III Utilization of Agricultural and Food Processing Wastes containing Carbohydrates

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Biological treatment of liquid effluents from food processing factories has been established for several years and is the principle on which lagooning, activated sludge, and trickling systems are based. Provided land is available, in many cases these processes are adequate and economic in removing the biochemical oxygen demand (BOD) of effluents. However, the by-product of these schemes is waste sludge, which must be transported away from factories and discharged, usually to land. Depending on the location in the U.K., cost of sludge removal may be 30—60 % of operating costs for the process, which, as land for disposal becomes less available, is likely to increase in the future. The conversion of BOD into useful sludge or other product, therefore, is attractive since disposal costs are no longer incurred and sale of the product provides a financial return which may offset at least part of the costs of effluent treatment.

Bioconversion of wastes is not confined to liquid effluents, but can be applied to solid waste materials such as straw, fruit peels, and vegetable matter. Ensilage and composting are simple, unsophisticated operations which have been practised for many years throughout the world. In contrast, in the Orient, solid fermentation processes are highly developed and established technologies used in the production of, for example, soy sauce and sake wine. Such sophisticated technology has not been exploited in the West, but there is realization that it may have applications in specific areas which include effluent treatment.

The range of products that can be produced by microbes from carbohydrates is diverse. Nevertheless the technology applied to waste treatment and utilization has been confined mainly to production of microbial biomass because fermentation and recovery of biomass is often simpler and capital costs comparatively low compared to other fermentation products. However, processes to produce fuel as methane or ethanol are becoming economically more attractive because of improvements in technology and the need for energy.

The objective in waste treatment is the removal of BOD, which in this context is assumed to be carbohydrate. Thus the objective of fermentation processes to produce biomass from waste is the total utilization of the carbohydrate component, and therefore fermentations are designed to run carbohydrate limited at dilution rates which will allow virtually complete substrate utilization. To achieve this, nutrients for biomass production must be supplied, as normally,

the effluent of itself does not act as a balanced growth medium. Oxygen, nitrogen, and phosphate are often only required but additions of trace elements such as potassium, magnesium, or iron may be necessary.

The production of microbial biomass from carbohydrate wastes[1] can be described in approximate terms by

$$\underset{2.0\text{ kg}}{C_6H_{12}O_6} + \underset{0.7\text{ kg}}{O_2} + \underset{0.1\text{ kg}}{N,P,K,Mg,S} \rightarrow \underset{1.0\text{ kg}}{\text{biomass}} + \underset{1.1\text{ kg}}{CO_2} + \underset{0.7\text{ kg}}{H_2O} + \underset{3\text{ kcals}}{\text{heat}}$$

The maximum yield constants, *i.e.* biomass produced/substrate consumed, on particular substrates are often similar for a wide range of organisms. For example, on glucose they are normally around 0.5 (Table 1). However, variations

Table 1 *Microbial yield coefficients from carbon substrates**

	Substrate		
Organism	*Glucose*	*Acetate*	*Ethanol*
Candida utilis	0.47	0.36	0.69
Saccharomyces cerevisiae	0.39—0.43		
Average for bacterial species	0.51	0.36	0.68
Pseudomonas fluorescens	0.39—0.66	0.28	0.48

*Units: g cell/g substrate

in yield can be considerable when comparing growth on different substrates or at different rates, temperatures, and other fermentation conditions.[2–7] The nutrient requirements for growth depend on the elemental composition of the cell. For example the nitrogen contents of moulds and yeasts are commonly 6—8 % of the dry weight of the cell whereas those of bacteria can be as high as 12—15 % (Table 2). Thus in balancing the medium correctly the amounts of nitrogen required will depend on the organism used.

Table 2 *Elemental composition of micro-organisms*

	Yeasts	*Bacteria*
Carbon	47 %	53 %
Hydrogen	6.5 %	8 %
Oxygen	31 %	19 %
Nitrogen	7.5 %	12 %
Ash	8 %	8 %

[1] B. D. Church, H. A. Nash, and W. Brosz, *Developments in Industrial Microbiology*, 1972, **13**, 30.
[2] A. J. Forage and R. C. Righelato, *Progress in Industrial Microbiology*, 1978, **14**, 59.
[3] A. J. Forage and R. C. Righelato, *Economic Microbiology*, 1979, **4** (in the press).
[4] W. P. Hempfling and S. E. Mainzer, *J. Bacteriology*, 1975, **123**, 1076.
[5] H. R. S. Mason and R. C. Righelato, *J. Applied Chemistry and Biotechnology*, 1976, **26**, 145.
[6] S. J. Pirt, *Proc. Royal Soc. B*, 1965, **163**, 224.
[7] H. K. Von Meyenburg, *Arch. Mikrobiol*, 1969, **66**, 289.

