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NON-CONVENTIONAL MICROBIAL FOOD
INDUSTRIAL AND ECONOMIC POSSIBILITIES
FOR CANADA

by

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#96



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The views and opinions expressed in this report are those of the authors and are not necessarily endorsed by the Department of Regional Industrial Expansion.

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1. INTRODUCTION

One practical way of improving present food production is to develop new foods from abundantly available raw materials that are presently not used for food production or are wasted. These materials may represent starchy or cellulosic plants, industrial and agricultural by-products, or bulk chemicals.

Non-conventional foods may fall into the following categories:

- a) Food materials obtained by chemical synthesis
- b) Fish and meat protein concentrates
- c) Leaf and other plant proteins
- d) Microbial or single cell proteins

There is a great potential for development of any of the above categories. This report is concerned with the category of microbial or "single cell" proteins-SCP. The work could be extended in the future to evaluate other categories as well.

To utilize microbes as a human food is not a novel idea, as man has used microbial food from ancient times. Products such as cheese, yogurt, bread, yeast spreads, pickles, soy sauce, vitamins, alcoholic beverages, to mention just a few, are familiar to all of us. Today, we are looking at microbes to produce not only the conventional food categories but also to make abundant quantities of high quality proteins badly needed for the exploding human population. They are capable of doing this in a very efficient way.

There are several outstanding advantages of microbial proteins over their agriculturally derived counterparts. These can be briefly summarized in the following:

1. Production of most of the microbial proteins is independent of agriculture, climatic conditions and high quality soil. Consequently, a plant producing single cell proteins could be erected anywhere in the world where basic energy for plant

operation and water are available. This is of particular interest to agriculturally deficient or unsuitable areas or "in situ" where a major protein deficiency exists.

2. The growth rate and consequently the mass doubling time of microorganisms is significantly in their favour over any other biological system. Microbes grow about 4000 times faster than cattle and 2000 times faster than poultry. The capability of fast production is of particular interest in stressful situations (wars, crop failures, etc.) as large quantities can be obtained in a matter of days, provided industrial capacities are available.
3. A wide variety of wastes or cheap and abundant substrates exist that can be converted into protein, combatting at the same time the widespread pollution of the environment. Molasses, waste sulfite liquors from pulp and paper mills, dairy waste, whey, brewery waste, various food industry wastes, waste gases, liquid hydrocarbons, cellulose and wood, garbage and municipal wastes can serve as microbial substrates. While many of the above substrates may not be suitable for production of SCP intended for direct human consumption, products can successfully be used for animal feeding.
4. The protein content of microorganisms is higher than that of most common foodstuffs. Fats and Carbohydrates are also present and contribute to the nutritional quality of SCP. The amino acid spectrum is comparable to recommended standards, and many microorganisms contain an excellent spectrum of essential amino acids. Sulfur-containing amino acids are generally not present in sufficient quantities.
5. Due to fast and efficient production and availability of low priced raw materials, microbial proteins may have an attractive price as compared to oilseed or other nonconventional proteins.

6. Microbes can be mutated and 'engineered' and their composition and capabilities changed to suit the requirements. This advantage opens a new and exciting era in microbial food technology. By tailoring the nutritional quality of SCP in terms of proportions of protein/fat/carbohydrate/vitamins and by increasing production rates, these 'newly engineering' microorganisms may become attractive for the food market.

Because of these advantages and other factors regarding nutritional characteristics of SCP, considerable research and development in this area is being undertaken in various parts of the world. Pilot plant and industrial operations do exist today. In most cases the product is manufactured for animal consumption rather than for direct human use. The reasons for this are primarily due to lack of sufficient information regarding all aspects of nutritional, biological and toxicological characteristics of this type of food.

1.1 NUTRITIONAL QUALITY OF SINGLE CELL PROTEINS

Significant amounts of yeast were consumed during World Wars I and II in Germany and Japan and were sold under the term food yeast. After the wars, the yeast consumption for human food decreased, but yeast is still being used as a nutritious food.

Yeast, bacteria, fungi and algae can contain 50 % and more protein, which makes these materials a natural protein concentrate. However, protein content as often reported has been determined on the basis of total nitrogen determined by the Kjeldahl method and employing a factor of 6.25 to arrive at the 'equivalent' protein content. Microorganisms, and particularly the faster growing species such as bacteria, contain a relatively high proportion of nucleic acids, and the non-protein nitrogen

acceptability?

may represent about 20 % or more of the total nitrogen.

Yeast has a broad spectrum of amino acids and is one of the richest sources of lysine. Sulfur-containing amino acids and methionine in particular are low. In general, the SCP food value is high (in some cases compared to casein), and after methionine addition it is very high. Some algae, like Spirulina, are quite attractive as a human food due to their very high protein content (up to 70 %), high digestibility and vitamins. Higher fungi such as morel mushrooms are also regarded as a valuable food, due to their quality protein, low nucleic acids and delicious flavour.

Depending on the substrate utilized for microbial growth, several nutritional effects may be imparted. Single cell proteins grown on industrial wastes may contain toxic organics or metals which have to be eliminated before any food or feed use of the derived SCP. As an example, SCP derived from pulp and paper mill waste liquors may contain lignin-sulfonates or other pulping chemicals, depending on the pulping process itself..

The nucleic acid content in tissue that is highly metabolically active, such as in microorganisms, is relatively high. It ranges from 8-25 g/100 grams of protein as compared to 4 g in liver, 2.2 g in fish and 1.1 g in wheat flour (Kharatyan, 1978). The problem with nucleic acids is that they are broken down to uric acids, whose increased levels in blood are suspected to be associated with kidney damage, bladder stones and gout formation. In producing SCP for human consumption, the nucleic acid levels have to be brought to an acceptable level. It should be noted that the enzyme uricase is present in animals but not in man. Uricase converts uric acid into the soluble and easily extractable metabolite allantoin. Therefore, high nucleic acids do not cause a problem in other animals. However, it may be the lack of uricase and high plasma levels of uric acid which play a decisive part in the superior intellectual development of man compared with other animals. There is some evidence that a positive correlation of serum uric acid and intelligence,

achievement oriented behavior, drive and range of activities exists.

There are various methods to eliminate or reduce nucleic acid levels in the final product. This can be accomplished by chemical extraction and separation of nucleic acids from the proteins or by enzymatic breakdown of nucleotides following their separation. In this way a purified protein enriched product (concentrate) can be obtained for direct human consumption.

The 'Commission on Biotechnology' of IUPAC (International Union of Pure and Applied Chemistry) has set guidelines for the required quality of microbial proteins for animal consumption (Dellweg 1980). It is clearly stated in these guidelines that no new protein source be allowed to be introduced for animal feeding before a thorough investigation is performed on the presence of toxic substances. The samples for such investigations must be derived from a fully stabilized process which assures production with full reproducibility. In this respect, reproducible quality data based on pilot plant testing must also be proven in the scaled-up production facility. The producer of SCP is required to fully disclose all data concerning the microorganisms used and the details of the process particularly as related to the safety and quality control data (e.g. organisms, substrates, nutrients, use of chemicals for foam breaking or product extraction etc.).

The commission recommends the following tests to be performed on the final product:

- a) determination of water content, lipids, proteins, fibers, ash and calorific value
- b) fats: identification and quantitative determination of fatty acids, glycerides, phospholipids and unsaponified materials
- c) nitrogen containing substances: identification and quantitative determination of total nitrogen, amino-acid nitrogen,

as well as the total profile of amino acids, purine and pyrimidine bases and the amount of DNA and RNA

- d) ash: minerals such as Na, K, Mg, P and Cl, trace elements, in particular those which are of toxicological importance (Pb, Hg and As) as well as nutritional physiological importance (Mn, Zn, Cu, Se, Co, Mo)
- e) all major carbohydrates should be qualitatively and quantitatively determined
- f) determination of all fat- and water-soluble vitamins.

Short term tests, such as 90 day tests with rats should be very useful in development stage of the production. The screening methods such as the in vitro Ames-test with the soluble part of SCP and the in vivo Dominant-Lethal-test are useful for determination of any mutagenic and potential carcinogenic effects as well conventional pathological and biochemical parameters. Assurance with proper controls must be given also to distinguish between any changes due to SCP or the 'balance of nutrients' that may be different from conventional diets administered to test animals.

At present the commission is actively involved in establishing guidelines for SCP to be used directly as human food as well. In this respect the commission is closely collaborating with the Protein Advisory Group of the WHO/FAO, IUPAC Food Commission and with a Japanese working group (Dellweg, 1980).

1.2 ACCEPTABILITY OF MICROBIAL PROTEINS

Concerning the nutritional quality of SCP, evidence based on animal feeding tests suggests that this product represents a nutritionally good and safe food. Of the organisms used, yeast and fungi seem to be most promising for human consumption. Some algae, like the blue-green Spirulina, have been eaten

by man for centuries and have shown to be of excellent nutritional quality.

One of the largest problems facing single cell production is public acceptance of this 'new' type of food. Man is generally a creature of habits, customs and prejudices, and they are particularly strong when dealing with food. People do not change eating habits, and the only way to introduce this new type of food is to use SCP to upgrade and fortify existing diets.

There are a number of possibilities of processing these proteins to fit this particular requirement. Microbial food is actually a natural product, and in many ways similar to plant proteins that populations are accustomed to eating. The acceptability problem is particularly large in areas where food is most needed. Man generally has difficulty in adapting to new situations. Take the potato for example: it took 200 years for the English to fully accept this product in their daily diets.

On a global scale, a vast majority of people in developing countries depend on diets which would be very unconventional on a European and American table. To mention just a few examples of these unfamiliar and exotic foods: tortillas in Latin America, chapatties of India, tempeh of Indonesia, mealie meal of South Africa, mague worms in Latin America, termites in Australia and Africa, locusts in the Middle East, and raw turtle eggs, frogs, monkeys, snakes, rats, grasshoppers, raw fish, raw octopus, raw chicken, etc..

On the other hand, millions of people in the world reject our familiar foods such as caviar, artichokes, truffles, limburger and roquefort cheese, snails, frog legs, whale steak, blood sausage, beef, meat in general, pork, alcoholic beverages, etc..

So the problem is not one-sided but complex and present in all human beings.

It takes time to educate any population to accept something new. We in North America have been sufficiently adjusted to accept a number of new industrial products if packaged and adver-

tised attractively. There is an increasing awareness of the chemical composition of the food we buy. This awareness is highly justified, but very often 'chemicals' are subjected to over-reacted scrutiny. Do we really always know what these ingredients are for and where they came from into the final product? Imagine a bagged or canned product listing these ingredients: carotene, anthocyanins, tangeretin, polygalacturonic acid, arginine, choline, asparagine, 3-keto-l-gulonolactone decylaldehyde, octyl alcohol, hesperidin, 2-hydroxipropanetricarboxylic acid, oxalic acid, methanol, formaldehyde, tyramine, synephrine. You would surely hesitate to have this mixture of chemicals on your table and by doing this deprive yourself and your family of the delicious taste of the Florida orange or of natural orange juice in the morning.

1.3 SOCIAL ASPECTS OF SCP UTILIZATION

One of the prime criteria to the success of single cell protein utilization is that the product must be psychologically acceptable to the consumer. While it seems that massive effort has gone into the investigation of the technical and nutritional aspects of SCP, it does not appear that a great wealth of information has been collected by the food industry in regards to the consumer acceptance of these products (Litchfield, 1977).

The force of consumer psychology must not be under-estimated. Katoh (1974) and Yanada (1976) both state that the delay of SCP production from n-alkanes in the early 1970's was imposed by the Japanese authorities as a result of consumer pressure. In addition, Rose (1979) views that the reason progress has been slow in the advent of new fermented foods is due to a tremendous built-in resistance in most countries of the world to proposals for changing the composition of their national dishes (no matter how slightly).

The three most important considerations likely to influence the introduction of SCP are, according to Foreman et al. (1974);

1. Safety or image of safety.

Apprehension among people with regards to the possible toxicity and safety of single cell proteins will create serious obstacles to utilization. For example, mushrooms that are grown on organic wastes are an acceptable commodity, however, rarely does the consumer identify these organisms or their substrates with the product they find palatable (Ritchie-Calders, 1976). To many, fungi is synonymous with decay, bacteria with disease, and algae with the green material found on stagnant ponds.

2. Government policies concerning SCP utilization.

These policies will be influenced by the desires for such things as food and/or feed self-sufficiency, a favorable trade balance, reduced dependance upon imports, stabilizing domestic food prices, as well as pressures from industrial, labour, and consumer groups.

3. Mass media communications.

Rapid and widespread education through the mass media concerning the acceptance and proven viability of SCP in one area will tend to enhance acceptance in other regions (Forman et al, 1974).

Consumer resistance may be avoided if the SCP product is used only as feed for livestock. While it may be true that most consumers do not realize what the sources of their meat products are fed, many retailers may still not risk their credibility in the event of any inadvertant publicity as to the quality of product they sell. Thus, the social pressure is not removed.

Finally, it is unfortunate that due to the public stigma within developed countries, few investors are willing to face the capital risks, long developmental lead times, and the costs of intensive promotional schemes necessary to transform SCP to

a psychologically appetizing product since it is the hungry people of the developing nations that will benefit most from this more lucrative technology (Ritchie + Calders, 1976).

The present study relates mainly to single cell protein production for animal use. Some products for direct human use (e.g. Attisholz product) are also discussed in the report.

The report comprises several sections and is basically divided into three parts:

- a) substrates and their potential
- b) selected industrial processes
- c) marketing and economics

In the first part an extensive discussion of potential substrates for SCP production is given. Emphasis was on such materials that are available in large quantity in Canada and world wide and at a low or no cost. World industrial developments are thoroughly presented and basically all available literature on the subject has been assembled.

In the second part several processes have been selected that represent SCP based on yeast algae, bacteria and fungi. Also, an attempt was made to elaborate processes based on substrates that may be of major interest for the Canadian situation such as pulp and paper mill waste liquor, methanol, agricultural waste and lignocellulosics. For any of these processes, background detailed data was lacking so that development of best estimated parameters was needed. Two groups of example processes in this part have been evaluated and presented. These are:

- i) examples of existing industrial operations such as the Attisholz and the Czechoslovak Academy of Sciences processes on pulp and paper mill waste liquors; and the Hoechst-Uhde process for production of SCP based on methanol.
- ii) elaboration of model processes for algae SCP based on poultry waste and fungal SCP based on agricultural waste. These processes are presented in detail as a preliminary design of these two alternatives using original calculations and interpretations.

The third part of the report is on marketing and economics. A general marketing strategy for the new protein product is presented. In the economic analysis, the Czechoslovak Academy of Sciences process for which a reasonable amount of data was available was considered in detail for various alternative modes. A special computer program was developed for this purpose and is fully presented in the report.

The report represents a detailed evaluation of the microbial protein production alternatives and an assessment of potential development for the Canadian environment.

2. SUBSTRATES AND THEIR POTENTIAL

2.1 SACCHARIDES

Saccharide containing feedstocks are advantageous for SCP production in that they require little preparation prior to the fermentation step other than perhaps extraction or concentration of the carbon substrate. Additional nutrients (i.e. N, P, K, trace factors) may be required to support microbial growth in some cases. These may be provided by individual compounds (i.e. $(\text{NH}_4)_2\text{SO}_4$ or KH_2PO_4) or by inexpensive complex fortifying agents such as corn steep liquor or malt.

2.1.1 MOLASSES

2.1.1.1 Sources

Originally considered a waste product of sucrose production, molasses has become an important sugar substrate for many types of fermentations. This material is primarily prepared from sugarcane (Saccharum sp.) or sugar beet feedstocks (Chenopodiaceae family).

Present global production of sugarcane exceeds 56×10^6 tpa (ECN Petrochemicals Supplement, 1979). It exhibits extremely high biomass yields of desirable composition and produces large quantities of fibrous residue (bagasse). This residue may be utilized as boiler fuel to power the unit processes of the operation. The crop may be harvested over an extended period of time due to its long growing season however, efficient cultivation is limited to tropical or semi-tropical areas.

