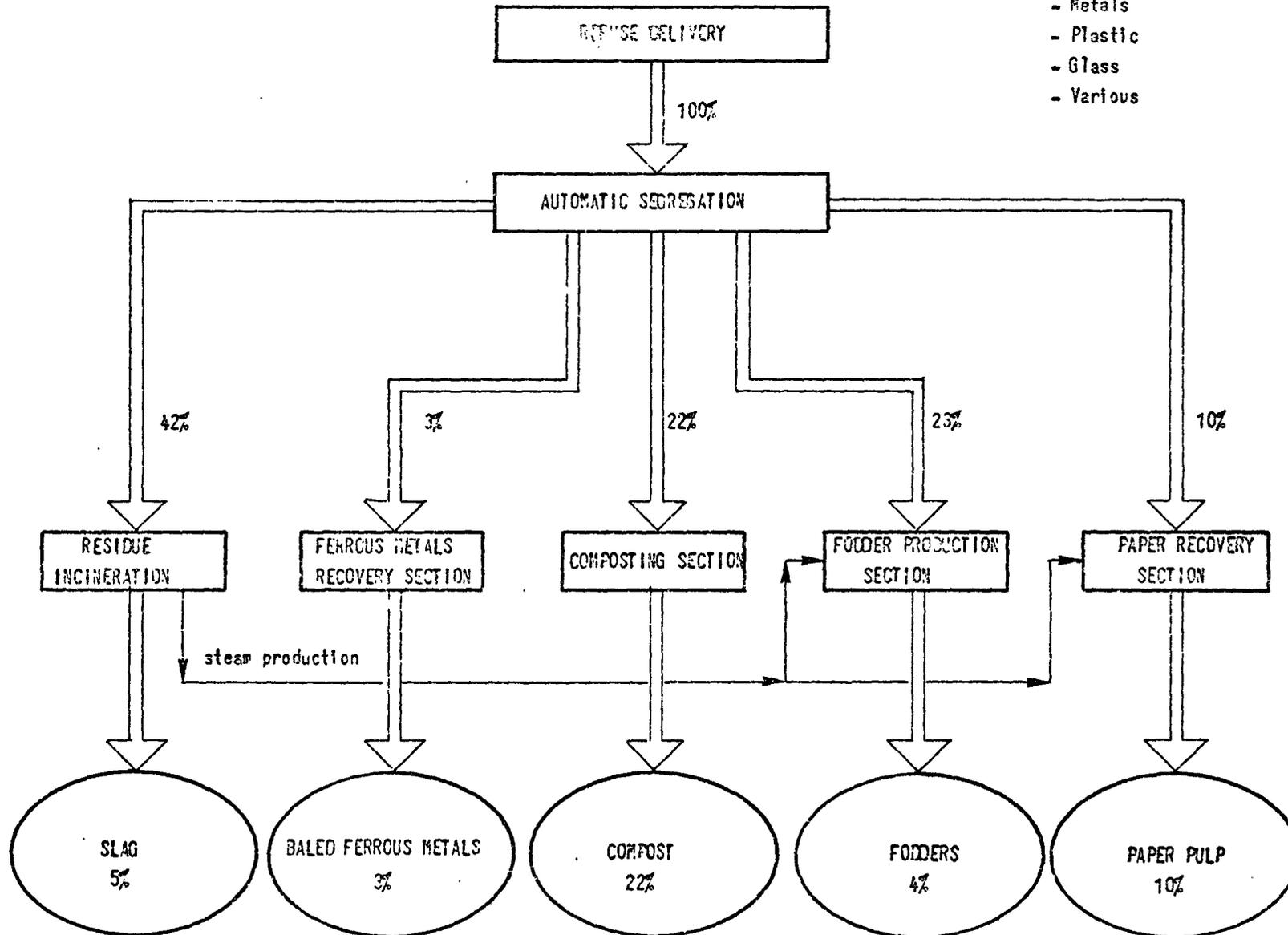
Cost data of the plant are furnished in \$ 1.6.

URBAN REFUSE

FIG.10 - RECYCLING PLANT: FLOW DIAGRAM

REFUSE COMPOSITION:

- Paper, carton, wood 30%
- Organic substances 45%
- Metals 3%
- Plastic 5%
- Glass 5%
- Various 12%



#### 4.4. Status of R & D

##### 4.4.1. Recovery of protein from pig slurry (SIBE)

A pilot plant was installed with the scope of developing and optimizing a process of recovery of proteic material from pig slurry.

The plant is able to treat the liquid waste from a breeding farm of 1,000 pigs and has been set up by the SIBE (Società Impianti Bio-Ecologica) at a farm in Valenza Po (Alessandria).

The products obtained by these biological processes of aerobic fermentation can be proposed as zootechnical foder and on two of these (residue no. 1 and 2) experiments have been carried out by the Institute of Zootechny and Science of Nutrition of the Università of Piacenza (\*).

The analytic data relative to this research are shown in Tables no. 19.

The microbic charge resulted as being quite low 30,000 germs/gram and no pathogenic micro-organisms were seen.

Toxicity experiments carried out on rats by inoculating under the ski extracts from the two products gave negative results.