2 Liquid Effluent

There are several commercial processes which produce microbial protein from liquid effluents. The longest established are those using molasses, sulphite waste liquor, and whey. Also, in Russia, several processes utilize the sugars from hydrolysed wood chips and corn trash.

Although the action of bacteria, moulds, and yeasts has been the basis of foods such as yoghurts, cheese, wine, and bread, only yeasts have been used to any extent for SCP production. The yeasts commonly used, species of *Candida* and *Saccharomyces*, cannot utilize polymeric carbohydrates such as starch and cellulose unless these are hydrolysed to their component sugars. Moulds, on the other hand, are able to catabolize a wider range of carbohydrates (Table 3) and

Table 3 *Growth of fungi on single-carbon sources*

Substrate	*Candida utilis*	*Kluyveromyces fragilis*	*Aspergillus niger* M1	*Fusarium moniliforme* M4
Glucose	+	+	+	+
Sucrose	+	+	+	+
Maltose	+	−	+	+
Cellobiose	+	+	+	+
Lactose	−	+	+	+
Starch	−	−	+	+
Cellulose	−	−	−	−

have been used increasingly where there are polymeric sugars or heterogeneous mixtures of substrates. For example, the recently developed Pekilo process uses a mould, *Paecilomyces varioti*, rather than yeast to treat sulphite waste liquor. The process is claimed to be more efficient than yeast processes because the mould consumes acetic acid also present in the waste which may be particularly high in effluents from hardwood pulping.[8]

The inability of *Candida* yeast to degrade starch has been cleverly overcome in the Symba process developed by the Swedish Sugar Company.[9] The process, which treats the effluent from potato processing is based on the symbiotic culture of the yeasts *Endomycopsis fibuliger* and *C. utilis*. Effluent is fed, after nutrient addition and sterilization, to a fermenter containing the amylase-producing *Endomycopsis* strain which degrades starch to sugars. The broth containing sugars, dextrins, and *Endomycopsis* cells is then fed continuously to a second fermenter containing *Candida* which has a faster growth rate and assimilates the sugars more rapidly than the *Endomycopsis* strain. Rate limitation of the system is the hydrolysis of starch to sugars. The process, which has operated since 1973, treats 20 m^3 h^{-1} effluent and removes 90 % of the BOD producing 250 kg h^{-1} yeast.

[8] K. Forss and K. Passinen, *Paperi ja Puu*, 1976, **9**, 608.
[9] H. Skogman, in 'Food from Waste', ed. G. G. Birch, K. J. Parker, and J. T. Worgan, Applied Science Publishers, London, 1976, p. 42.

Tate and Lyle Ltd. have applied yeast fermentation technology to the treatment of confectionery effluent produced by Geo. Bassett Holdings Ltd. at their factory in Sheffield.[10] An effluent stream with COD values of about 35000 mg l^{-1} has been isolated from the remaining discharge waters which contain comparatively low COD levels. The COD is due mainly to the presence of sucrose and glucose both assimilable by *Candida utilis* (Table 4). Pilot plant work has demon-

Table 4 *Analysis of confectionery waste solids*

	% w/w
Sucrose	55
Glucose	16
Starch	22
Gelatine	3.5
Caramel	2
Organic acids	1
Coconut	0.5

strated that a continuous fermentation process operating at dilution rate 0.25—0.3 h^{-1} removes 74 % of the COD (Table 5) with, on the commercial scale, the

Table 5 *COD removal from confectionery effluent*

	COD mg l^{-1}	*% COD removal*	*COD/BOD*
Pre-fermentation	33827 ± 2548		1.4
Post-fermentation	7795 ± 1353	74 ± 4	1.7

daily production of 1.5 ton dry yeast product. The approximate fermentation materials balance was described by

34 g COD + 9.2 g O_2 → 11 g + 9 g COD + 15.4 g CO_2 + 4.7 g H_2O + 31 kcal
effluent + nutrients — yeast product (48 % crude protein) — Liquor discharged

The process will treat 140 m^3 effluent per day and will go on stream in 1979. Comparison of operating costs with an alternative effluent treatment scheme showed an economic advantage of using the process with a projected saving of over £60000 p.a.[9]

In many respects the above processes are comparatively sophisticated in terms of the equipment used and the degree of control at the fermentation step. They are run with pH, foam, and temperature control to maintain maximum efficiency.

In the last ten years or so there has been a move to develop low-technology processes. As the term implies, these processes require minimal control during

[10] A. J. Forage, *Process Biochemistry*, 1978, **13**, 8.

fermentation and as simple and cheap a plant as possible. Most low technology processes use moulds as microbes of choice because of their ability to grow on a wider range of substrates and at comparatively lower pH values than yeasts or bacteria. It is often claimed that growth at low pH values selects against contamination and thereby removes the need for sterilization which can be a significant part of the operating costs. A quoted further advantage of using moulds is that because of their filamentous form, they can be separated from broth using rotary vacuum filters rather than centrifuges, which are more efficient in dewatering, producing a cake containing less than 80 % moisture. Subsequent drying of separated biomass is therefore less costly.