The sugar beet is a more versatile crop than sugarcane since it can tolerate a wide range of soil and climatic conditions. This allows its growth throughout the Southern U.S., Europe, Africa, Australia, and New Zealand. Global production capacity for this crop is about 36×10^6 tpa (Faust and Präve, 1980). Additional benefits from sugar beet production arise from a high yield of

crop co-products such as beet tops and extracted pulp. The pulp is bulky and palatable which exhibits a high feed value in wet or dry form. The tops may be used for soil cover.

2.1.1.2 Processing

Cane and beet molasses are prepared in much the same manner. The new feedstock is crushed and after extraction of the juice, the inorganic component of the juice is precipitated with alkali (Prouty, 1980). After multi-effect evaporation of the juice to separate the sucrose product, molasses remains as a non-crystallizable residue. This heavy viscous material is easily stored for extended periods in a concentrated form.

2.1.1.3 Characteristics

Molasses is composed of sucrose and invert sugars, dextrose, and levulose with a total carbohydrate concentration of about 50 % (w/v). (See Table 1)

Table 1: Analysis of beet- and cane-molasses.

| Content (%) | Beet molasses | Cane molasses |
|--------------------|---------------|---------------|
| Sucrose | 48.5 | 33.4 |
| Raffinose | 1.0 | - |
| Invert | 1.0 | 21.2 |
| Ash | 10.8 | 9.8 |
| Organic non-sugars | 20.7 | 19.6 |
| Nitrogen | 1.5-2.0 | |
| Water | 18.0 | 16.0 |

(Source: Rhodes and Fletcher, 1966)

This feedstock also contains sufficient organic and inorganic nutrients such that little, if any, fortification is required prior to fermentation. Nitrogen is of great importance to microbial growth and as can be seen from Figure 1, low grade molasses will not support an efficient process due to low nitrogen content. This nitrogen exists in the form of protein, amino acids, amides, ammonium salts, nitrates, and others (Sobkowicz, 1976). Also important is the ash content as it has been found that K_2O concentrations of less than 4.2 % limit yeast growth (Malanowska and Labendzinski, 1969).

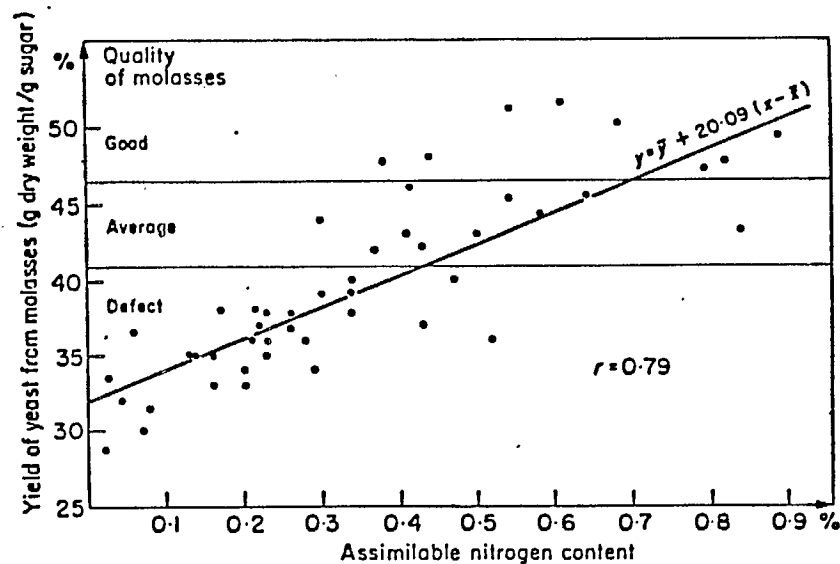


Fig. 1 Relationship between the amount of assimilable nitrogen and yield of the yeast.

(Source: Sobkowicz, 1976)

Organic acids may be formed in molasses as a result of sucrose degradation at high temperatures and pH (Schiweck and Hubert, 1973). Many of these volatile acids (formic through to valeric) are inhibitory to yeast metabolism and at sufficient concentrations may halt it completely.

2.1.2 FRUIT CROPS

A number of fruits exhibit high concentrations of fructose and sucrose. Examples are grapes, peaches, apricots, and pineapples. Though the potential of this raw material is relatively high, its use as a feedstock for microbial protein production is limited due to a significant market value for direct human consumption. However, fruits are extremely perishable by nature and use of spoiled material for SCP conversion may be economically feasible though these wastes would be highly variable in quantity and sugar content.

2.1.3 SUCCULENT CROPS

Arid and semi-arid regions constitute approximately 30 % of the total global land area (4.36×10^9 ha; Walton, 1969). These regions exhibit the lowest net annual primary productivity of all agricultural ecosystems (estimated at 0.01 - 0.025 kg/m²/yr). As such, agronomic use of these lands has, up to the present, been limited (Bassham, 1981).

Plants which display crassulacean acid metabolism (CAM) exhibit a photosynthetic system which is extremely efficient in regards to water requirements. In fact, when compared to other agricultural crops on a basis of biomass production per unit existing biomass, these succulent plants exhibit above average productivity.

CAM organisms which contain high concentrations of fermentable carbohydrates include various cactii (i.e. Agave sp., Opuntia sp.) and other plants. Productivity estimates have placed the potential of these crops at 50,000 kg/ha-yr (Nobel, 1980). Batch and continuous fermentation of various yeast strains on agave juice have been shown to be equivalent in the kinetics and cell composition as the same organisms grown on molasses and sugarcane liquor (Sánchez-Marroquin, 1977). However, the fermentation required the addition of ammonium salts or corn steep liquor.

2.1.4 INDUSTRIAL AND FOOD PROCESSING WASTES

Most often, effluents arising from various industrial and food operations require some form of treatment to minimize the

environmental impact of their disposal. This fermentation to produce microbial biomass from these feedstocks will also yield a side credit. The biological oxygen demand (BOD) of the waste stream would be reduced to a level where simple discharge of the fermented broth is possible.

However, characteristics of these waste streams are extremely disadvantageous to SCP processes. Effluents are produced on a relatively small scale and in widely scattered locations. In addition, fermentable carbohydrate concentrations are usually quite low.

2.1.4.1 Waste Sulfite Liquors

Waste sulfite liquors (WSL) constitute the aqueous streams which are produced during the pulping treatment of wood using acid bisulphite. This treatment serves to solubilize lignin and degrade hemicelluloses to their constituent pentose and hexose monomers. About 9,180 l. of WSL are generated per ton of pulp produced, which yields a yearly global production capacity of 100×10^6 metric tons of this material (Forage and Righelato, 1979).

The carbohydrate content in WSL amounts to 2-4 % which depends upon the type of wood processed and the digestion process utilized. Due to this high carbohydrate content the WSL is highly polluting ($BOD_5 \sim 50000$ ppm) if released to the environment. By growing microorganisms in this liquor the BoD can be reduced by 90 % with the added production of microbial protein.

The composition of the sugars in the liquor varies depending upon wood species and type. A representative composition of WSL from spruce is shown in Table 2.

Table 2: Chemical Composition of Organic Dry Substance in a spent Spruce Sulphite Liquor

| | % |
|--|------|
| Lignosulphonic acids | 43 |
| Hemilignin compounds | 12 |
| Incompletely hydrolysed hemicellulose compounds and uronic acids | 7 |
| Monosaccharides | |
| D-Glucose | 2.6 |
| D-Xylose | 4.6 |
| D-Mannose | 11.0 |
| D-Galactose | 2.6 |
| L-Arabinose | 0.9 |
| Acetic acid | 6 |
| Aldonic acids and substances not investigated | 10 |

(Source: Detroy and Hesselstine, 1978)

Prior to utilization of WSL, steam or aeration stripping at low pH (1.5 - 3.0) is necessary to remove SO_2 which would otherwise inhibit microbial growth. The pH is then adjusted to the desired level and the media supplemented with various nutrients or corn steep liquor. An advantage to be gained in an industrial SCP process utilizing this feedstock is the lack of sterilization requirements since feed coming directly from the digesters will have undergone 257°F - 320°F and 90-110 psi for 6 to 12 hours (Kosaric et al., 1973).

2.1.4.2 Whey

Whey is the liquid effluent generated by the cheese and casein manufacturing industries. Annual production of this material has been estimated at 74 million tons (FAO, 1974). Since the BoD_5 value of this waste can reach 60,000 - 70,000 ppm then some type of treatment is necessary prior to disposal. Conventional methods for disposal are cost prohibitive.

Negaard (1975) has estimated that for a medium sized facility, equipment necessary for BoD reduction would cost upwards of 10 million (\$U.S.). However, the suitable composition and relative purity of this waste stream would make it highly amenable for conversion to microbial biomass and subsequent BoD reduction.

Sweet whey is produced as a by-product in the manufacture of hard and soft cheeses while sour whey is generated from the production of cottage cheese. The characteristic differences between these two waste streams are shown in Table 3. These differences arise from the use of lactic acid bacteria for the production of cottage cheese.

Table 3 : Comparison of sweet and sour-whey composition.

| Components | | Composition in | |
|--------------------------|------|-------------------|------------------|
| | | sweet whey (%) | sour whey (%) |
| Dry matter | | 6-7 | 5-6 |
| Ash | | 0.5-0.7 | 0.7-0.8 |
| Crude protein of this | | | |
| genuine protein nitrogen | 52.5 | 43.9 | |
| peptide nitrogen | 31.3 | 33.1 | |
| amino-acid nitrogen | 2.5 | 6.1 | |
| creatin nitrogen | 2.6 | 2.5 | |
| ammonia nitrogen | 1.0 | 2.3 | |
| urea nitrogen | 9.1 | 10.3 | |
| purine nitrogen | 1.0 | 1.8 | |
| Lactose | | 4.5-5.0 | 3.8-4.2 |
| Lactic acid | | traces | up to 0.8 |
| Citric acid | | 0.1 | 0.1 |
| pH | | 4.5-6.7 | 3.9-4.5 |

(Source: Drews, 1975)

Pre-treatment of this feedstock involves deproteinization to reduce excessive foaming due to the presence of lactalbumin and lactoglobulin in the broth. This concentrated protein would yield an important by-product credit. The deproteinized whey could then be concentrated by evaporation for stable storage over long periods (Bechtle and Claydon, 1971).

Supplementation of the media with nitrogen, phosphorus, and vitamins is necessary prior to fermentation. A number of organisms are capable of growth on lactose (Meyrath and Bayer, 1979). The most efficient microbe in the metabolism of this sugar is Kluyveromyces fragilis. This organism is used in the Bel Fromageries Process as shown in Figure 2.

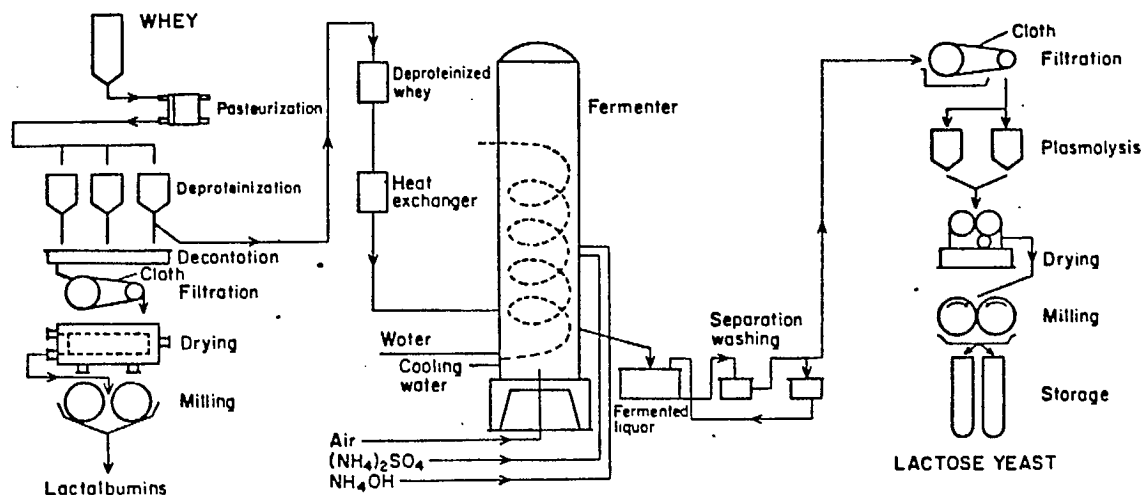


Fig. 2 : Flow diagram showing production of whey protein and *Kluyveromyces lactis* biomass in the Bel Fromageries process.

(Source: Meyrath and Bayer, 1979)

This process involves the immediate treatment of whey as it is produced from the cheese plant. Non-assimilable whey proteins are separated with a 75 % recovery and the deproteinized whey is adjusted to a lactose concentration of 3.4 %. After addition of mineral salts, fermentation of *K. fragilis* is undertaken on a continuous basis.

2.1.4.3 Peat Hydrolysates

In many European countries peat is used extensively as a fuel source. For this end use, natural wet peat must be pre-treated by the wet carbonization process to facilitate its mechanical dewatering. In the wet carbonization process, raw material is heated in a reactor to temperatures of 150°C - 540°C for 5 to 60 minutes. This increases the heating value of the peat as well as producing an extractable liquid phase as waste, which contains up to 5% total carbohydrates (mainly glucose, xylose, galactose, rhamnose, mannose, and arabinose). On an industrial scale, this waste would contribute to the overall cost of the process due to a need to lower the BoD value.

Recent work by Boa and LeDuy (1981) as well as Martin (1982) have shown peat hydrolyzates to be favourable substrates for the growth of various fungi. With the addition of ammonium, potassium, and magnesium salts, batch production of 10.4 g/l biomass has been achieved (24 % protein) with a carbohydrate reduction of 70 % (Boa and Le Duy, 1981).

2.1.4.4 Various Food Processing Wastes

Though food wastes are excellent microbial nutrients, their highly diverse nature in terms of volume and saccharide concentration makes their utilization for large scale SCP production difficult.

In general, they are characterized by a high organic content and BoD values which may range from 100 to 100,000 ppm. Canning industries usually generate BoD₅ values of 2,500 - 7,000 ppm for medium scale operations. Suspended solids may be as high as 12,000 ppm for some waste streams while others contain almost zero. The pH values are highly variable from acidic waste streams of pH 3.5 to alkaline sources of pH 11. The need for the addition of mineral or trace nutrients may or may not exist (Kosaric, 1976).

Table 4 shows some of the representative fruit and vegetable processing wastes generated in the U.S. Though the potential

of some of these waste residues seem great, factors involved in their availability may prove difficult to overcome. These factors include their highly disperse points of origin, their seasonal aspects of production (75% of total processing is completed in 4 months of each year), and extensive competition with established feed and by-product markets which provide greater cash return for producers of the waste.

Table 4 : Representative Fruit and Vegetable Processing
Wastes in the United States

| Product and Process Employed | Major Season | Residual Wet Weight tons/annum | Residual Saccharide Content tons d.w. | Percent Disposed (not used as by-product) |
|---|--------------|--------------------------------|---------------------------------------|---|
| apple peel and core (69%) crush (31%) | Aug.-Jan. | 284,400 42,600 | 38,376 5,924 | 31 |
| citrus crush (100%) | all year | 3,555,640 | 944,378 | 3 |
| grape crush (100%) | Aug.-Nov. | 438,000 | 54,750 | 20 |
| peach peel and pit (100%) | July-Oct. | 192,500 | 86,625 | 68 |
| tomatoe Peel (10%) crush (90%) | July-Nov. | 70,200 | 1,543 | 78 |

(Source: Cooper, 1976)

2.2 POLYSACCHARIDE BASED FEEDSTOCKS

Material composed of high concentrations of long chain carbohydrate polymers are abundant in nature. The result is a high availability of these substrates; however, technological difficulties arise due to the need for hydrolysis of this sugar source prior to, or during the aerobic fermentation. Therefore, the credit for an inexpensive substrate (especially cellulose) is often nullified due to additional pretreatment costs.

2.2.1 LIGNOCELLULOSIC MATERIAL

World production and present stocks of cellulose based biomass is by far larger than any other carbohydrate source. By approximating the average value for incident solar energy reaching the earth surface at $876,960 \times 10^{15}$ kcal/annum (Holdren and Ehrlich, 1974), global photosynthesis (with an efficiency of 0.07 %) could convert 614×10^{15} kcal to cellulose material. This would provide an overall yield of 1.8×10^{12} of lignocellulose material, $\sim 40\%$ of which is cellulose (Whittaker, 1970). At present, only 0.5 % of this total biomass is removed on a global basis. (Ghose and Ghosh, 1978).