Using Table n. 20 it is possible to examine and compare the aminoacid compositions of the two proteic residues with those of soya flour, Torula yeast and fish meal.

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(\*) Piva G., Santi E., Marica A, "New Proteic sources of zootechnical use", Faculty of Agriculture, University of Piacenza.

Tab. 19

Content of food principles of two samples of fermentation residue from the recycling of pig-sties liquid waste

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|                          | Residue no. 1<br>----- | Residue no. 2<br>----- |     |
|--------------------------|------------------------|------------------------|-----|
| Water                    | 4.41                   | 1.62%                  |     |
| Raw protides             | 31.33                  | 40.20%                 | ss  |
| Ether extract            | 6.13                   | 9.50%                  | ss  |
| Ash                      | 23.77                  | 10.49%                 | ss  |
| Raw fibres               | 9.49                   | 7.89%                  | ss  |
| Non-nitrogenous extracts | 29.28                  | 31.92%                 | ss  |
| Calcium                  | 4.48                   | 0.93%                  | tq  |
| Phosphorus               | 2.26                   | 1.19%                  | tq  |
| Magnesium                | 0.32                   | 0.22%                  | tq  |
| Zinc                     | 1,097                  | 377                    | ppm |
| Copper                   | 174                    | 77                     | ppm |
| Manganese                | 420                    | 227                    | ppm |
| Iron                     | 1,737                  | 745                    | ppm |
| Sodium                   | n. d.                  | 3.187                  | ppm |
| Potassium                | n. d.                  | 9.437                  | ppm |
| Chlorine                 | traces                 | traces                 |     |
| pH at dil. 1:10          | 6.85                   | 6.70                   |     |

Tab. 20

Content of essential aminoacids in some flours

|               | Soya<br>----- | Torula<br>----- | Fish<br>----- | Residue 1<br>----- | Residue 2<br>----- |
|---------------|---------------|-----------------|---------------|--------------------|--------------------|
| Lysin         | 6.22          | 6.75            | 8.00          | 5.53               | 5.67               |
| Methionine    | 1.58          | 1.49            | 3.00          | 1.51               | 2.26               |
| Cystine       | 1.10          | 1.42            | 1.00          | 0.43               | 1.60               |
| Tryptophan    | 1.56          | 1.59            | 1.10          | 0.88               | 1.18               |
| Arginine      | 7.80          | 4.40            | 6.00          | 3.44               | 3.81               |
| Histidine     | 2.75          | 2.08            | 2.50          | 1.72               | 3.23               |
| Isoleucine    | 4.46          | 4.66            | 6.00          | 4.17               | 4.56               |
| Leucine       | 7.66          | 7.06            | 7.50          | 7.42               | 7.42               |
| Phenylalanine | 5.18          | 4.76            | 4.50          | 3.83               | 4.84               |
| Tyrosine      | 3.40          | 3.21            | 3.10          | 2.62               | 3.53               |
| Threonine     | 3.94          | 4.92            | 4.00          | 3.97               | 4.92               |
| Valine        | 4.64          | 5.22            | 5.00          | 4.42               | 5.18               |

Actually, experiments are being carried out on chickens, the diet of which contains residue number 1 of fermentation in complete substitution for the Torula flour (10% of the diet).

- Pilot scale process

The residue from pig-farms collected after centrifugation of the biological sludge added to a known volume of water are sent to a tank to be converted into hydrolyzed. The hydrolyzed, after being introduced into an aerobic covered digester (thermostated at 25-30°C, equipped with agitator, automatic control of pH, with the possibility of blowing sterile air) are sowed with a symbiotic charge of micro-organisms capable of metabolizing rapidly these media. Centrifugation and biomass separation is then carried out.

- Economic evaluation

According to data supplied by the technicians of the SIBE, 14 grams of protein per litre of treated water can be obtained, for a value of 25,000 lit/quintal.

The investment cost for a plant of 60,000 pigs is 500 million Lit. For 10,000 animals, it is 200 million Lit.

Referring to a plant for 10,000 animals, it results that a plant of this kind with an expense of 300,000 Lit/d (including amortizement over 8 years) allows the recovery of protein at 750,000 Lit/d. These proteins would cause a decrease in the consumption of soya of 80% (they are mixed with a quantity of soya equal to 20%). The price of soya is 25,000 Lit/q + VAT.

4. 4. 2. Recovery of protein from cattle manure and straw stalks

In Italy, it is estimated that 97,000 tons/year of refuse (excretion and urine) from cattle breeding (\*) are available and that a potential of 7,000,000 tons/year of wheat straw remain available for zootechny (\*\*).

The recycling of manure mixed with dry forage in zootechnical breeding farms as fodder not only aids the disposal problems of this refuse, where it is not possible to use it as an organic fertilizer if the agricultural surfaces available to intensive cattle raising for meat are limited, but it also forms a material of a certain nutritive value.

In spite of the fact that the Law in Italy forbids the re-use of animal residue for fodder purposes, the Experimental Institute of Zootechny in Rome has proposed to the CNR (National Council of Research) a research subject along these lines which has been included in the finalized project "Agricultural mechanization", sub-project, "Mechanization of the harvesting, preservation distribution of forage".

The research in object proposes to optimize an ensilage process from straw stalks and cattle manure in order to obtain fodder to be used in zootechny.