An example of a low-technology process is that developed by the Denver Research Institute to treat the starchy effluent from maize corn processing at a Green Giant factory in Minnesota.[1] The process utilizes the moulds *Trichoderma viride* or *Gliocladium deliquescens*. Effluent is supplemented with phosphate and nitrogen and fed continuously without sterilization into aeration ponds or oxidation ditches containing the mould. The process is operated with a residence time of 20 h and removes 95 % of the BOD with the production of 1g l^{-1} mould.

Several other low technology processes are at the pilot plant stage of development including the Central American Research Institute process for treating coffee waste water using *Aspergillus oryzae*[11] and that developed by the Rowett Institute, Aberdeen on upgrading the protein content of barley grains used in ruminant feeding.[12] Both processes use simplified, conventionally designed fermenters with minimal control and are operated on a continuous or semi-continuous draw–fill basis.

3 Solid Wastes

In many Third World Countries large amounts of solid fruit and vegetable wastes such as the wastes from citrus and pineapple canning factories, green bananas, and those from cassava processing are dumped on land and left to rot. A significant proportion, 30—70 %, of the dry weight of these wastes is carbohydrate other than cellulose, which is readily assimilable by moulds. In some cases these materials if fed directly as is, or dried, would act as valuable energy sources to animals and occasionally they are processed as such. However, in countries which wish to build up their non-ruminant animal farming, and where the need is for protein feeds, processing of the waste by submerged or solid fermentation to produce animal feed protein could be economically feasible. Although protein yields and productivities tend to be lower, due to poor mass transfer rates experienced in fermenting solid matrices, solid substrate fermentation processes are simpler and require less energy input, particularly at the separation and drying stage, compared to the requirements for submerged culture process.

4 Solid Fermentation

Because of its particulate nature, usually only part of the assimilable carbohydrate

[11] C. Rolz, in 'SCP II', ed. S. R. Tannenbaum and D. I. C. Wang, MIT Press, Cambridge, Mass., 1975, p. 273.

[12] A. E. Reade and R. H. Smith, *J. Applied Chemistry and Biotechnology*, 1975, **25**, 785.

is immediately available to the micro-organism. An important step in the process therefore is comminution of the solid into a form which increases the availability of the substrate, yet does not render the material difficult to handle as a solid matrix. Care must be taken also to ensure that the inoculum and nutrients required for growth are evenly dispersed in the solid mass in order to obtain rapid even growth and penetration and maximum substrate utilization. The invasive growth pattern of moulds makes them the preferred microbes for solid fermentations. Their filamentous form allows them to grow into the interparticle spaces and also to invade the particles, break down their structure, and thereby utilize more substrate than yeasts or bacteria. A disadvantage is that if bulk mixing is necessary to dissipate heat and aid gas transfer, the action may shear the mycelial strands. Therefore mixing is usually carried out periodically imposing a minimum of shear.

Tate and Lyle Ltd. have applied the technology to citrus peel, the solid residue from the fruit and juice canning industry at a pilot plant in Belize, Central America. Citrus peel was minced, spread on trays, and dried to 60 % moisture. Nitrogen as urea or ammonium sulphate and phosphate was mixed with the peel and the mass inoculated with spores of the mould *Aspergillus niger*. The material was laid out on to trays and incubated at 30 °C, the ambient temperature for much of the year, for 30—36 hours after which the temperature of the incubator was increased to dry the material. The process gave a final product containing 20—25 % crude protein. The process was applied successfully to the fermentation of whole green bananas a high volume waste in that area.

Solid fermentation processes have been applied also to cassava[13] and in the treatment of animal wastes. In America animal wastes are a problem because of the size of feedlot units. Several approaches to animal waste utilization are in progress, but projects employing solid fermentation technology are investigating the ensiling of pig waste–corn mixtures and cattle feedlot waste–liquid-free grain mixtures and feeding the product back to the animals.[14] An area in which solid fermentation processes may be applicable is the treatment of the putrescible fraction of domestic waste, provided that there is adequate separation from non-desirable materials such as glass.

5 Future

The constraints on SCP production are economic rather than technical. Situations have been identified where the technology can be applied to the treatment of effluents and as methods to treat wastes these are shown to be more economical than the conventional alternate schemes. Since legislative pressure backed by higher treatment charges levied by local authorities is increasing, the market for these types of processes will grow. In parts of the world where calorific feeds are surplus to requirements and there is need for protein, fermentation of solid carbohydrate wastes could be economically viable.

[13] M. Raimbault, *Proc. of Fifth Intern. Conf. on Global Impacts of Applied Microbiology*, 21—26 Nov., 1977, Bangkok, Thailand.
[14] R. A. Rhodes and W. L. Orton, *Trans. ASAE*, 1975, **18**, 728.