2.2.1.1 Availability of Lignocellulosics

2.2.1.1.1 Waste materials

a) *Agriculturally derived materials*

As can be seen from Table 5, global production of crop and logging residues is extensive. It has been estimated that the U.S. is responsible for the yearly generation of 540 million tons of this residual material (Pimentel et al., 1978) while Canada accounts for about 90 million tons (Moo-Young et al., 1979). However, though the potential of this feedstock is great, there exist a number of important considerations which must be taken into account in the appraisal of its feasibility.

Table 5 : Global production of representative crops and their residues on an annual basis

| Residue Source | Global Production Area (ha x 1,000) | Global Production Mass (t x 1,000) | Residue Yield (t/ha) | Global Residue Production (t x 1,000) | Composition of residue | | | Potential Glucose yield (t x 1,000) |
|---------------------|-------------------------------------|------------------------------------|----------------------|---------------------------------------|------------------------|----------|--------|-------------------------------------|
| | | | | | Hexosan | Pentosan | Lignin | |
| Wheat | 224,111 | 363,945 | 3.0 | 672,333 | 33-51 | 23-38 | 18-21 | 208,420-342,890 |
| Rice | 137,395 | 329,358 | 7.4 | 1,016,700 | 32-53 | 21-24 | 12-25 | 325,340-538,850 |
| Corn | 112,346 | 311,030 | 5.6 | 629,140 | 35 | 15 | 15 | 220,000 |
| Barley | 89,109 | 165,157 | 3.5 | 311,880 | - | - | - | - |
| Soybeans | 45,264 | 63,308 | 3.0 | 135,790 | - | - | - | - |
| Sugarcane | 11,667 | 57,850 | 1.8 | 21,000 | 45-55 | 25-27 | 19-21 | 9,450-11,550 |
| Oats | 32,250 | 54,374 | 4.0 | 129,000 | 33.1 | 29.5 | 13.5 | 43,473 |
| Sorghum | 43,269 | 52,800 | 1.2 | 51,920 | - | - | - | - |
| Cotton | 32,861 | 38,181 | 0.5 | 16,430 | - | - | - | - |
| Rye | 15,970 | 28,485 | 2.3 | 36,730 | - | - | - | - |
| Logging (U.S. only) | - | - | - | 53,000 | 25 | 50 | 25 | 13,250 |

(Sources: Grethlein, 1978; Kosaric et al., 1981; Pimentel et al., 1981, Rivers and Emert, 1980; Sitton et al., 1979; Thorne and Thorne, 1979)

This material at present has been considered to have a zero or net negative value but if its utilization becomes profitable, a cost for this substrate is expected to be assigned. Also other competing applications must be taken into account. For example, as of 1966 over a million tons of corn cobs were processed on a yearly basis in the U.S. for the production of abrasives, packing materials, shingles, and dynamite. (Walden, 1966).

In addition, there exists a valid concern over the diminishing fertility of global croplands due to intensive farming operations. (Brown, 1978; Tanner, 1982). In the United States, present agricultural operations are estimated to exceed erosion tolerance limits in two-thirds of all cases (Gupta et al, 1976) and by an average value of twice that of acceptable standards (Brink et al., 1977). A number of beneficial effects are gained by the return of crop residues to the soil after harvest. These include erosion control, nutrient returns, structure stabilization (tilth), reduction of bulk density, as well as a host of others (Thorne and Thorne, 1979).

Analytical methods have been developed to quantitate the effects of residue removal on soil erosion due to wind and water (Posselius et al., 1980) however, it is much more difficult to assess parameters such as soil tilth and organic loading. Pimentel (1981) estimated the effect of removal of 60 % total corn stover from an agricultural operation employing conventional tillage. He found that in a 30 year time span, a 37 % increase in energy (for fertilizer and tillage) would be required to produce the same crop yield.

The collection of residues and their inherent seasonal production also may present problems in specific cases. For example, logging residues are widely scattered in a number of small-scale operations. In the U.S., only 9 % of the total logging wastes have been estimated to be readily accessible (Anderson, 1972).

b) *Domestically derived wastes*

Municipal refuse is the greatest source of cellulosic wastes in the developed countries. In the U.S., about 28 million tons of such refuse is produced on a daily basis in the urban centres.

The main advantages in the utilization of domestic wastes are that collection systems have already been developed for substrate recovery, there is a disposal credit in its use, and due to its highly processed state the cellulose component is easily hydrolyzed. However, the heterogeneity and dirtiness of the residue requires an efficient mechanical sorting technology for the separation of the cellulose rich component. These approaches to materials separation have been outlined elsewhere (Sloneker, 1976; Birch et al., 1979).

Once separation has been accomplished, use of this material must compete economically with its recycle potential to the parent process.

c) *Industrially derived wastes*

Pulp and paper, as well as food processing industries account for the major contribution of industrial effluents which contain high quantities of cellulosic material.

Waste solid residues are generated at almost all stages of the pulp and paper process. At present, the greatest proportion of these sludges are burned or disposed by landfill after settling in lagoons and dewatering processes. As such, centralized collection has already been established.

Depending upon the wood species, pulping method, and bleaching processes used, the cellulose content of waste sludges is approximately 25 - 45 % (w/w) (Pamment et al., 1979). A favorable aspect of this material is that the pulping process causes a severe disruption of cellulose and acts to de-lignify the original wood. Therefore, pretreatment requirement prior to fermentation is limited to size reduction by milling or nullified altogether (Andren, 1976). Inhibition of microbial growth may occur with these sludges due to appreciable amounts of insoluble inorganic materials such as china clay, lime mud and sand (Pamment et al., 1979).

Fruit and vegetable processing industries also produce large quantities of cellulosic wastes. Characteristic residues are shown in Table 6. A number of factors inherent to this industry

will make large scale utilization of its waste streams difficult if not impossible. This is a consequence of the highly disperse points of origin for each effluent, seasonal production characteristics (which lead to high amortisation costs), and the extreme variability in the composition and characteristics of both solid and liquid wastes (Carroad and Wilke, 1978).

Table 6 : Representative food processing wastes and crude fibre content arising from each in the U.S.

| Foodstuff | Residuals Wet Weight (tons/annum) | Crude Fibre Content (tons dry wt.) |
|----------------|--------------------------------------|---------------------------------------|
| apple | 327,000 | 4,413 |
| citrus | 3,555,640 | 204,805 |
| grape | 438,000 | 56,940 |
| peach | 192,500 | 28,875 |
| beet | 98,666 | 3,946 |
| white potatoes | 2,595,400 | 36,335 |
| tomatoe | 70,200 | 11,051 |

(Source: Cooper et al., 1976)

2.2.1.1.2 Silviculture

Rather than employ waste cellulose as a carbohydrate source for the production of single cell protein, intensive farming of various tree crops for their use as a feedstock in the process is a possible alternative. The main advantage in such an operation is that harvesting and processing may be undertaken in a year-round cycle which would eliminate the detrimental characteristics of seasonal production (Ferchak et al., 1981). The plant species may be chosen to

optimize the local climatic, economic, and technological conditions.

In general, hardwoods are usually a more favorable choice than softwoods since they display rapid juvenile growth, fit short rotation criteria, and usually contain higher concentrations of cellulose. However, hardwoods do not exhibit as great a site adaptability since they require fertile, well drained soils (Cheremisinoff, 1980). Yield of cellulosic based biomass is about 5 - 12 tons per acre for present silviculture methods (Salo and Henry, 1979).

2.2.1.2 Characteristics of Lignocellulosics

2.2.1.2.1 Composition

The approximate composition of lignocellulosic feedstocks is shown in Table 7.

Table 7: Average composition of lignocellulosic materials

| Content | Percentage (w/w d.w.) |
|---------------|-----------------------|
| Cellulose | 40 |
| Hemicellulose | 27 |
| Lignin | 14 |
| Protein | 4 |
| Ash | 8 |
| Nitrogen | 0.7 |
| Phosphorous | 0.09 |
| Potassium | 1.3 |

Cellulose is a linear homopolymer of anhydroglucose units linked by β -(1,4)-glucosidic bonds. The macromolecule varies to a great extent in its length. For instance, newsprint ex-

hibits an average DP (degree of polymerization) of about 1,000 while cotton is found to have a DP of about 10,000 (Callihan et al., 1979).

Closely associated with cellulose in the plant cell wall is hemicellulose and lignin. Hemicelluloses are heteropolymers of galactose, mannose, xylose, arabinose, and various other sugars and their uronic acids. For the economic viability of the SCP process, it should also include the assimilation of the hemicellulosic portion as well as the cellulose. Thus, the preferred organisms have a broad specificity for utilizable carbohydrate.

The lignin component of lignocellulosics is highly responsible for the recalcitrance of cellulose to hydrolysis. This macromolecule is phenolic in character being composed of varying relative amounts of three different monomeric alcohols. The relative proportions of each is dependent upon the source (Adler, 1977). The lignin matrix forms a protective sheath which tightly surrounds the cellulose microfibrils.

2.2.1.2.2 Cellulose structure

While lignin accounts for some degree of protection to the microfibrils, the conformation of native cellulose is also highly effective in limiting hydrolysis.

The generally accepted model at present is known as the folding chain model (Chang, 1971). The cellulose molecule is visualized folding back and forth along the fibrillar axis as shown in Fig. 3 .

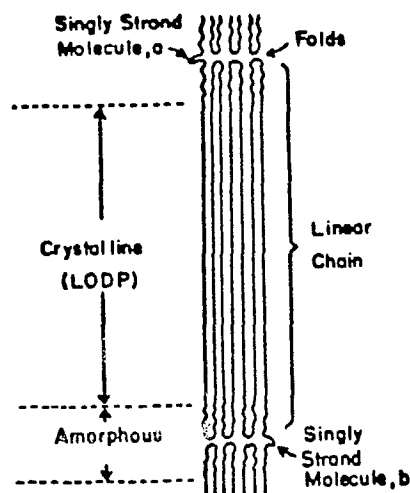


Fig. 3: Folding chain model for cellulose.
(Source: Chang et al., 1981)

One repeating unit (termed platellite) corresponds to about 1,000 DP and is designated as the chain length between points a and b in Fig. 3. Platellite units are joined by single stranded glucose chains which are easily hydrolyzed.

Regions which are easily hydrolyzed along the cellulose chain (amorphous regions) are thought to be comprised of areas rich in deflected β -glucosidic linkages found at each fold. The crystalline, linear regions are extremely resistant to hydrolysis.

Crystalline units are further packed to form elementary fibrils which are further associated into microfibrils about 250 Å wide and infinitely long. It is these microfibrils that are tightly surrounded by the lignin matrix.

2.2.1.3 Pretreatment Processes

A number of physical and/or chemical methods have been developed which enhance cellulose hydrolysis. This is achieved by the separation of cellulose from its protective lignin sheath as well as increasing the surface area of the crystallite by size reduction or swelling.

2.2.1.3.1 Physical methods

a) *Milling*

Compression (Spano et al., 1979) and ball milling (Wilke and Mitra, 1975) have both been found to be effective in removal of the lignin barrier, increasing the bulk density, and disruption of cellulose crystallinity. Ball milling to sizes of 400 mesh and less was sufficient to enhance cellulose hydrolysis with no other pretreatment, however, milling to this small scale may not be economically feasible (Nystrom, 1975).

b) *Steam explosion*

This pretreatment involves the pressurized heating of the lignocellulosic material at a temperature sufficient to soften the lignin and depolymerize hemicellulose. Upon completion of the heating cycle, the reaction vessel is abruptly

discharged to atmospheric pressure which illicit an "explosion" effect upon the woody cells (Wayman, 1980). Spano et al. (1979) have found this method to be quite effective in the pretreatment of agricultural residues and hardwoods, however, softwoods and municipal wastes did not exhibit enhanced cellulose hydrolysis.

2.2.1.3.2 Chemical methods

a) *Solvents*

Solvents not only serve to dissociate cellulose from its protective lignin but also destroy the crystalline structure of native cellulose through its successive dissolution and regeneration to a highly active form. A number of researchers (Ladisch, 1978; Tsao, 1978) have reported highly successful results with a solvent known as Cadoxen (alkaline solution of ethylene diamine and cadmium oxide). A major advantage of this solvent is its ability to be recycled. Figure 4 illustrates the increased susceptibility of Cadoxen pretreated crop residues to enzymatic hydrolysis. Other solvents include H_3PO_4 (Welseth, 1952) and alkali (Trivedi, 1980).

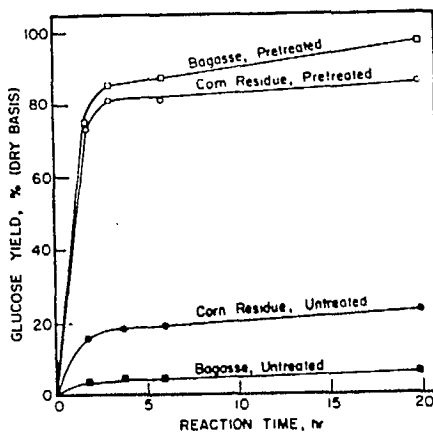


Fig. 4 : Hydrolysis Timecourses of Cadoxen Pretreated Residues.
(Source: Tsao, 1978)

b) *Swelling agents*

Intracrystalline swelling requires a chemical agent capable of breaking down the H-bonding of adjacent glucose molecules in the cellulose matrix. These swelling agents include concentrated NaOH, organic bases, and certain metal salts (i.e. SnCl_4) (Chang et al., 1981).

2.2.1.3.3 Miscellaneous pretreatment methods

A number of other pretreatment methods are available for the enhanced hydrolysis of cellulose. These include pulping (Baker et al., 1975), heating (Millett and Goedken, 1965), freezing (Bykov and Frolov, 1961), irradiation (Saeman et al, 1952), and the use of lignin-consuming microbes (Crawford and Crawford, 1980). Many of these alternative pretreatment methods are too energy intensive to be considered on a large-scale SCP process. Microorganisms capable of lignin assimilation would be especially useful in an SCP process based on cellulose since greater biomass yields may be available per unit mass of feedstock due to a more complete utilization of substrate.

2.2.1.4 Feedstock Utilization Alternatives

2.2.1.4.1 Indirect conversion to SCP

a) *Acid hydrolysis of lignocellulosics*

Cellulose may be hydrolyzed to its constituent monomers by concentrated or dilute acid. Concentrated acid is advantageous in that the kinetics of hydrolysis are not dependent upon the structural details or degree of crystallinity of the feedstock. Thus, rapid hydrolysis of up to 90 % of potential glucose may be achieved (Grethlein, 1978). Disadvantages with concentrated acid include the need for recycle of the catalyst due to high costs and extensive decomposition of glucose as it is liberated due to the harsh conditions.

To minimize the effects of catalyst cost and product degradation, dilute acid has been utilized for cellulose hydrolysis. However, in general, the rate of hydrolysis is much less than that for concentrated acids. It has been shown that

the kinetics of hydrolysis will increase using dilute acid if under very high temperatures ($\sim 500^{\circ}\text{C}$) and for short times (Yu and Miller, 1980) yet this leads to high energy consumption and increased process costs.

b) *Enzyme hydrolysis*

Cellulose enzymes are basically composed of three components (Bisaria and Ghose, 1981) classified as;

- endo- β -(1,4)-glucanases:

this group is composed of several components which are postulated to cleave internal β -(1,4)-glucosidic linkages

- exo- β -(1,4)-glucanases:

consists of two groups of enzymes which cleave glucose or cellobiose residues from the non-reducing end of the chain

- β -(1,4) glucosidases:

hydrolyze cellobiose and short chain oligosaccharides to glucose

A number of mechanisms have been postulated for cellulase attack on cellulose (Wood, 1980; Reese, 1977). The generally accepted mechanism involves the swelling of crystalline cellulose by a special "hydrogen bondase" of the endo-glucanase class (C_1). The cleavage of internal glycosidic bonds is achieved by other endo-glucanases (C_x) followed by the synergistic attack of exo-glucanases. Final hydrolysis of the product oligosaccharides is achieved by β -glucosidase. Figure 5 shows a schematic summary of this mechanism.