The experiments will be carried out in silos and parame-

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(\*) Siniscalchi, Boschi "Management of Agricultural and Agro-Industrial Wastes", UNEP-FAO Seminar, Rome 18-21 January 1977

(\*\*) Triolo, "Straw, Availability, consumption perspectives", IXth Annual ATI CELCA Assembly, Florence 28-30th October 1976.

ters such as pH, production of lactic acid, volatile fatty acids, the proteic fraction will be very strictly controlled. The nutritive value of the ensilaged material will be estimated. Particular attention will be paid to the research for pathogenic micro-flora which could eventually be present and the toxic catabolites.

Feeding experiments with the ensilaged product will be carried out on calves from 6+ 15 months old.

At the actual moment, the optimal proportions of mixing straw and excretion are being examined as well as the eventual addition of a certain quantity of ground cereals to encourage the lactic fermentation and increase the nutritive value of the end product.

#### 4. 4. 3. Protein recovery trough fermentation routes from industrial liquid waste

##### 4.4.3.1. Oil mills

###### - Raw materials

In Italy, the average production of olives is about 2,000,000 - 2,500,000 tons/year which, on an average corresponds to 1,200,000 - 1,800,000 cu. m./y of wastewater and 100,000,000 - 200,000,000 kg/y of oxidable organic substances given a COD of 50,000 - 100,000 mg/l (BOD 40,000 - 90,000 mg/l). (see table 21)

The wastewater is of a seasonal nature.

Tab. 21

Average characteristics of technological streams  
from press plants

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|                       |                           |
|-----------------------|---------------------------|
| BOD                   | 40 + 90 g/l               |
| COD                   | 50 + 100 "                |
| pH                    | 4 + 5                     |
| Total solids at 105°C | 50 + 200 g/l              |
| Total nitrogen        | 3 "                       |
| Saponifiable esters   | 0,3 + 0,4 "               |
| Carbohydrates         | 20 + 80 "                 |
| Free organic acids    | 5 + 10 "                  |
| Ash                   | 188 g/kg of solid matters |
| Sugars                | 598 g/kg " "              |

- Process of controlled fermentation

The experiments were carried out at the Tecneco laboratories in glass fermenters of 2 l , thermostated at 30°C and equipped with agitator and air blower devices, on diluted effluents (at 25-33%), acidified (pH 3-4) added of nutrients (N) and inoculated with various yeast strains.

For initial sugary concentrations between 1.5 and 2% and a period varying from 30 to 48 hours, the experiments brought about the production of yeast biomasses of 100-160 g/l of centrifugated wet weight as well as the reduction of TOC of 30-50%.

- End products

The food value of this biomass (10 g/l dry weight, of which 45-50% are protein) is very high because of the high index of essential aminoacids (especially lysin ) and the very high vitamin content (especially of group B).

Its use (at least for man) is however limited because of the high content of nucleic acids.

4.4.3.2. Distilleries -

- Raw materials

In Italy, there are about 500 distilleries; 95%, however, are small and only 3% can really be compared to an average industry.

The wastewater is of seasonal nature, geographical-

ly scattered, high polluting charge (COD 25,000 + 50,000 mg/l and BOD 20 + 25,000 mg/l) as well as a low pH, intense colour and high temperatures and concentrations of suspended solids (see tab. 15).

- Process of controlled fermentation

The experiment was carried out at the Tecneco laboratories in flasks of 500 ml, put into shakers both for agitation and aeration and heated to 30°C, in which the diluted effluent from fruit and dregs of pressed grapes distillation were inoculated with one of the three yeast strains: *Candida utilis major*, *Candida lipolytica* and *Rhodotorula glutinis*.

This method brought to a biomass rendering of about 6 g/l of protein, and a removal of COD of 80+90% (after 7 days) from the dregs of pressed grapes.

- End products

The biomass, separated by centrifugation, has a high vitamin content (especially of group B) and is utilizable as animal fodder (or food for humans after reduction of the nucleic acid content).

TAB. 22

CHARACTERISTICS OF DISTILLERY WASTEWATERS USED IN THE EXPERIMENTS

|                              | UNIT        | FROM FRUIT<br>DISTILLATION | FROM DREGS OF<br>GRAPES DISTILLATION |
|------------------------------|-------------|----------------------------|--------------------------------------|
| pH                           | -           | 4.2                        | 4.2                                  |
| COD                          | mg/l        | 31,400                     | 24,750                               |
| BOD <sub>5</sub>             | "           | 17,100                     | 12,600                               |
| BOD <sub>20</sub>            | "           | 25,036                     | 19,630                               |
| Sugars                       | g/l glucose | 7.10                       | 12.60                                |
| Suspended solids<br>at 105°C | g/l         | 19.06                      | 26.76                                |
| Suspended solids at 600°C    | "           | 1.50                       | 1.16                                 |
| Residue at 105°C             | "           | 31.09                      | 59.59                                |
| Residue at 600°C             | "           | 3.61                       | 24.65                                |
| Sludge from centrif.         | "           | 221.00                     | 66.70                                |
| Sludge waste content         | % wt        | 94.3                       | 78.1                                 |
| Total N                      | mg/l        | 202.70                     | 152.30                               |
| Chlorides (Cl <sup>-</sup> ) |             | 123                        | 117                                  |