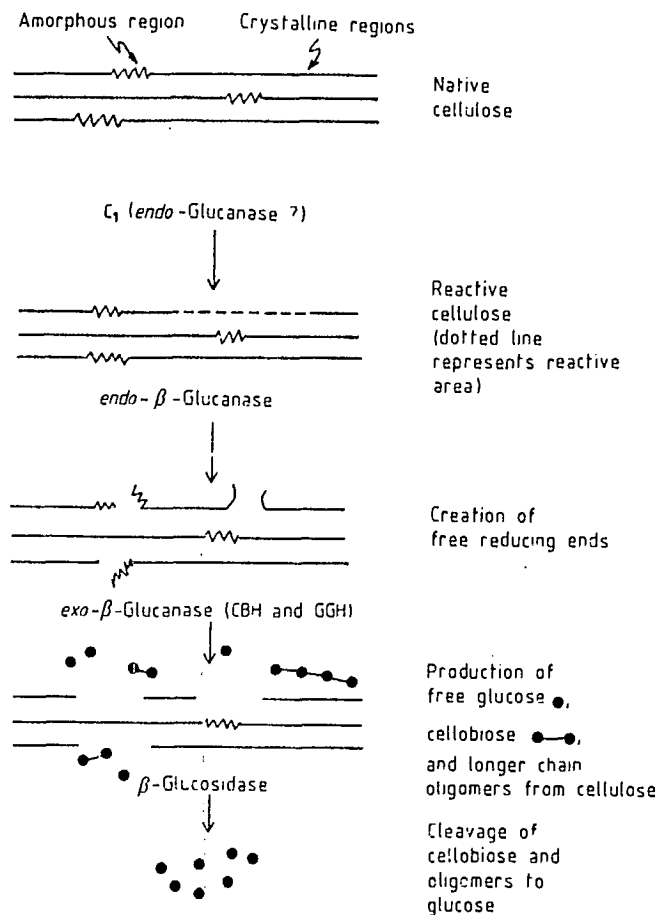


Fig. 5 : Schematic representation of the synergistic action of enzymes involved in cellulose degradation. Note role of C_1 in crystalline swelling.
 CBH = cellobiohydrolase
 GGH = glucanase glucohydrolase
 (Source : Bisaria and Ghose, 1981)

A great many microorganisms are capable of cellulase production, however, many are deficient in one or more components of the enzyme complex which are necessary for the biodegradation of crystalline cellulose. The most extensively studied microbe capable of the enzymatic hydrolysis of native cellulose is Trichoderma reesei (viride) (Gallo, 1982; Ghosh et al., 1982). This organism has been mutagenized by a number of artificial methods (i.e. linear accelerator, nitrosoguanidine) which has yielded many constitutive mutants capable of hypercellulase production. As well, research in immobilized cellulase components

have led to cost savings by the recycle of enzyme (Montenecourt 1981).

c) *SCP production from cellulose hydrolyzates*

The synthesis of SCP from lignocellulosics which have been pre-hydrolyzed (whether achieved by acid or exogenous enzymes) reduces the required fermentation technology to a simple case of monosaccharide assimilation by the microorganism.

Recent work by Ivarson and Morita (1982) and Miller et al. (1984) have shown that growth of an acid tolerant fungus (*Scytalidium acidophilum*) on acid hydrolysates of various waste papers is extremely efficient. A total of 97 % of carbohydrates (hexosans and pentosans) were assimilated by this fungus and converted to biomass with yields up to 56 % (see Table 8).

Table 8 : Weights, percent yields, and protein contents of fungal mycelia derived from fermentation of waste paper hydrolysates. ^{a)}

| Culture | Paper | No. of runs | Dry wt of mycelia (g/flask) | % of yield (% of sugar consumed) | % Protein ^{b)} |
|---------|-----------|-------------|-----------------------------|----------------------------------|-------------------------|
| Shake | Newspaper | 4 | 4.32 ± 0.13 ^{c)} | 37.7 ± 1.2 | 35.38 ± 1.13 |
| | Magazine | 4 | 3.79 ± 0.03 | 38.4 ± 0.6 | 34.88 ± 0.38 |
| | Bonded | 5 | 5.55 ± 0.81 | 37.2 ± 1.1 | 35.18 ± 1.34 |
| Aerated | Newspaper | 4 | 4.72 ± 0.22 | 43.0 ± 3.0 | 44.24 ± 2.85 |
| | Magazine | 4 | 4.85 ± 0.15 | 48.9 ± 1.8 | 47.31 ± 1.13 |
| | Bonded | 4 | 8.20 ± 0.41 | 55.5 ± 2.6 | 45.76 ± 5.02 |

a) All preparations were fermented for 12 days.

b) Determined as described in the text (N x 6.25).

c) Mean ± standard deviation.

(Source: Ivarson and Morita, 1982)

Due to the acid tolerant nature of this organism, neutralization requirements after hydrolysis are minimized. The yields obtained compare favorably with *C. utilis* growth on a number of wood hydrolysates (Reese et al., 1972) where yields of 45 - 52 % were observed.

Simultaneous saccharification and SCP fermentation has been carried out by the use of two organisms in co-culture. Peitersen (1975) utilized NaOH pretreated barley straw as a substrate for T. viride growth over 24-32 hours. At this point, the fermentation was inoculated with either S.cerevisiae or C. utilis. The presence of the yeast was not found to illicit a more extensive utilization of the polysaccharide, however, it increased the rate of protein and enzyme production by the mold about 2-fold. This is due to assimilation of glucose by the yeast as soon as it is freed from the cellulose chain and therefore decreasing the effects of product inhibition on β -glucosidase. As well, it is possible that cell lysis of the yeast provides a source of easily metabolized compounds for T. viride growth.

2.2.1.4.2 Direct conversion to SCP

Growth of microorganisms capable of metabolizing cellulose directly has also been extensively studied.

Low technology processes of this type are important for use in the developing countries. These include the growth of mold on steam pre-treated residues such as peanut presscake, coconut presscake, soybean hypocotyledons, and other agricultural wastes. The final product is known as tempeh (Advisory Committee on Technological Innovation, 1981). In Taiwan, rice straw is extensively utilized for the growth of the common mushroom as a food source for human consumption.

Processes which require a greater technological expertise include variations on pretreatment and fermentation methods. The Waterloo process (Moo-Young et al., 1979) involves the use of a mesophilic fungus (Chaetomium cellulolyticum) grown on various agricultural and industrial wastes of cellulosic character which have been pretreated with NaOH.

A cellulolytic bacteria of genus Cellulomonas is the organism used in the Louisiana State University Process (Callihan and Clemmer, 1979). This microbe is cultivated on hot alkali treated feedstock. Economic analysis of the LSU process has shown that for a production capacity of 100 tonnes of biomass per year,

the total product cost would be about \$ 0.22 (U.S.) per kg of protein.

2.2.2 STARCH CONTAINING FEEDSTOCKS

2.2.2.1 Availability of Starch Materials

2.2.2.1.1 Waste materials

Food processing effluents which contain large quantities of starch are shown in Table 9 . In the United States, approximately 410,000 tons (dry wt.) of waste starch are generated per year from the processing of white potatoes alone (Cooper, 1976). For economical SCP production from these wastes, the dry matter content of the effluent should not be less than 2 % (Skogman, 1976). However, characteristics of these industries would make an effluent stream of this concentration difficult to obtain.

Table 9 : Products which produce wastes with high starch content

| | |
|---------------------|--|
| Potato products: | french fries granules cobes flakes hashed brown dehydrated chips |
| Rice products: | instant rice |
| Starch grains: | wheat maize barley |
| Vegetable products: | carrots red beets sweet potato |
| Bakery products: | dough bread crackers |

(Source: Skogman, 1976)

2.2.2.1.2 Starch crops

a) *Cereal grains*

The production of starch containing grains such as wheat, barley, and corn is extensive on a global scale. In addition, these materials offer the advantage of easy year-round storage. However, the cost of food grade starches makes them economically non-viable for use as an SCP substrate. This economic balance may change with the intensive cultivation of nutritionally poorer but higher yielding strains of sorghum (MacLennan, 1975).

Sweet sorghum (*Sorghum bicolor* sp.) is an extremely hardy crop which is highly adaptable to a large range of soil and climatic conditions. Fermentable sugar and starch content of this crop may amount to greater than 40 % of total dry weight of the plant (see Fig. 6). Yields are highly dependant upon local conditions. Based on U.S. growing trials, the average yield was found to be 66 tonnes/ha which was reached in a time span of 97 to 135 days (Nathan, 1978).

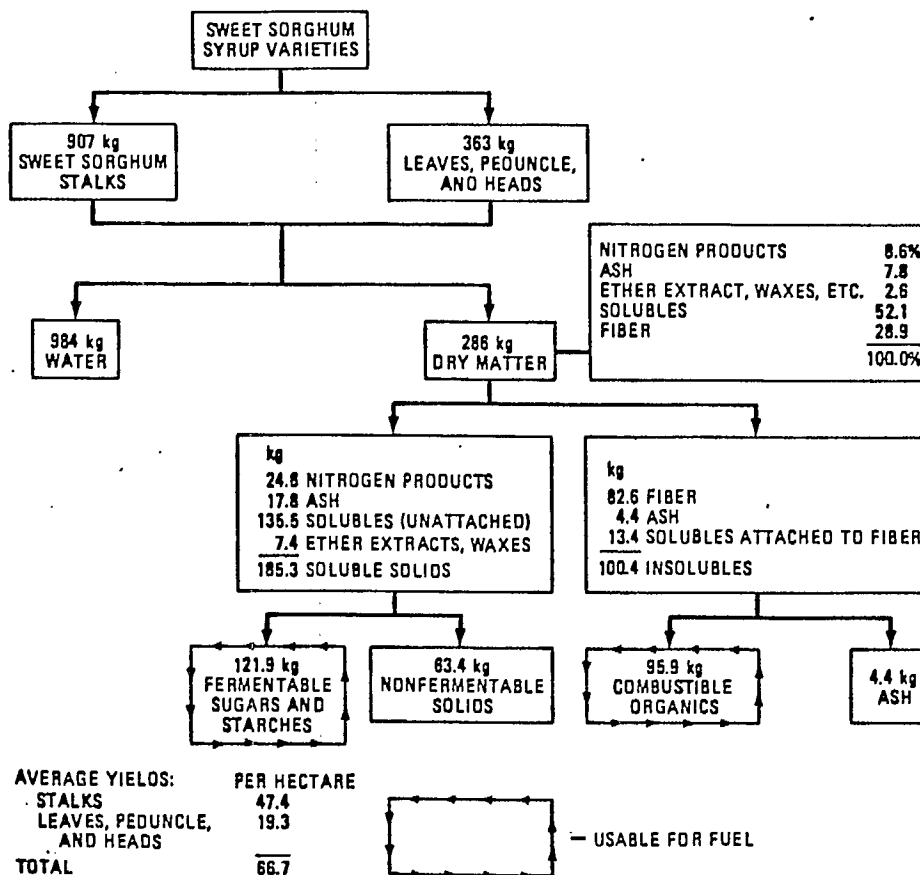


Fig. 6 : Estimated approximate composition of sweet sorghum, syrup varieties. (Source: Nathan, 1978)

b) *Starch containing root crops*

Though potatoes contain an extremely high proportion of starch, its value as a feedstock for SCP production is limited for the same reasons as for grain crops. Low quality starch containing tubers which grow in high yields include cassava and the Jerusalem artichoke.

Present annual production of cassava on a global basis amounts to approximately 100×10^6 tons (Jackson, 1976). This root crop is capable of substantial growth on relatively low quality land under climatic conditions which may be unfavorable to other crops. At present it is produced mainly in tropical areas. The average composition of cassava is shown in Table 10. This feedstock contains all nutrients required to support microbial growth. On medium quality soil, yields of cassava range from 13-15 tons/ha. However, under optimal conditions this value may reach 40-60 tons/ha (de Menezes, 1978).

Table 10: Composition of Cassava Tubers

| <u>Component</u> | <u>% (w/w d.w.)</u> |
|------------------|---------------------|
| starch | 80 - 89 |
| total sugars | 3.6 - 6.2 |
| reducing sugars | 0.1 - 2.8 |
| pentosans | 0.1 - 1.1 |
| fibre | 1.7 - 3.8 |
| protein | 2.1 - 6.2 |
| fat | 0.2 - 0.7 |
| ash | 0.9 - 2.4 |

(Source: de Menezes, 1978)

The Jerusalem artichoke, though not technically a starch containing plant has been included in this category. The major carbohydrate of this member of the sunflower family is inulin which is a linear chain of linked fructose monomers with a degree of polymerization of about 35. Glucose molecules are

covalently bound to each end of the polyfructofuranose chain. The overall ratio of fructose to glucose is approximately 5 : 1 (Kiersten, 1980). The total carbohydrate concentration of the tuber is, on average, 18 % on a wet basis (Chubey, 1981) with a yield of 45 tons/ha on light soils. In addition, this plant is extremely resistant to disease, insect damage and cold.

2.2.2.2 Conformation of starch

In its native state, starch is comprised of amylose and amylopectin. Amylose is primarily a straight chain homopolymer of α -(1-4) linked anhydroglucose units. The molecular weight of this molecule may reach 500,000. The linkage characteristics of amylopectin are the same as amylose along its main chain. In addition, amylopectin contains many branched chains joined by α -(1-6) linkages to the main chain of length 20 - 25 glucose residues. As such, the molecular weight of amylopectin can reach 100 million.

2.2.2.3 Feedstock utilization alternatives

2.2.2.3.1 Indirect conversion to SCP

Acid may be utilized to hydrolyze starch to its constituent sugars prior to aerobic fermentation. However, as for cellulose, this harsh treatment not only leads to degradation of fermentable sugars but also produces a number of inhibitory compounds which would prove detrimental to microbial growth.

Enzymatic hydrolysis methods hold practical advantages over the use of acid. The conditions for the process are much less severe since it is performed under atmospheric pressure and mesophilic temperatures. As well, the enzymatic saccharification of starch is well understood being extensively utilized in food and brewing processes. After pressure cooking the feedstock to solubilize the starch component, the saccharifying enzymes are added. These proteins are endo and exo-acting catalysts which act upon the glucose linkages. They may be derived from molds (the amylo process) or from germinating barley (the malt process) (Maiorella et al., 1981). The batch hydrolysis process completes the conversion of starch to maltose and dextrans within

one to two hours. Modifications which include continuous cooking and flash conversion have been developed which are able to complete this hydrolysis in a matter of minutes. As well, utilization of dialysis membranes for the continuous saccharification of starch with containment of enzymes has been successfully demonstrated (Nielson, 1980).

As with cellulose, the indirect production of SCP from starch has also involved the co-culture of microbes. Perhaps the most familiar operation of this type is known as the Symba-Process first introduced on a commercial scale in Sweden nine years ago. The Symba-Process is based on a symbiotic association of two microorganisms, Candida utilis and Endomycopsis fibuliger. The latter produces amylose necessary to saccharify the starch to glucose which is then metabolized by C. utilis to yield a high protein product. Fermentation proceeds on a continuous basis with the saccharifying organism grown on fresh starch feedstock in a preliminary vessel (endofermenter in Fig. 7) and subsequent yeast culture in the symbiosis fermenter. For such an operation with a capacity of 20 m³/hr of potato processing waste water (3 % d.w.), 300 kg of yeast may be produced with a subsequent BoD reduction for the effluent of 90 % (Skogman, 1976).