Tab. 23 - SUMMARY OF PROTEIN RECOVERY R & D PROCESSES

| Process                             | Fermenter Type       | Substratum            | Microorganism   | Status      |
|-------------------------------------|----------------------|-----------------------|---|-------------|
| SIBE                                | Aerobic Fermenter    | Pig slurry            | Bacteria  | Pilot-scale |
| ISTITUTO SPERIMENT. ZOOTECNICA ROMA | Silo                 | Straw + cattle manure | Bacteria  | Research    |
| TECHCO                              | Stirred tank reactor | Oil mill wastewater   | Candida utilis<br>major<br><br>Candida lipolytica<br><br>Torulopsis farata<br>Rhodotorula glutinis<br><br>Rhodotorula rubra<br>Saccharomyces fragilis | Research    |
| TECHCO                              | Shake batch reactor  | Distillery wastewater | Candida utilis<br>major<br><br>Candida lipolytica<br>Rhodotorula glutinis   | Research    |

#### 4.5 Economic evaluations

Since there is a lack of references in Italy for economic evaluations, it seems useful to mention some of the most significant conclusions of the studies or experiments carried out in other Countries.

At this present stage, it can be affirmed that the production of SCP from organic waste is technically feasible.

It can be considered economically convenient at certain conditions (marketing, cost of disposal of refuse, etc.). In every case, the plants should be large sized.

For the production of proteins from solid town refuse, according to a study by the Ionics Inc. (o), the cost of production of proteins results competitive with the price of proteins available on the market (soya, fish-meal, etc.) for plants with capacity superior to 500 t/d.

The plant includes the hydrolysis section (in which the following operations are carried out: hydrolysis, flash vaporization, neutralization, centrifugation), the fermentation section (cultivated micro-organism: Candida Utilis) and separation of the product.

The assumed cost referring to fodder at the time of the study was the equivalent of Lit. 60-100/kg (with a proteic content equal to 45-60%).

According to another more recent study (oo), the minimum economic capacity is the quantity of refuse produced by 1,500,000 inhabitants of the plant that is 1,000-1,100 t/d.

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- (o) Report to Public Health Service (contract n. pH 86-67-204) U. S. A.
  - (oo) Wolfson Laboratory-Interplan LMD: "Conversion of Organic Waste into Marketable Protein", U. K. 1974.

The corresponding quantity of SCP produced is 50,000 t/y which could be put into the market at a selling price of 80-100 Lg./t (that is Lit. 120-150/kg).

Since the actual price of soya on the Italian market is Lit. 250/kg (VAT not included) it would seem possible smaller economical dimension of the plant.

In the same study, there are estimates that for the production of SCP from town sludge; SCP could be produced at 100 Lg./t (that is Lit. 150/kg) for treatment plants which serve at least 1,5 - 2 million inhabitants.

4.6. Competitive processes

4.6.1. Recovery of protein using acid and alkaline hydrolysis processes (SICIT)

- Raw materials

The SICIT the Italian Chemical Industrial Company Chiampo (Vicenza) started, some years ago, the production and commerce of hydrolyzed proteins obtained from tanning scraps, flesh scraps, various butchers' residue.

- End products (\*)

As in tab. 24.

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(\*) Bonsembiante, Parigi, Bini, "The utilisation of protein hydrolyzed of animal origin in Milkreplacer for calves", Proceeding of II International Milkreplacer Symposium, 1972, Published by National Renderes Association, Brussels, Belgium.

Tab. 24

Chemical and nutritive characteristics  
of hydrolyzed proteins

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|               |                      |
|---------------|----------------------|
| Moisture      | 5 %                  |
| Proteic rate  | 80 + 95 % (N x 6.25) |
| Lipids        | 1 %                  |
| Ash           | 6 + 20 %             |
| Carbohydrates | absent               |
| Ca            | 1 + 5 %              |
| P             | 0.1 %                |
| Na            | 0.6 + 1.4 %          |
| Cl            | 0.2 + 1.3 %          |
| S             | 0.4 + 0.6 %          |
| Mg            | 0.02 + 0.05 %        |
| K             | 0.02 + 0.05 %        |
| Fe            | 75 + 100 ppm         |
| Cu-Zn-Mn      | traces               |

The protein of the hydrolizates has a complex content of essential aminoacids which is about half of that of milk.

The most stressed deficiency concerns threonine, methionine, cystine, valine, isoleucine and leucine, while in the proteins of the hydrolizates, only tryptophane and arginine are contained in amounts superior to those of milk.

The protein of hydrolyzates is particularly rich in aminoacids which are not essential, such as alanine, proline and glycine.

The hydrolyzed protein described above is used as a partial replacer of the protein contained in milk in the breeding of calves for white meat.

The research which led to the carrying out of industrial plants, was carried out for the SICIT at the Institute of Zootechny of the University of Padova and Florence on a total of 90 sucking calves of various breeds, all of male sex.

The controls carried out on each animal concerning the growth, consumption of powdered milk, index of food conversion meat yield, evaluation of the colour of the meat (reflect spectrophotometry), systematic control of the hemoglobin, number of leucocytes and erythrocytes, azotemia, transaminases of serum, rate of glycemia.

- Description of the process

The products mentioned are obtained by making the raw material undergo acid and alkaline hydrolysis after a sterilization treatment.

The resulting solution containing peptons, polypeptides and free aminoacids is stabilized, concentrated and de-watered using the "spray" method after elimination of undesirable mineral elements.

During the hydrolytic process, the conditions of temperature, pH, oxygenation, contact times are controlled with the aim of avoiding the formation of undesirable products which would decrease the biological and nutritive value of the product.

The hydrolyzed proteins are in a form of soluble white powder, slightly hygroscopic, free from pathogenic microorganisms.

- Economic evaluations

In Italy, according to data supplied by the Ministry of Foreign Trade, the development of breeding farms for calves for white meat has caused the importation, in 1970, of more than 1,600,000 quintals of skimmed powdered milk as well as a quantity of milk replacers which, according to estimates made by the Assalzoo, amount to about 1,700,000 quintals.

On the basis of the results of the research mentioned, it seems possible to state that the hydrolizates of animal protein from waste could contribute, at least

partially, to solve the problem of finding new and more economic sources of proteins which can substitute those contained in milk.

These hydrolyzates, even though deficient in aminoacids, are well tolerated even during the first weeks in life of a calf although they have a negative effect on its growth.

In the second fattening period, the milk replacers have allowed levels of growth to be reached which are comparable with those of the animals of control and so indicating a real possibility of lowering feeding costs.

#### 4.6.2. Utilization of dried poultry manure in zootechny feeding

Poultry litter represents an economical source of nitrogen above all for ruminants.

The great spreading of industrial fowl rearing has accentuated the problem of the removal and elimination of poultry manure, for which research and studies have been begun for some time by the Institute of Zootechny at the Universities of Florence (o) and Padova (oo) in order to evaluate the possibility of re-using

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(\*) Geri, "Alimentazione Animale", Year XII, no. 12, December 1968.

Geri, Sattini, Olivetti, extracted from "Alimentazione Animale", Year XIV, no. 5 September-October 1970.

(\*\*) Parigi, Bini, "Alimentazione Animale" year XIII, no. 5, September-October 1969.

the poultry litter in the food diets of animals.

Table 25 shows the chemical composition of various poultry manure dried and sterilized using different methods.

The experiments carried out on sheep and young pigs has underlined that such products can become part of the food mixture of the animals.

The optimal dose of poultry litter to be introduced into the mixture is yet to be determined as well as an evaluation of the economic aspects connected to this re-use.

TAB. 25

CHEMICAL COMPOSITION OF VARIOUS POULTRY MANURE SAMPLES

|  | Pure lyophilized<br>poultry manure<br>(substratum no.1) | Pure poultry ma-<br>nure dried in o-<br>ven at 70°C for<br>12 hours | Pure poultry ma-<br>nure dried in o-<br>ven at 140°C for<br>1 hour | Pure poultry ma-<br>nure dried in o-<br>ven at 100°C for<br>30 mins | Pure poultry ma-<br>nure dried in in-<br>dustrial drier<br>at 140°C for 1 h. | Pure poultry ma-<br>nure dried in in-<br>dustrial drier<br>at more than 200°C<br>for 15 mins |
|--|---|---|--|---|--|--|
| Dry substances %   | 92.38   | 94.43   | 54.30  | 93.63   | 92.34  | 93.17  |
| <u>Composition per-<br/>centage of dry<br/>substances:</u> |   |   |  |   |  |  |
| - Raw protein  | 37.40   | 38.10   | 38.33  | 38.68   | 21.46  | 21.25  |
| - Raw fats   | 1.73  | 2.00  | 2.01   | 1.86  | 2.22   | 1.31   |
| - Raw fibres   | 12.12   | 12.62   | 12.64  | 12.32   | 14.30  | 13.65  |
| - Non-nitrogenous extracts                                 | 30.69   | 28.41   | 28.51  | 28.18   | 34.68  | 28.07  |
| - Ash  | 18.06   | 18.87   | 18.51  | 18.36   | 27.32  | 35.72  |

#### 4.7 Research proposals

The contrast with the Ministry Ordinance dated 10/5/1973 concerning the initiative in Italy, of the re-use of animal residue for fodder purposes cannot go un-noticed.

Some of these researches were before the Ordinance (use of pig-slurry according to the SIBE project and use of poultry litter according to research of the Institute of Zootechny of the Universities of Padova and Florence) which however, has put a stop to the possibility of commercial development in Italy of these processes.

It is significant the fact that the research on the recovery of proteins from cattle manure presented by the Experimental Institute of Zootechny, in Rome, was carried out after the Government Ordinance.

This probably derives from the fact that this draw back of legal nature does not exist in other Countries where recovery forms of this type are applied and so demonstrate that there is a scientific value of these processes.

However, in putting forward proposals for research, the legal side of the question cannot be overlooked.

Therefore, keeping in mind this and also the quantity of refuse produced in Italy and its availability and/or distribution, the interest of the recovery of SCP, from the point of view of the authors of this study, is limited to the following type of refuse:

- agricultural-forestry refuse
- refuse and sludge from food industries
- town refuse

Numerous schemes using a variety of substrata, species, fermenters, methods of purification and separation for the recovery of proteins have been proposed.