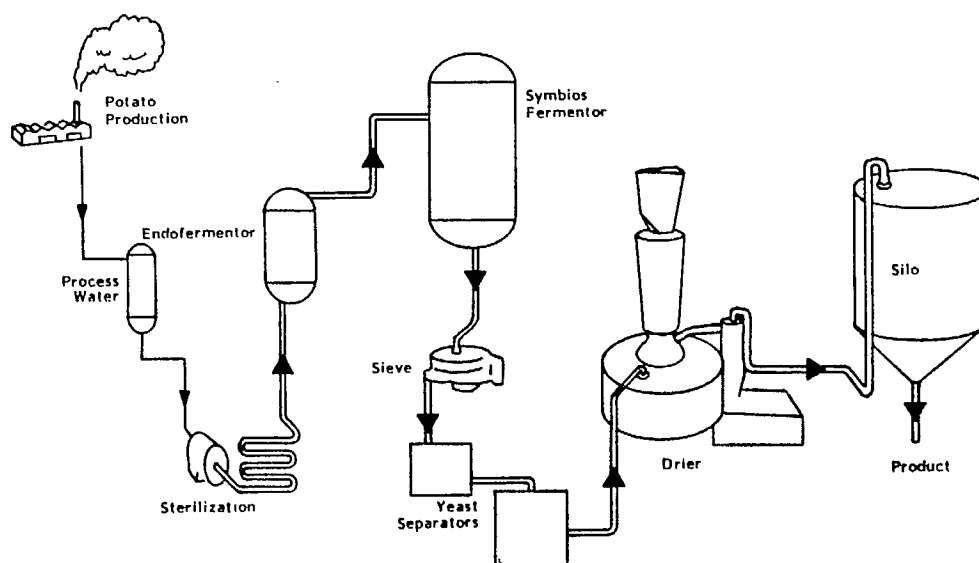


Fig. 7: Flow diagram for the Symba process.

(Source: Skogman, 1976)

2.2.2.3.2 Direct conversion to SCP

Organisms able to hydrolyze starch and rapidly metabolize the glucose to yield high quantities of biomass are mainly of the fungi class though some molds are also capable of this.

A low technology process is being developed by the University of Guelph (Canada) and Centro International de Agricultura Tropical (Columbia) which involves the aseptic fermentation of cassava starch with a thermophilic strain of Aspergillus fumigatus. Cooling costs are low due to the use of a thermophilic organism and the final product was found to contain 47 % crude protein (Gregory et al., 1976). A similar process has been developed (Reade and Smith, 1975) which utilizes A. oryzae on barley grains for protein fortification purposes. Protein yields were found to be low when the system was scaled up to a 1 m³ fermenter.

2.3 PETROCHEMICAL FEEDSTOCK

Processes which involved the synthesis of SCP from petrochemical feedstocks were first introduced in the early 1960's (Litchfield, 1977). The principle advantages seen in the utilization of these materials was their availability in large quantities and constant quality in many parts of the world. As well, their availability is independent of climate and environmental fluctuations. With the imposition of the OPEC oil embargo in 1973, world prices of hydrocarbon stocks rapidly increased while the costs of feed fell or at least remained relatively stable (Levi et al., 1979). This has resulted in a general re-evaluation as to the potential of this carbon source for SCP production. At the present time there exists an oil surplus in many parts of the world, however, this supply is subject to rapid fluctuations.

2.3.1 PARAFFINIC FEEDSTOCKS

The n-paraffins (e.g. n-alkanes) are saturated straight chain hydrocarbons which range in a homologous series from C_5 to C_{25} in length (Levi et al., 1979). These compounds are readily assimilated by a large range of fungi, yeast, and bacteria, although paraffins at length of n-octane or less have been found to be toxic to the cell (Gill and Ratledge, 1972).

Crude oil contains 6 - 10 % n-alkanes (Dimmling, 1978) though this value may range in extremes from 0 to 30 % depending upon the source. North African crudes are generally higher in composition while those from Venezuela and the North Sea contain significantly less n-alkanes and are higher in naphthene and aromatic hydrocarbon content which are not readily metabolized by microbes (International Petroleum Encyclopedia, 1972).

Upon distillation, two cuts are obtained which are high in paraffin concentration (see Table 11). Kerosine comes off the waxy crude first and has a range of C_{10} to C_{14} alkanes. The gas-oil component (i.e. diesel fuel) has a higher concentration

of n-paraffins (10 - 25 %) which correspond to C₁₄ - C₁₇ homologues (Litchfield, 1977).

Table 11: Carbon number distribution of n-paraffins 'cuts'
(% w/w)

| Alkane | Typical 'kerosine-range' | Typical 'gas-oil range' | 'Wide-range' cut from waxy crude |
|-------------------|-----------------------------|----------------------------|-------------------------------------|
| n-C ₁₀ | 6 | - | trace |
| n-C ₁₁ | 39 | trace | trace |
| n-C ₁₂ | 42 | trace | 1 |
| n-C ₁₃ | 13 | 4 | 2 |
| n-C ₁₄ | trace | 28 | 3 |
| n-C ₁₅ | - | 29 | 5 |
| n-C ₁₆ | - | 22 | 8 |
| n-C ₁₇ | - | 12 | 14 |
| n-C ₁₈ | - | 4 | 21 |
| n-C ₁₉ | - | 1 | 19 |
| n-C ₂₀ | - | - | 13 |
| n-C ₂₁ | - | - | 9 |
| n-C ₂₂ | - | - | 4 |
| n-C ₂₃ | - | - | 1 |
| n-C ₂₄ | - | - | trace |

(Source: Levi et al., 1979)

Originally, the utilization of the entire gas-oil fraction was considered to be the most attractive for SCP production (Dimmling, 1978) since fermentation of this substrate would remove the n-paraffins (dewaxing effect). This would provide a credit since it would lower the pour point of the oil and decrease clouding effects. The dewaxed effluent would be returned

to the refinery for further processing with a 10 % reduction in volume. This processing scheme exhibits a number of technological difficulties. Gas-oil contains a number of aromatic polycyclic impurities which have potent carcinogenic effects (Zaki, 1981). For this reason sophisticated and expensive methods need to be utilized to separate and wash biomass from the spent medium (i.e. phase separation with solvents; Bonavita, 1972). In addition, increases in sulphur concentrations of the fermented broth may induce pollution problems (Litchfield, 1977). In spite of these and other problems, Schwedt (GDR) has gone ahead with this process on a scale of 60,000 tons per annum.

Purified n-paraffins may be utilized as substrate instead of gas-oil mixtures. This would eliminate the problems discussed above, however, since the hydrocarbon accounts from 40 to 60 % of total SCP cost (Cooney et al., 1980) the use of a purified feedstock will greatly affect the economics of the process. The amount of n-paraffins produced on a global basis is shown in Table 12. These are utilized in such commercial products as detergents, paints, and solvents. In Western Europe alone, only 4 % of the total n-paraffin potential is utilized (Dimmling, 1978). Separation processes by which n-alkanes are purified are primarily molecular sieve adsorption techniques which yield a 97.5 - 99 % pure $C_{10} - C_{23}$ product (Levi, 1979). As such, SCP processing is much less complex usually involving two-stage centrifugation and spray drying (Kanepron Product Bulletin, 1973).

Fodder yeast (*Candida* sp.) is produced in an amount of one million tons per year in the USSR (Gradova, 1984). The carbon source for the yeast growth are purified liquid petroleum paraffins. The product contains a minimum of 60 % protein, 5 % of lipids and 7 - 10 % of mineral substances. Its assimilation by animals is reported at 85 - 95 %. Reported amino acids on a crude protein basis are lysine 3.5 - 6.5 %; methionine 0.8 - 1.7 %; tryptophane 0.1 - 3.0 % and arginine 4.0 - 6.0 %.

Table 12: n-Paraffins production capacities

| | | |
|------------------|--|----------------------------|
| FRG | Veba BP Erdölr Raffinerie Emsland | 145.000 tpa |
| Italy | Saras Liquichimica | 775.000 tpa |
| UK | BP | 50.000 tpa |
| Spain | Petresa | 100.000 tpa |
| Western Europe | | 1.070.000 tpa |
| GDR | Schwedt | 70.000 tpa |
| USSR Bulgaria | 10 plants 1 plant | 1.300.000 tpa projected |
| USA | Continental Oil Exxon UCC Shell Texaco | 450.000 tpa |
| Japan | Mitsui-Texaco Nippon-Petrochemical Nippon Mining | 170.000 tpa |

(Source: Dimmling and Seipenbusch, 1978)

There exist a number of technical problems with respect to the fermentation of n-paraffins. First and foremost is that of substrate mass transfer from the broth to the growing cells. These hydrocarbons are not miscible with water and therefore they are finely dispersed in the fermenter by means of mechanical agitation or violent aeration. These techniques are highly energy intensive. Air-lift agitation is favored due to a maximum emulsion with a lower energy consumption (Bennett et al., 1969). Another technical problem is the highly exogonic nature of hydrocarbon fermentations. Large amounts of cooling water are required though the use of thermotolerant organisms may help alleviate this effect. Due to the reduced state of the substrate,

high levels of dissolved oxygen are necessary for efficient growth. A mineral medium must be provided to these fermentations which would contain assimilable nitrogen and phosphorus as well as trace minerals and growth factors. A comparison between SCP processes based on gas-oil and n-paraffins is shown in Table 13.

Table 13: Comparison of SCP Processes

| Characteristic | Process | |
|-----------------------------------|--|---|
| | Gas oil | n-Paraffins |
| Sterility | Non-aseptic | aseptic or non-aseptic |
| Fermenter | Airlift or agitated | airlift or agitated |
| Feedstock | Gas oil, 300-380 C BR (C ₁₅ -C ₃₀) (10-25%) | n-paraffins, C ₁₀ -C ₂₃ |
| Feed stock utilization | 65-75% of n-paraffins utilized | fully metabolized |
| Temperature | 30 - 40 ° C | 30 - 32° C |
| pH | 2.9 - 4.0 | 3.0 - 4.0 |
| Product recovery and purification | Centrifugation, countercurrent solvent leaching, solvent recovery | Centrifugation |

(Source: Litchfield, 1977)

2.3.2 C₁ AND C₂ FEEDSTOCKS

2.3.2.1 Methane

Natural gas is found in many locations of the world as an associated or unassociated product of the oil retrieval and refining industries. Though the main component of this material is methane, a number of other gaseous alkanes are present in varying proportions (Hamer, 1979).

The conversion of natural gas to SCP may be performed by direct or indirect means. The direct methods entail the culture of methane-utilizing bacteria on aqueous solutions of nutrient with the gaseous carbon source bubbled through the broth. The methane is oxidized to methanol and then formic acid which is finally decarboxylated to CO₂.

Due to the gaseous nature of the feedstock, the level of substrate conversion is always less than 100 %. However, with the use of gas fired drying and steam production equipment, non-utilized methane may be used as a fuel in these unit processes. Advantages with the use of gaseous feedstocks over n-paraffins is the lack of residue left in the final product.

Higher yields of biomass are available from the bacterial assimilation of methane than any other feedstock (approximately one kg of biomass per kg substrate), however, problems with fermenter cooling are critical. Energy release as observed in the metabolism of various substrates is shown in Table 14. As can be seen, methane fermentations can produce 4 times as much heat as common sugar substrates.

Table 14: Heat Production During Growth on Selected Carbon Energy Sources

| <u>Substrate</u> | <u>Heat Production (kcal / l-hr)</u> |
|------------------|--|
| Glucose | 24 |
| Methanol | 32 |
| Hexadecane | 42 |
| Methane | 109 |

2.3.2.2 Methanol

Methanol can be synthesized from a variety of waste materials such as natural gas, naphtha, heavy fuel oils, coal, and cellulose. Thus, this substrate is particularly important to SCP processes due to the great flexibility in its production characteristics.

The total global capacity for methanol production is approximately 10,500,000 tpa (Dimmling and Seipenbusch, 1978). Production capacities on the basis of geo-political location is shown in Table 15. Apart from present uses in the chemical industry, methanol is also gaining importance as a fuel additive in combustion engines. Recent projections (Anon., 1979) on world wide demand of methanol indicate that a growth rate of 7 % per year is expected until at least 1984. [?]

It is probable that this feedstock will follow general energy price developments in the future (Ericsson et al., 1981), however, increases in its cost over the past few years (see Figure 8) have (at least in part) been attributed to speculation on its value as an SCP substrate.

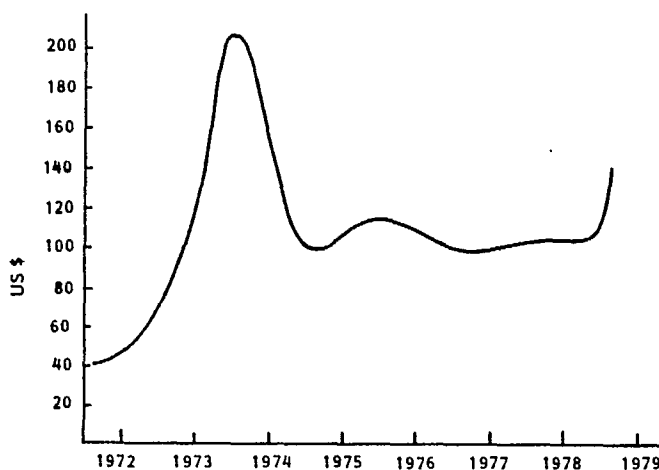


Fig. 8 : Price of methanol 1972-1979 (CIF Rotterdam).

(Source: Ericsson et al., 1981)

On a technical basis, methanol exhibits many advantages. It is completely miscible with water and as such, no mass transfer limitations are observed. Storage and safety characteristics are rather simple though some precautions are necessary since it is a toxic substance to humans. No residues are associated with the cell mass upon leaving the fermenter. Methanol is available in high states of purity (greater than 99.5 %; Cooney et al., 1975) and therefore constant concentrations may be supplied to

Table 15: Methanol production capacities

| | | |
|----------------|---|----------------|
| FRG | BASF ELF UK Wesseling Veba | 1,650,000 tpa |
| France | Methanolacq Ugine-Kuhlmann Courrieres-Kuhlmann Onia SCC | 550,000 tpa |
| Holland | AKZO/DDM Konam NV (AKZO) | 400,000 tpa |
| Italy | Montedison ANIC Pozzi (ENI) SIR | 500,000 tpa |
| UK | ICI | 570,000 tpa |
| Finland | Typpi Oy | 350,000 tpa |
| Norway | Norsk Hydro | |
| Spain | Albanos-Sevilla | |
| Austria | Hiag | |
| Western Europe | | 3,970,000 tpa |
| USA | Allied Chemical Borden Chemical Celanese CSC Du Pont Escambia Georgia Pacific Corp Hercules | 4,800,000 tpa |
| Japan | Kyowa Gas Chem Mitsubishi Saitetsu Kagaku Mitsui Toatsu Chem Higashi Nihon Methanol Nishi Nihon Methanol | 1,800,000 tpa |
| | | 10,570,000 tpa |

(Source: Dimmling and Seipenbusch, 1978)

the fermenter.

Since methanol is already partially oxidized, heat generated from the fermentation is less intense than for methane. Oxygen requirements are also lower. Cell yields from methanol are intermediate to those achieved using methane and carbohydrates.

One of the main advantages of methanol over ethanol is in the price of the two raw materials, when SCP production is concerned. Methanol sells for US \$ 0,86/gal (FOB Los Angeles) whereas the ethanol price is US \$ 1,70/gal (East USA) (Chemical Marketing Report, May, 3, 1982).

As can be seen from Table 16 (Cooney, 1975) ethanol gives a higher yield in SCP, but for similar heat load at a given productivity the price of ethanol should be US \$ 1,30/gal ($0,86 \times \frac{0,75}{0,50}$) in order to be as economical as methanol. Also the operating temperature of the fermenter is higher with methanol (37 - 40°C) than with ethanol (30 - 32°C) and the raw protein content is also higher being about 80 % as opposed to 55 - 60 % with ethanol (Dimmling and Seipenbusch, 1978).