Experiment on industrial scale is lacking.

This is all the more necessary the larger the size of the plant (required for the economical running of the processes).

It is probable that no large community, industry or combine which has the contingent problem of the disposal of residues, will consent to carry out plants of 500 t/d or more if it does not have a well-tested technology with evident results.

It is also probable that the diffusion of such processes would be more successful if they could be applied to small or middle size plants adoptable by average industries or communities.

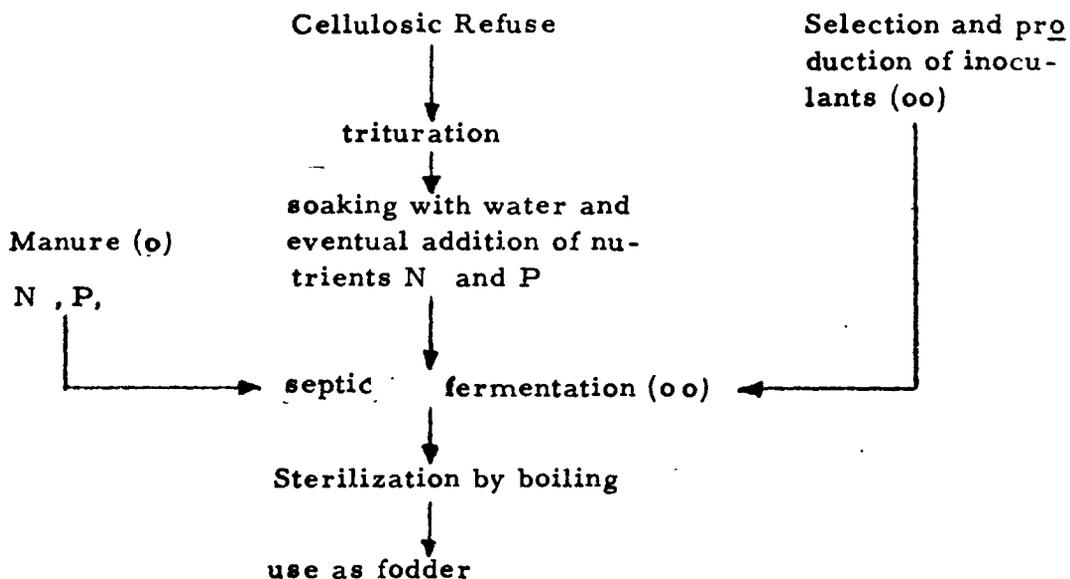
The suggested research programmes aim to give a demonstrative answer to this questions.

#### 4.7.1 Production of fodder from cellulosic waste in integrated farms

This proposal is relative to the production of fodder from cellulosic waste in farms integrated with zootechnical breeding.

The research connected with that of anaerobic digestion could bring about the formation of self supporting farms.

The production lay-out could be as follows:



(o) In the case of legal obstacles, N or P of inorganic origin can be used

(oo) Stages to be developed

In particular, there are two stages to work out:

- selection and production of a microbic flora which grows in the fermenter and competes with natural flora
- designing and carrying out of a simple technical fermenter which is automatic, has low investment costs and which requires few interventions.

An estimated sum of 500 million has been put forward for this research which is to last 4 years.

#### 4. 7. 2 Recovery of protein by fermentation from wastewater sludge

This proposal foresees the utilization of surplus sludge from biological purification plants for the production of proteins.

The principal reason for which the highest form of biological energy accumulated in activated sludge is not exploited is because of its great heterogeneity and variability which makes it unsuitable to construct a proteic food with constant and definite characteristics, in spite of its well-balanced content of aminoacids.

Since, in order to obtain an homogeneous biomass of a definite quality, besides having effluents of acceptable quality, it is necessary to conduct the biological purifications as a true industrial fermentation, it is possible, taking as example the well-known ecological food chains (activated sludge, rumen), to encourage the ingestion of bacteria responsible of the purification of the water by a ciliated protozoa, selected and inoculated in massive quantities (for examples *Tetrahymena pyriformis*, already present normally in the populations of the sludge, as in rumens).

This micro-organism has particularly interesting characteristics for its composition of proteins, nucleic acids, lipids, carbohydrates and essential aminoacids (in concentrations superior even to pork meat), as well as having a high biological value, good digestibility and high coefficient of proteic use.

The use of Protozoa (as well as those of bacteria, algae, yeasts and fungi) besides allowing a more easily controlled production than that agricultural which depends on geographic and climatic factors, brings about a higher level of production since their speed of growth and multiplication is much more superior to that of plants and animals (in the region of hours instead of months and years).

Where the economic evaluation is concerned of the exploitation of wasted activated sludge, an estimate made by Battelle Research Centre of Geneva on the costs which must be met for the two alternative of elimination, that is incineration and fermentation, for the treatment of a waste of 1,000 cu. m. /d with a BOD of 10,000 mg/l allows the conclusion to be made that the utilization of sludge is more advantageous, able to reduce the overall costs of elimination in a remarkable manner (from about 200 Lit/kg BOD removed to about 130 Lit. ).