Table 16 : Comparison of oxygen demand and heat loads for the production of SCP from various materials

| compound | substrate yield (g cell/g substrate) | Oxygen yield (g cell/g O ₂) | Oxygen demand ^{a)} (mmol/l·hr) | Heat load ^{b)} (kcal/l·hr) |
|-----------|---|--|--|--|
| Methanol | 0.5 | 0.6 | 182 | 22 |
| Ethanol | 0.75 | 0.7 | 156 | 19 |
| n-Alkanes | 1.0 | 0.47 | 232 | 28 |
| Methane | 0.62 | 0.20 | 549 | 66 |

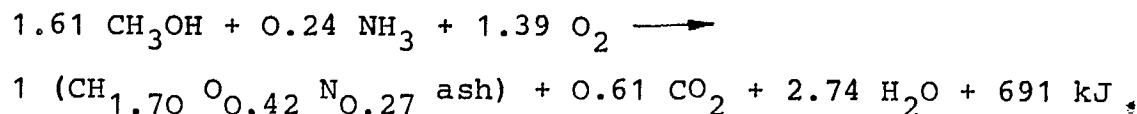
a) Based on a productivity of 3.5 g cell / l·hr

b) Calculated from the correlation of Cooney et al. (1968): heat load = 0.12 (oxygen demand)

(From Cooney 1975)

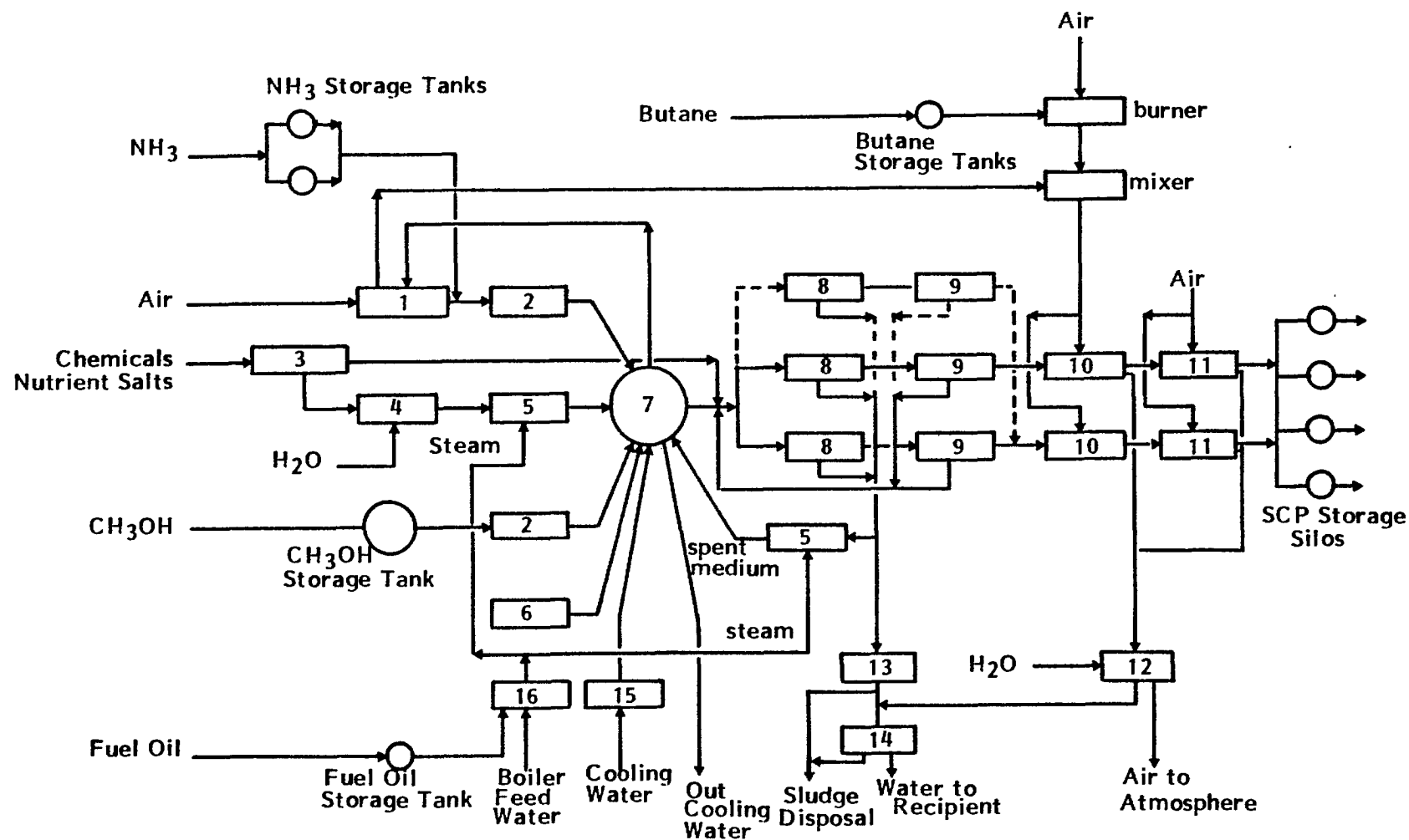
Several processes have been developed for the production of SCP from methanol. The Norprotein Process developed by the Norwegian Chemical Group Norsk Hydro A.S. and the Swedish food company A.B. Marabon (Mogren, 1979; Ericsson et al., 1981) involves the use of an obligate methylotroph bacterium, Methylomonas methanolica. The fermentation is operated aseptically. Cell productivity has been found to reach 25 g / l-hr under experimental conditions, however, oxygen transfer in a large scale fermenter will most probably limit this value. The price of the SCP product has been estimated to be about \$ 0,39 (US) based on a plant of capacity 100,000 tpa. This value is subjected to a number of highly diverse variables (Ericsson et al., 1981).

The SCP is produced according to the following equation:



(Mogren, 1979). "Norprotein" is said to contain 81 % crude protein, and to have a good balance of amino acid. A process flowsheet is shown in Figure 9. The fermenter operates under strictly sterile conditions and at a pressure of 4 atm. Air and gaseous ammonia, sterilized by filtration, are fed to the fermenter, together with an aqueous medium of nutrient salts and chemicals, sterilized by steam. The energy of the exit gases from the fermenter is recuperated in a turbine coupled to the air compressors; the exit gases are mixed with heated air and used to dry the product. The harvesting step consists of three parallel lines, only two in operation leaving one free for cleaning. The flocculation / filtration concentrates the culture to a dry weight of about 20 %, by heating the suspension followed by the addition of acid to lower the pH. Further dewatering by filtration increases the cell concentrate to 25 - 30 % dry weight. Eighty percent of the spent medium is recycled after being sterilized. The non-recirculated part of the medium is taken to a conventional waste-water treatment system. The cell suspension is dried in two parallel spray-dryers so that the final product contains particles larger

Figure 9 : The Norprotein SCP-process



- | | | | |
|--|---------------------------|-------------------------------------|-----------------------------------|
| 1. Air Compressor and Recuperation Turbine | 5. Sterilization | 10. Drying | 14. Chemical Wastewater Treatment |
| 2. Sterile Filtration | 6. Inoculum Fermentor | 11. Cooling | 15. Pumping Station |
| 3. Nutrient Salts and Chemical Storage | 7. Fermentor | 12. Air Purification | 16. Steam Producer |
| 4. Medium Preparation | 8. Flocculation/Flotation | 13. Biological Wastewater Treatment | |
| | 9. Filtration | | |

than 200 μm in diameter. The air from the dryers, containing some cells, is cleaned thoroughly in a multistep Venturi scrubber system. The product is cooled, stored, and supplied to the feed mixing industry as a bulk powder. Norprotein did an extensive economic study and concluded that, in 1979, it was not commercially viable to start SCP production from methanol in Scandinavia (Ericsson et al., 1981).

Imperial Chemical Industries (ICI) of Great Britain utilize a *Pseudomonas* sp. bacterium (*Methylophilus methylotrophus*) for SCP from methanol. The major technical innovation of the ICI process involves the design and development of their pressure cycle fermenter (see Figure 10). This fermenter is of an air-lift / tower design such that oxygen injected at the base is rapidly dissolved due to hydrostatic pressure. Thus, the mass-transfer characteristics of this system are extremely favorable. Excessive heats of fermentation are removed by a cooler located at the base of the fermenter. Cooling requirements are limited since the organism is thermotolerant (Slater, 1974).

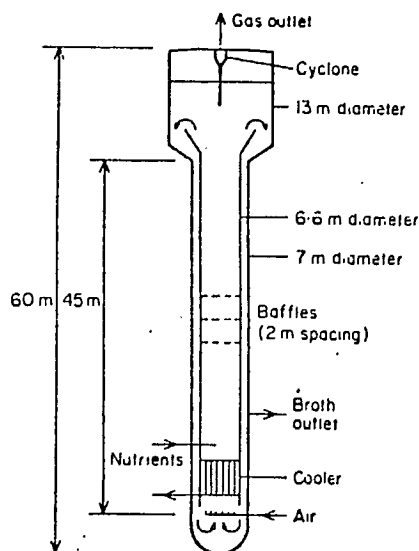


Fig. 10: Schematic Diagram of ICI Pressure Cycle Fermenter

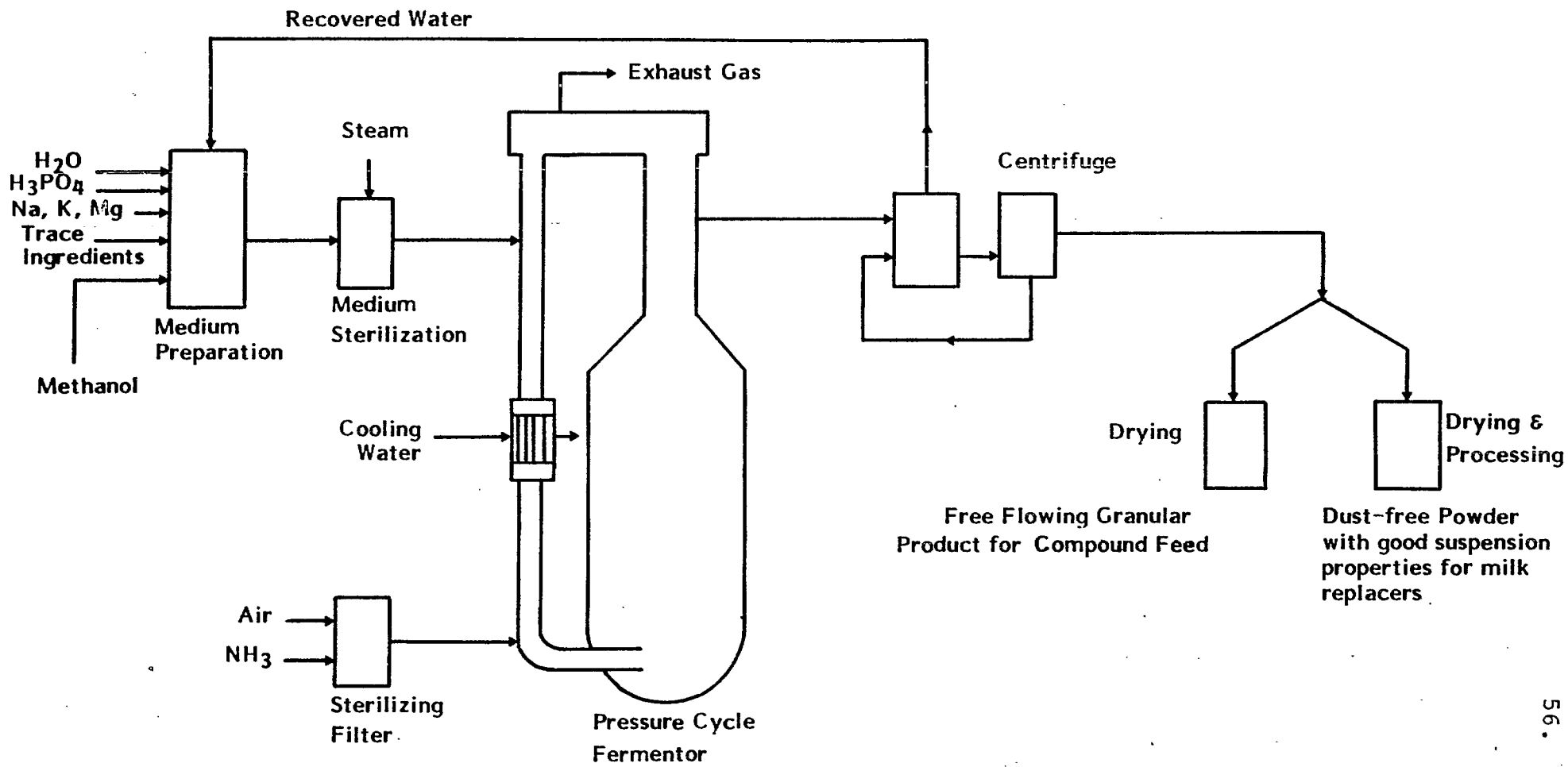
(Source: Hamer, 1979)

An industrial plant was designed and constructed by John Brown Engineers and Constructors Ltd. (Portsmouth) in Billingham, UK, with a capacity of 60,000 metric tons/year of SCP. The product (Pruteen, 72 - 77 % protein) is sold at US \$ 675 - 750 / metric ton. (The present selling price of soy bean is US \$ 212 / mt.) The schematics of the ICI process are presented in Fig. 11 .

The ICI strategy is to sell SCP in specialty markets. Specialty markets have existed already for a number of years. As an example Pure Culture Products, a wholly-owned subsidiary of Amoco Chemicals (Hutchinson, Minn.), has been selling SCP since 1975 to some 200 food companies in U.S., Canada and Europe for US \$ 1,20 to US \$ 1,30 / Lb, as a flavouring for processed meats. The Pure Culture SCP is the yeast Candida utilis continuously grown on ethanol made from corn or wheat starch that meets U.S. Pharmacopeia standards. Added to ethanol are such yeast nutrients as phosphoric acid, potassium hydroxide and magnesium sulfate. The product is centrifuged, pasteurized and spray-dried (Zanetti, 1984).

Phillips Petroleum Co (Bartlesville, Okla.) has also developed a process for continuous growth of yeast on methanol (as well as on ethanol and sugar substrates; Wegner, 1983). Yeast strains were derived from genera Candida, Pichia, Hansenula and Torulopsis. The major advantage of this process is that fermenter effluent (SCP and unused nutrient) is spray-dried directly without being concentrated by centrifuging or filtration, thus eliminating the need for treating recycle or waste streams. The schematic of the process is shown in Fig. 12. The product has been proven safe with rats, pigs, chickens, ducks, fish and ruminants.

Fig. 11: Schematics of the ICI SCP production from methanol



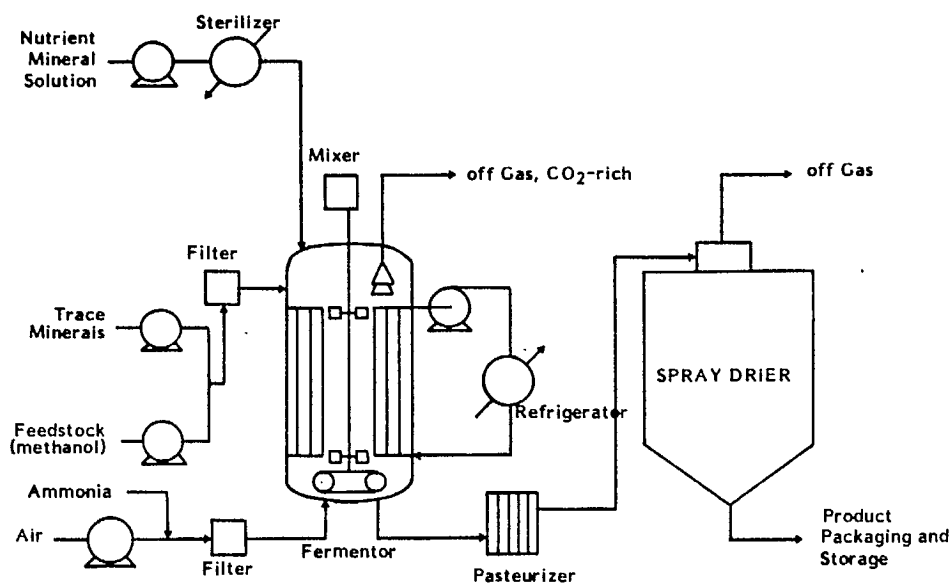


Fig. 12 : The Phillips process for production of SCP from methanol.

(Source: Wegner, 1983)

Hoechst/Uhde (FRG) has developed and tested a process for production of SCP from methanol by a bacterium Methylomonas clara (Präve et al., 1975; Präve and Faust, 1979; Puhar and Fiechter, 1980; Faust et al., 1980; Schlingmann et al., 1980). The process is described in more detail in section 3.2 of this report.

2.3.2.3 Ethanol

This feedstock offers all the technical advantages seen for methanol with a more extensive range of microbes capable of its assimilation. As well, since the feedstock is non-toxic special treatment is not required for biomass purification.

Ethyl alcohol may be produced by the catalytic oxidation of

ethylene or by anaerobic fermentation. World capacities for synthetic ethanol production are about 2 million tons per year while that for fermentation based ethanol is just under half that value (Dimmling and Seipenbusch, 1978). The global production of this feedstock is likely to change rapidly over the next few years.