The same mentioned process for the production of protein by protozoa from waste sludge can be directly applied to the treatment of the liquid effluents particularly those of the agro-food industry which have high pollution levels and which in general do not contain either pathogenic micro-organisms or heavy metals (whey, vegetation water from oil mills, effluent of distilleries, paper mills, etc. ).

This process scheme was used by the Battelle Research, Centre.

#### 4.7.3 Production of proteins from solid town refuse

According to the feasibility studies (para. 4.5), it seems that the production of SCP from town refuse by fermentation is convenient for quantities superior to 500 t/d.

However, given the actual price of soya, it is probable that this limit is inferior.

As for the production of ethyl alcohol from refuse (para. 3.7), this process should have a profit.

It therefore seems that a research along the following lines would be useful:

- verifying and bringing up-to-date existing studies and costs mentioned, specifying of critic parameters and of the sensitivity of the connected costs.

- experiments on industrial scale of the "critical" stages of the proposed processes.

SUMMARY OF CONTACTS ESTABLISHED

| <u>COMPANY/ORGANIZATION</u>  | <u>CONTACT(S)</u>                    | <u>VEHICLE (*)</u> |
|--|--------------------------------------|--------------------|
| 1. <u>Research Institutes, Laboratories, Universities</u>  |                                      |                    |
| Istituto di Zootechnica<br>Facoltà di Agraria<br>Università di PIACENZA                            | Prof. Piva Gianfranco<br>Prof. Silva | V                  |
| Istituto di Zootechnica<br>Facoltà di Agraria<br>Università di FIRENZE<br>Via delle Cascine, 5     | Prof. Geri                           | V                  |
| Istituto di Zootechnica<br>Facoltà di Agraria<br>Università di PADOVA                              | Prof. Bonsembiante                   | V                  |
| Scienze della produzione animale<br>Facoltà di Agraria di BOLOGNA<br>Reggio Emilia - Via Crispi, 3 | Prof. Chiappini                      | V                  |
| Stazione sperimentale per le<br>industrie degli oli e dei grassi<br>Via Colombo, 79 - MILANO       | Dott. Arpino<br>Dott. Lanzani        | V                  |
| Istituto sperimentale per la<br>zootecnia<br>Via Onofrio Panvino, 11 - ROMA                        | Prof. Malossini                      | V                  |
| Istituto sperimentale di agronomia<br>di MODENA<br>Via Caduti in Guerra, 134                       | Prof. Valentino Boschi               | V                  |
| Ente Nazionale Carta e Cellulosa<br>Centro sperimentale agricolo e<br>forestale - ROMA             | Prof. Scaramuzzi                     | T                  |
| Ente Risi<br>Centro Ricerche<br>Mortara (PV)   | Dr. Ranghino                         | T                  |

(\*) L = letter, questionnaire

T = telephone

V = visit

|  |                   |       |
|--|-------------------|-------|
| IRSA - CNR<br>ROMA   | Ing. Di Pinto     | T     |
| Istituto Nazionale per piante<br>da legno "G. Piccarolo"<br>Corso Casale 476 - TORINO                        | Dr. Jodice        | V     |
| Prodeco S.p.A.<br>Laboratorio di RAVENNA   | Dr. Fantei        | V     |
| <br>   |                   |       |
| II. <u>Category Associations</u>   |                   |       |
| Associazione Nazionale Industriali<br>Distillatori Alcool e Acqueviti<br>Via Barberini, 86 - ROMA            | Dr. Ing. Lombardi | T     |
| Associazione Nazionale Industriali<br>delle Conserve Alimentari Vegetali<br>Piazza dei Martiri, 58<br>NAPOLI | Dr. Cabib         | T - L |
| Associazione Nazionale fra gli<br>Industriali dello zucchero<br>Via Bosco, 57 - GENOVA                       |                   | T     |
| <br>   |                   |       |
| III. <u>Public Bodies, Corporations</u>  |                   |       |
| Ente Nazionale Carta e Cellulosa<br>Via Veneto - FABRIANO (AN)   | Ing. Baldo        | V     |
| Ente Risi<br>Ufficio Mercato Interno<br>MILANO   | Sig. Scarone      | T     |
| Ministero Agricoltura e Foreste<br>ROMA  | Dr. Siniscalchi   | V     |

|  |                  |   |
|--|------------------|---|
| Comune di PISTOIA<br>PISTOIA                                   | Ing. Papini      | V |
| Comune di ROMA<br>ROMA   | Ing. Rosicarelli | T |
| Comune di POTENZA<br>POTENZA                                   | Ing. L. Grimaldi | V |
| Comune di RAVENNA<br>RAVENNA - P.zza del Popolo 1              |                  | L |
| Comune di COMACCHIO<br>COMACCHIO - Via Folegatti, 5            |                  | L |
| Comune di BARI<br>BARI - C.so V. Emanuele, 84                  |                  | L |
| Comune di SALERNO<br>SALERNO - Via Roma                        |                  | L |
| Comune di TRIESTE<br>TRIESTE - P.zza dell'Unità d'Italia, 4    |                  | L |
| Comune di MILANO<br>MILANO - Piazza Scala, 2                   |                  | L |
| Comune di FOGGIA<br>FOGGIA - Corso Garibaldi                   |                  | L |
| Comune di TERAMO<br>TERAMO - Piazza Orsini                     |                  | L |
| Comune di CROTONE<br>CROTONE (CZ)                              |                  | L |
| Comune di REGGIO EMILIA<br>REGGIO EMILIA - P.zza Prampolini, 1 |                  | L |