2.3.3 WASTE PETROCHEMICALS

2.3.3.1 Flare Gases

It has been estimated (Slater, 1974) that methane containing flare gases burned off at various sites throughout the globe could be used to produce about 70 million tons of methanol per year.

Due to the diverse composition of flare gases and a high time-dependant variability for the relative amount of each component (Gaspar, 1982) in the mixture, this feedstock may pose great technical problems in its effective utilization.

2.3.3.2 Plastics

A novel process was reported by Karthigeson and Brown (1981) whereby waste plastics from domestic refuse were converted to SCP using pyrolysis followed by fermentation. The composition of pyrolysates vary depending upon the source material. Generally, large concentrations of straight chain hydrocarbons (C_8 to C_{40}) were obtained. Cell yields were generally low using the yeast, Candida tropicalis. Protein content of the organism was 17 % less than normal. Technical difficulties include agitation and mass-transfer problems due to the waxy nature of the pyrolysate.

In spite of these drawbacks, the potential of this feedstock warrants further research.

2.3.4 COMPARISON OF PETROCHEMICAL FEEDSTOCK FOR SCP PRODUCTION

Tables 17 and 18 compare various petrochemical feedstocks on the basis of potential cell yields, protein yields, and

substrate costs.

When the price of substrate is adjusted to normalize for protein yield, it can be seen that methane and methanol offer the most attractive economic characteristics based on substrate cost. Comparisons of this type are difficult and it is important that processing requirements be taken into account before a complete analysis can be made.

Table 17 : Cell conversion yields on various substrates

| Carbon source | Organism | Temperature (°C) | Cell yield (gm cell/gm substrate) | Reference |
|---------------|-----------------------------|------------------|-----------------------------------|------------------------------|
| n-Paraffins | <u>Pseudomonas</u> sp. | 30 | 1.07 | Wodzinski and Johnson (1968) |
| | <u>Nocardia</u> sp. | 30 | 0.98 | Wodzinski and Johnson (1968) |
| | <u>Candida intermedia</u> | 30 | 0.83 | Miller and Johnson (1967) |
| Methane | Mixed bacteria | 40 | 0.62 | Sheehan and Johnson (1971) |
| Methanol | <u>Candida boidinii</u> | 28 | 0.29 | Sahm and Wagner (1972) |
| | <u>Hansenula polymorpha</u> | 37 | 0.37 | Levine and Cooney (1973) |
| | Mixed bacteria | 56 | 0.42 | Snedecor and Cooney (1974) |
| | <u>Pseudomonas</u> C | 32 | 0.54 | Goldberg et al. (1976) |
| Ethanol | <u>Candida utilis</u> | 30 | 0.68 | Johnson (1967) |
| Glucose | <u>Candida utilis</u> | 30 | 0.51 | Johnson (1969) |

Table 18 : Cell cost in the production of SCP

| Substrate | Organism | Protein yield ^a (gm/gm substrate) | Substrate cost ^b (c/kg substrate) | Substrate cost (c/kg SCP) |
|----------------------|----------|--|--|---------------------------|
| n-Paraffin | Yeast | 0.50 | 33 | 66 |
| | Bacteria | 0.80 | | 41 |
| Methane ^c | Bacteria | 0.50 | 10 | 20 |
| Methanol | Yeast | 0.20 | 15 | 75 |
| | Bacteria | 0.40 | | 38 |
| Ethanol | Yeast | 0.41 | 41 | 100 |
| | Bacteria | 0.54 | | 76 |
| Glucose | Yeast | 0.31 | 17 | 55 |
| | Bacteria | 0.41 | | 41 |

^a For this comparison, the crude protein (N x 6.25) for yeast and bacteria has been taken as 0.60 and 0.80 gm/gm, respectively.

^b Cost data for March, 1979. Source: Chemical Marketing Reporter.

^c Cost calculation based on a methane fuel value of \$ 2 per million Btu.

2.4 MISCELLANEOUS FEEDSTOCKS

2.4.1 MUNICIPAL AND AGRICULTURAL ORGANIC WASTES

The production of municipal sewage in North America is approximately 200×10^6 tons per year. Waste manures arising from various animal industries are about 10 times this value at 2×10^9 tpa (Hesseltine, 1977) Cattle manure accounts for 70 % of this production.

The high BoD content of these wastes ($\sim 20,000$ mg/l) make it extremely important that they be treated before disposal. At present, animal manures are spread on land and thus are causing serious environmental problems.

Well developed collection systems which lead to pre-existing treatment plants are favorable characteristics for the utilization of human waste. Livestock manure is also easily collected in the case of feedlot operations, however, its characteristic low moisture content and high concentrations of inorganic materials such as grit, cement dust and gravel could make separation procedures necessary (Cowley and Wase, 1981).

A number of processes are capable of converting manure to high protein feedstuffs. Due to the high nitrogen content of this material (see Table 19) its potential in the production of algae biomass is extensive. Biomass productivity of up to $82,000$ kg/ha-yr (45 - 65 % protein) is possible under high rate algae pond techniques (Advisory Committee on Technological Innovation, 1981). However, these HRAP processes are limited to geographical regions with high daily incident solar energy.

Ensilage of manure with dry fodder is a successful method of fortifying the feed with protein. The organisms in this case involve large undefined populations of bacterial anaerobes, coliforms, spores, yeast, and fungi which are naturally found in faeces and urine. It has been found that treatment of cracked corn by this technique enhanced the total organic nitrogen of the final product by levels of 12 % (Rhodes and Orton, 1975).

Table 19: Composition of Cattle and Pig Wastes (% d.m.)

| Component | Cattle (%) | Pig (%) |
|-----------------|------------|---------|
| Volatile solids | 81.0 | 76.0 |
| Cellulose | 24.5 | 7.5 |
| Lignin | 12.7 | 3.0 |
| Total Nitrogen | 1.204 | 1.918 |
| Crude Protein | 7.53 | 11.96 |
| C/N Ratio | 39.02 | 22.98 |

(Source: Jain, 1982)

It is not likely that these organic wastes will be extensively utilized for SCP production in the near future. Despite the obvious psychological difficulties consumers would exhibit for this SCP product there is also concern of contamination by pathogenic organisms (Salmonella or Brucella), trace heavy metals (Cu or Zn), and various disinfectants used to wash out the feedlot (chlorinated or phenolic hydrocarbons) (Cowley and Wase, 1981).

2.4.2 MINE WASTES

The mining industry produces a large volume of hydrogen sulfide which is considered as a troublesome waste and not economically useful as a feedstock for conversion to any other sulfur compounds. It is most often incinerated and discharged to the atmosphere as sulfur dioxide where it contributes to the pollutant load. At present the U.S. produces ~ 49.3 mmt of SO₂ per year (EPA, 1973).

Recently it has been discovered (Jannasch, 1979; Jannasch and Wirson, 1979) that large populations of mussels, clams, and other sea organisms are able to successfully feed on non-defined cultures of chemolithotrophic bacteria. These microbes

are able to utilize H_2S emitted from deep sea vents on the ocean floor. Thus, interest has been shown in the utilization of waste H_2S as an energy source for bacterial growth which in turn will support the commercial production of shellfish. The advantage of such a system resides in its freedom from the requirement for light since hydrogen sulfide provides the reducing energy to produce carbohydrate from CO_2 and H_2O . The system is found to be self-inoculating with common sea water providing all nutrients except H_2S , O_2 , and a nitrogen source.

2.4.3 OIL AND FATS OF ANIMAL ORIGIN

Animal fats and greases are produced at the slaughterhouse, packinghouse, and processing stages of the meat and poultry industries. These greases and fats are closely associated with highly organic nitrogenous wastewater and as such, BOD_5 values of up to 3,000 ppm are obtained (Kosaric, 1976). Vass et al. (1976) have described a process by which the grease derived from pork slaughtering operations is added to an inorganic mineral media at a concentration of 1 % w/v. Subsequent aerobic fermentation with the yeast Candida utilis has led to the complete conversion of the grease to biomass within 10 - 12 hours. The yield of dry yeast relative to pork fat was found to be 75 %.

3. SELECTED INDUSTRIAL PROCESSES

3.1 SCP FROM WASTE SULFITE LIQUORS

As already mentioned in section 2, waste sulfite liquors can be used as a substrate for SCP production. These liquors represent a highly polluting waste stream from pulp and paper industry which has to be treated before discharge. Today's practice in most industries is either to concentrate the liquor to about 30 - 50 % solids and then burn the organics (predominantly lignin sulfonates) in a conventional or fluidized bed furnace (e.g. Copeland process). Biological treatments employing activated sludge processes and aerated lagoons are also practiced. However, by using these liquors as a raw material for SCP production an economic benefit is obtained as a product with nutritional quality is produced from a waste.

One problem, however, in using the WSL for large scale SCP production is the present availability of this liquor. Because of stringent environmental protection laws, many pulp and paper mills are converting their processes either to Kraft (sulfate) or to "Zero discharge" process modifications. This situation particularly happened in Finland where already a well developed SCP production from WSL was established (PEKILO process). Most of their facilities are now closed as the waste liquor is no more available.

Another problem in microbiological treatment of waste sulfite liquors is also the fact that the bulk of organics in lignosulfonates are not metabolized. This means that the effluents from such an SCP plant will still have to be treated for elimination of lignosulfonates which may be toxic to the environment even though their presence in water does not increase the BoD level (Biochemical Oxygen Demand).

Even with these drawbacks, WSL is considered in many countries as a viable substrate for SCP production. Two processes are discussed here - one operating in Attisholz, Switzerland and the other developed by the Czechoslovak Academy of Sciences and being presently constructed in Czechoslovakia.

Both of the above considered two processes utilize yeast. Yeast has been a preferred microorganism for production of protein particularly because of its good nutritional value, digestibility and general acceptance and use as food for humans for centuries (e.g. baker's yeast).

3.1.1 CELLULOSE ATTISHOLZ (SWITZERLAND) PROCESS

Cellulose Attisholz AG has produced yeast on a continuous basis since 1943 from waste sulfite liquor. Until 1975 yeast was manufactured for use in a variety of animal feeds. The production of food grade yeast for human consumption commenced on a commercial scale in February 1976. The food grade yeast is at present produced in addition to the feed grades on an alternating basis. Torula utilis is grown on a mixture of beech spent sulfite liquor and spruce vinasse (after alcohol fermentation). The process flowsheet is presented in Figure 13.

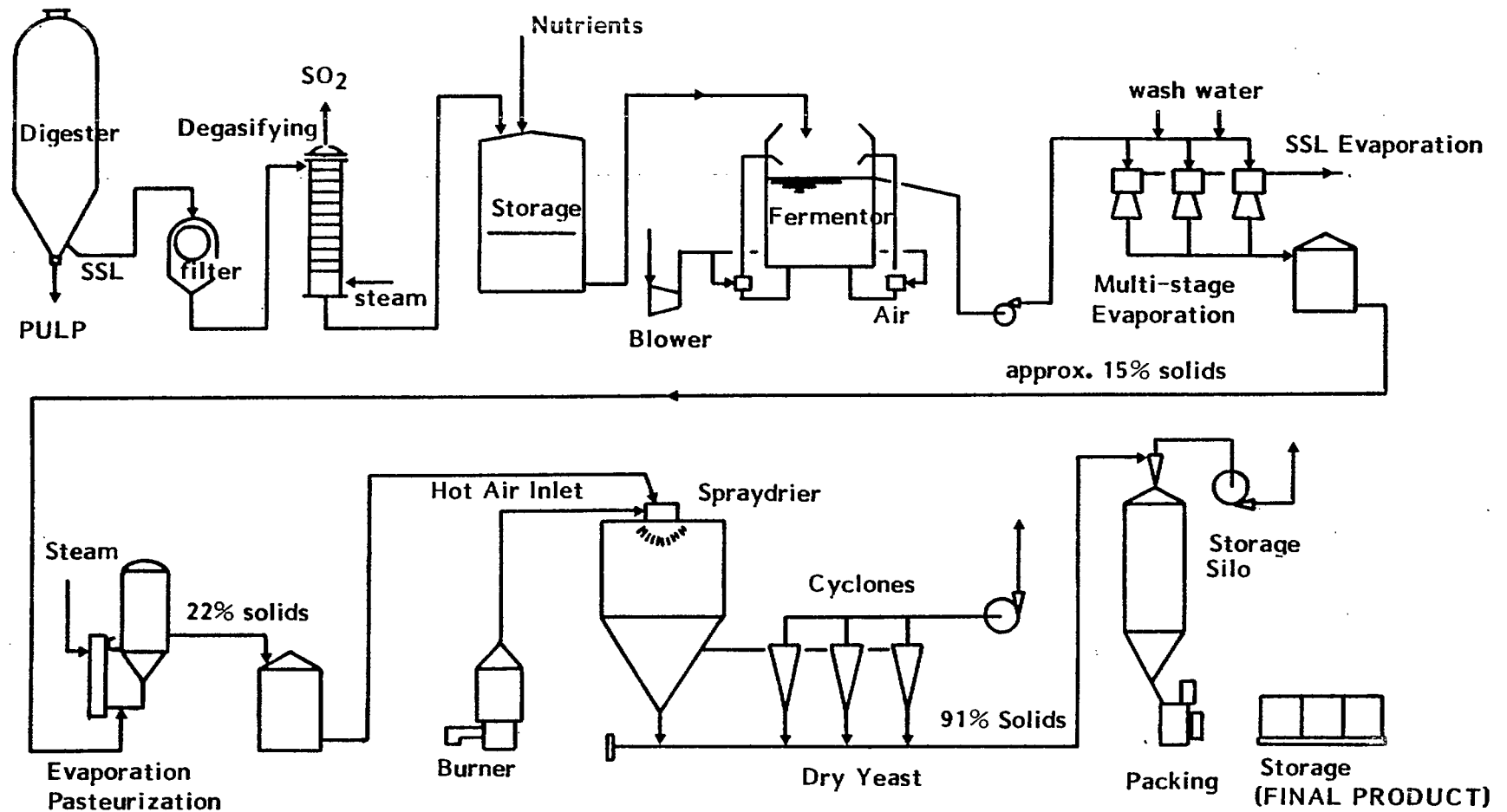
In the process, both sulfite waste liquors pass a rigorous thermal pretreatment in a degasifying column, followed by a continuous neutralization and an addition of all nutrients, which are necessary for a satisfactory growth of yeast.

The yeast fermenter, essentially, consists of a number of circulatory tubes, which are placed outside and around the central vat in a radial manner. These tubes are equipped with air injectors. They serve several important purposes:

Air injection takes place at the lower part of the tubes and brings about circulation (air-lift pump system). At the same time, the substrate is enriched with sufficient quantities of air-oxygen. Furthermore, the radial inflow of the circulated substrate ensures a thorough homogeneous mixing as well as a maximum expulsion rate of the carbon dioxide formed during fermentation. Besides, this type of aeration and circulation system provides a simple, effective and economic solution to the cooling problem arising from the strongly exothermic yeast fermentation process.

The fully developed, matured growth yeast is withdrawn from the fermenter by a continuous overflow system. It is, then,

Fig. 13: Production line for the manufacture of food-grade and feed-grade yeast



isolated from the depleted substrate by separators. A multi-wash of several stages follows, using water. The resulting yeast cream is passed to a two-stage evaporation plant, where it is subjected to thermolysis, simultaneously effecting pasteurization. After evaporation, a solids content of 22 % is achieved. Finally, the thickened yeast cream is dried to a marketable, finely powdered product, using a spray drier.

At present, Cellulose Attisholz AG produces more than 7,000 t per year of high quality yeast for human consumption as well as for domestic animal feeds. The yeast represents a product of highly nourishing properties due to its raw protein content of 53 - 55 % and other valuable components, such as trace elements and vitamins. The dried yeast is a beige to light brown powder with the typical odour and taste of yeast.

The technical data on the process are shown on the accompanying tables 20, 21 and 22.