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|--|---|
| Comune di S. VINCENZO<br>S. VINCENZO (LI) - Via B. Alliata, 4  | L |
| Comune di MONZA<br>MONZA (MI) - P.zza Trento e Trieste         | L |
| Comune di NAPOLI<br>NAPOLI - P.zza Municipio - Pal. S. Giacomo | L |
| Comune di RICCIONE<br>RICCIONE (FO) - Via V. Emanuele, 2       | L |
| Comune di UDINE<br>UDINE - Via N. Lionello                     | L |
| Comune di LUCCA<br>LUCCA - Palazzo Orsetti                     | L |
| Comune di ROZZANO<br>ROZZANO (MI) - Piazza del Comune          | L |
| Comune di CERVIA<br>CERVIA (RA) - P.zza Garibaldi, 1           | L |
| Comune di VENEZIA<br>VENEZIA - Cà Farsetti                     | L |
| Comune di APRILIA<br>APRILIA (LT) - Via Augusto, 34            | L |
| Comune di COLLEGNO<br>COLLEGNO (TO) - P.zza della Repubblica   | L |
| Comune di FALLONICA<br>FALLONICA (GR)                          | L |
| Comune di COMO<br>COMO - Via V. Emanuele II, 97                | L |
| Comune di PADOVA<br>PADOVA                                     | L |

Comune di COSENZA  
COSENZA - P.zza dei Bruzi

L

Comune di GORIZIA  
GORIZIA - P.zza del Municipio, 1

L

Comune di BOLOGNA  
BOLOGNA - P.zza Maggiore, 6

L

IV. Building firms of depollution plants

Snamprogetti S.p.A.  
Via E. Ronazini, 32  
MONTEROTONDO (Roma)

Dr. Degen  
Dr. Firriisi

V

DONDI/SIDERPOL  
Viale delle Industrie, 9  
ROVIGO

Ing. Farinatti

V

FIAT ENGINEERING  
Via Giacosa, 38 - TORINO

Ing. R. Fox

V

ENI  
P.zza E. Mattei, 1  
ROMA EUR

Dr. Forquet

V

CASTAGNETTI S.p.A.  
Via Fabbrichetta, 65  
GRUGLIASCO (TO)

Sig. G. Soave

T,L

EIMCO ENVIRONMENTAL S.p.A.  
Via Pasubio - TRIBIANO (MI)

Dr. Guerrieri

T,L

PANELLI S.p.A.  
Via G. Bruno, 29 - ALESSANDRIA

Geom. Gorrini

T,L

PASSAVANT S.p.A.  
Via D. Chiesa, 80  
NOVATE MILANESE (MI)

Ing. Moretti

T,L

|  |                                 |     |
|--|---------------------------------|-----|
| DE BARTOLOMEIS S.p.A.<br>Via Settembrini, 7<br>MILANO                            | Ing. Della Ianna                | T,L |
| ITALBA S.p.A.<br>Via Macchi, 26 - MILANO   | Ing. Paruscio                   | T,L |
| DEGREMONT ITALIA S.p.A.<br>Via Crocefisso, 27<br>MILANO                          | Ing. Gelli                      | T,L |
| ECOIMPIANTI S.p.A.<br>SENIGALLIA (AN)  | Dr. Bettini                     | T,L |
| SIBE (Soc. Impianti Bio-ecologia)<br>Azienda Agricola - Orano<br>VALENZA PO (AL) | Dr. Reverso                     | V   |
| <u>V. Manufacturing Industries (producers and/or utilizers of organic waste)</u> |                                 |     |
| ORINOCO S.p.A.<br>Via Salvini, 3 - MILANO  | Dr. Ing. U. Seni                | V   |
| Zuccherificio di ARGELATO (FO)   | Dr. Gliozzi                     | V   |
| SICIT S.p.A.<br>(Soc. Industrie Chimiche Italiane)<br>CHIAMPO (VI)               | Dr. F. Nurizzo                  | V   |
| FOMET<br>S. PIETRO DI MORUBIO (VR)   | Sig. Capperari<br>Dr. Butterini | V   |
| GLUTAMMATO<br>BOTTRIGHE (RO)   |                                 | V   |
| ITALCAMPO<br>S. AGATA B. (BR)  | Dr. Cavazza                     | V   |
| SOC. ITALIANA FURFUROLO<br>VALLE LOMELLINA (PV)                                  |                                 | T   |
| CARTIERE BURGO<br>Stabilimento di MANTOVA  | Dr. Jodice                      | V   |
| STAR<br>AGRATE BRIANZA (MI)  | Ing. Bozzano                    | T   |
| ANIC<br>S. DONATO MILANESE (MI)  | Ing. Chiarito                   | V   |
| <u>VI. Consulting Company</u>  |                                 |     |
| STICED<br>Via XX Settembre, 27<br>MILANO   | Dr. Ing. Cucchetti              | V   |