TABLE 20
 TECHNICAL DATA SHEET OF ATTISHOLZ YEAST PLANTS

| | | |
|-----------------|--------------------------|--|
| <u>Example:</u> | Basic substrate | = Spent sulfite liquor (Ca-, Mg-, NH ₄ - or Na-bisulfite) |
| | Product | = Food-grade yeast / Feed-grade yeast |
| | Days of operation | = 350 days per year (or 365 d/y, depending on plant design) |
| | Yeast production | = 7,000 t / year |
| | Pulp production (spruce) | = 50,000 - 70,000 t / year |
| | Total costs of plant | = Approx. US \$ 10 - 12 mio. / Turn-key project, prices 1976 (prices depend on industrial infrastructure and other conditions at construction site) |
| | Space requirements | = Approx. 45 m x 80 m. This includes space requirements for complete plant installation, storage tanks and warehouses. |

Approximate Requirements and Consumptions per Ton of Yeast Produced

| | | | |
|-----------------------|--|-----------------------------|--------------------------------|
| <u>Raw Materials:</u> | - 500 kg ammonia (25 % by wt.) | <u>Auxiliary Materials:</u> | - Water (mostly cooling water) |
| | - 280 kg superphosphate (16 % P ₂ O ₅) | | - Defoamer |
| | - 50 kg potassium chloride (60 % K ₂ O) | | - Cleaning chemicals |
| | - 20 kg magnesium sulfate (MgSO ₄ · 7 H ₂ O) | | |

Energy Consumptions

| | | |
|--|---|---|
| <u>1. Heat</u> | <u>2. Cooling</u> | <u>3. Electrical Power</u> |
| Most heat is required for SSL-pretreatment, yeast evaporation and yeast drying. Smaller amounts of heat are used for solution preparation of some chemicals. | The heat of reaction amounts to 2.5 - 3.0 mio. Kcal/h. In most cases, this is removed by cooling water. As an alternative, cooling is aided by refrigeration. Depending on climatic conditions, the most economical type is chosen. | Fermenter aeration and yeast separation require most of the electrical energy. A small fraction is used by spray drying and pumps. The average specific energy consumption is 1.5 kWh/kg yeast. |

Personnel Requirements (Western European conditions / largely automated operations)

| | | |
|--------------------------|-------------------------|--|
| 1 Yeast plant supervisor | 1 Laboratory technician | 15 Men (includes day workers and all shifts) |
|--------------------------|-------------------------|--|

Table 21 : Analysis of Yeast (Food Grade) AttisholzAnalysis (average values)

| | |
|-------------------------------------|-----------|
| Protein (dry matter content) | 54 - 57 % |
| N-free extract (carbohydrates) | 28 - 35 % |
| Ash content at 650°C | 7 % |
| Moisture content at 105°C (max. 8%) | 7 % |
| Fat content | 6 - 8 % |
| Fiber | 3 - 7 % |

Mineral salts and trace elements

| | |
|---|---------|
| Phosphorus (P ₂ O ₅) | 3.9 % |
| Potassium (K ₂ O) | 1.8 % |
| Calcium (CaO) | 0.7 % |
| Magnesium (MgO) | 0.3 % |
| Iron (Fe) | 0.02 % |
| Zinc (Zn) | 100 ppm |
| Copper (Cu) | 8 ppm |

Amino acids

| | | | |
|---------------|--------|------------------|--------|
| Lysine | 3.94 % | Cystine | 0.58 % |
| Methionine | 0.63 % | Alanine | 3.72 % |
| Leucine | 3.56 % | Arginine | 2.74 % |
| Isoleucine | 2.26 % | Asparaginic acid | 5.05 % |
| Phenylalanine | 2.18 % | Glutamic acid | 9.71 % |
| Threonine | 2.40 % | Glycine | 2.27 % |
| Tryptophane | 0.72 % | Proline | 2.02 % |
| Valine | 2.61 % | Serine | 2.27 % |
| Histidine | 1.02 % | Tyrosine | 1.73 % |

Vitamins

| | |
|---------------------|---------|
| Thiamine (B1) | 35 ppm |
| Riboflavin (B2) | 53 ppm |
| Pyridoxine (B6) | 9 ppm |
| Nicotinic acid (PP) | 347 ppm |

Microbiological analysis

| | |
|--------------------------|---------------------|
| Mesophilic aerobic germs | max. 10,000 per 1 g |
| <u>E.coli</u> | absent in 1 g |
| <u>Salmonella</u> | absent in 100 g |

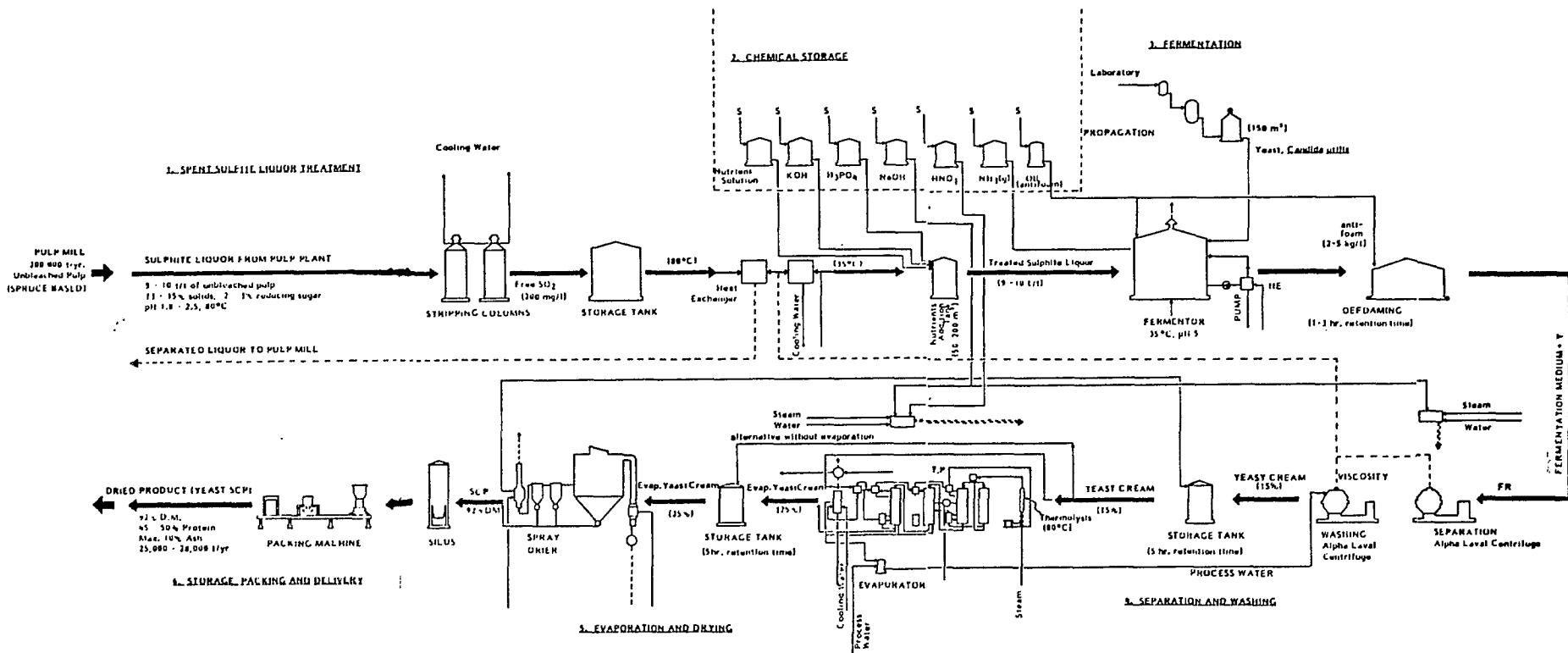
Table 22 : Attisholz Feed-Grade Yeast (Torula utilis)Average Analyses Values

| | |
|---|---------------|
| Dry substance | 90 % |
| Crude protein (based on dry substance) | 51 - 53 % |
| Crude fat: Total lipid | 6 % |
| Determined directly with petroleum ether | 0.1 % |
| Determined acc. to Berntrop | 3 - 4 % |
| Ash content | 7.5 % |
| Crude fiber | 1 - 2 % |
| Phosphorus (P ₂ O ₅) | 4 % |
| Potassium (K ₂ O) | 1.8 % |
| Calcium (CaO) | 0.7 % |
| Magnesium (MgO) | 0.3 % |
| Iron (Fe) | 0.02 % |
| Arsenic (As) | 0.7 ppm |
| Mercury (Hg) | 0.2 ppm |
| Lead (Pb) | 0.6 ppm |
| Cadmium (Cd) | 0.5 ppm |
| Copper (Cu) | 4.0 ppm |
| Vitamins: B1 | 1.5 mg/100 g |
| B2 | 5.3 mg/100 g |
| B6 | 2.8 mg/100 g |
| PP | 52.4 mg/100 g |

3.1.2 CZECHOSLOVAK ACADEMY OF SCIENCES PROCESS

A process for conversion of waste sulfite liquor to yeast SCP (Candida utilis) was developed by the Microbiology Institute of the Czechoslovak Academy of Sciences in Prague. A plant utilizing this process is presently being constructed for a spruce base pulp and paper mill in Paskov, Czechoslovakia that has an estimated capacity of 200,000 t of unbleached pulp per year. The pulping is an acid bisulfite Mg-base process utilizing 2 million tons of wood per year. The process flowsheet with a material balance is presented in Figure 14.

Fig. 14: The Czechoslovak Academy of Sciences SCP Process



The sulfite liquor from the pulp plant containing about 2 - 3 % of reducing sugars is first stripped of SO_2 in stripping columns or by use of the 4th stage of an existing multiple stage vacuum evaporator system. The stripped liquor, free of SO_2 , is further introduced into a storage tank having a residence time of approximately 10 hours. After cooling (from 80°C to 35°C) in a heat exchanger, nutrients in the form of KH_2PO_4 , H_3PO_2 , and $\text{NH}_3(\text{g})$ are added along with a complex nutrient solution of undisclosed composition.

The fermentation is performed by Candida utilis which is separately prepared in a 150 m^3 reactor. There are three main fermenters each of 800 m^3 capacity operating at 35°C and $\text{pH} \sim 5$.

The fermenter is, during operation, considered to be filled only to $1/3$ of its total volume. The fermentation liquid is aerated at a rate of $0.7 - 1.3 \text{ kg O}_2/\text{kg yeast}$ which is kept in the fermenter at a concentration of 1.5 - 2%. The specific productivity is estimated at $2.5 - 5.5 \text{ kg yeast}/\text{m}^3 \text{ liquid volume per hour}$ at a dilution rate of $D = 0.25 - 0.35 \text{ h}^{-1}$. An oil base antifoam is added as needed and the foam is collected in the defoaming tank which has a residence time of 1 - 3 hours.

The fermented medium passes through Alpha-Laval centrifuges for separation of the liquor which is then introduced into a five stage multiple effect evaporator and concentrated to about 50 % solids prior to incineration in a conventional recovery furnace.

The yeast product is further washed with process water in another Alpha-Laval centrifuge from which a yeast cream at about 15 % concentration is continuously withdrawn. Another storage tank allows for about 5 hours retention time prior to introduction of the cream into yeast evaporators from which a 25 % yeast cream is withdrawn.

The 25 % yeast cream is further dehydrated in a conventional spray drier from which a dried product (min 92 % DM) is further transported to the storage, packaging and expedition department.

The dried product contains 45 - 50 % protein. The nutritional quality of this protein product was separately tested by six state research institutes (from 1974 - 1978; total cost ~ 60 mill. Czech. Crowns).

The nutritional quality of the product was tested on animals such as laying hens, chicken, broilers, ducks, fish (carp), pigs and young pigs. With these animals, the following parameters were evaluated:

- growth
- egg laying
- young pig production
- fertility
- state of health
- analytical data of products
- organoleptic characteristics of products
- presence of unwanted materials in the product
- biological properties of tested animals and their offspring.

The yeast was added to the diets in the amount of 3 - 10% and in some cases also at a level of 15%. The yeast was used as a substitute of fish meal or soya meal.

The results showed that the addition of yeast in the amount of 10% had the same effect as the diets containing the equivalent amounts of fish- and/or soya meal. (Diets, however, for younger animals contained only 3-5% yeast depending also on the kind of tested animals). No adverse effects were noticed either on the animals or their products (meat, liver, eggs). The product quality is shown in Tables 23 and 24.

The present market price of yeast in Czechoslovakia is as follows (supplied by CSA Sci.):

| | |
|--------------------------|---------------|
| Feed yeast from molasses | 2,500 Kcs/ton |
| Feed yeast from ethanol | 9,500 Kcs/ton |
| Feed yeast from WSL | 6,500 Kcs/ton |

An economic analysis on this process and product is made as an example for our economics and marketing analysis, which is presented in detail in the economics section of this report.

In general, the process in Paskov is a conventional SCP-process the innovation being primarily in treatment of the liquor prior to fermentation. By using the existing pulp and paper mill multiple stage evaporators for stripping the liquor of SO₂, a savings in total energy and steam consumption should be accomplished.

Table 23 : Feed yeast from WSL and from ethanolChemical composition

| | Ethanol yeast | WSL yeast | | |
|--|------------------|--------------|-----|------|
| Dry matter | 92.75 | 93.66 | | |
| N-material (Raw protein) | 48.62 | 48.69 | | |
| Fibers | 0.10 | 2.34 | | |
| Fat | 0.21 | 0.25 | | |
| Ash | 7.38 | 9.65 | | |
| Extractive N-free materials | 36.45 | 32.82 | | |
| Dry substance | 92.41 | 93.56 | | |
| N-materials | 48.97 | 48.94 | | |
| Fat | 4.93 | 4.32 | | |
| Ash | 6.53 | 8.33 | | |
| Protein | 45.61 | 46.52 | | |
| Unsaponified | 1.7-7.65 | 4.5-12.36 | | |
| Heavy metals (ppm/DM) | | | | |
| As | 2 | 2 | | |
| Pb | 1.4 | 2.1 | | |
| Ca | 5.0 | 5.0 | | |
| Benzpyrene (10^{-12} /DM) | 1.2 | 1 | | |
| Accumulator of benzpyrene in eggs (10^{-12} /DM) | 5 % | 10 % | 5 % | 10 % |
| Control 100 | 202 | 208 | 203 | 206 |

(Source: Inst. of Microbiology, CSAV-Prague)

Table 24 : Amino acid composition in the feed yeast from ethanol and WSL (% in dry matter)

| <u>Amino acid</u> | <u>Ethanol yeast</u> | <u>WSL yeast</u> |
|-----------------------------|----------------------|------------------|
| Tanrin | - | - |
| Methionine sulfoxyd | trace | trace |
| Hydroxy proline | - | - |
| Aspartic acid | 4,903 | 3,965 |
| Threonine | 3,104 | 2,595 |
| Serine | 2,945 | 2,568 |
| Glutamic acid | 7,104 | 6,425 |
| Proline | 1,257 | 1,203 |
| Glycine | 2,095 | 2,018 |
| α -alanine | 3,064 | 2,848 |
| Cystine | | |
| α -aminobutyric acid | | |
| Valine | 2,652 | 2,785 |
| Methionine | 0,641 | 0,589 |
| Isoleucine | 2,267 | 2,108 |
| Leucine | 3,571 | 3,827 |
| Tyrosine | 2,036 | 1,761 |
| Phenylalanine | 2,141 | 1,967 |
| γ -aminobutyric acid | 0,143 | 0,067 |
| Lysine | 3,677 | 3,414 |
| Ornithine | 0,217 | 0,182 |
| Ethanolamine | - | - |
| NH ₃ | 0,927 | 0,670 |
| Histidine | 0,852 | 0,848 |
| Arginine | 2,029 | 2,278 |
| β -alanine | | |

(Source: Inst. of Microbiology, CSAV, Prague)

3.2 SCP FROM METHANOL

3.2.1 BACTERIAL BIOMASS AS A PROTEIN SOURCE

While yeast has been used directly for human food in various forms (particularly during 1st and 2nd World Wars in Europe) this was not the case with bacteria. Therefore, when concerning bacterial biomass, animal feed supplements would have to be considered at least in the first phase of introduction of this product to the market. There are both advantages and disadvantages when using bacteria for SCP and these are evaluated below.

Numerous non-pathogenic bacteria can utilize a wide variety of oxidized and reduced forms of carbon or nitrogen, and can withstand marked variations in growth conditions (ex. thermophilic bacteria). Some other advantages are:

- a) bacteria can grow four times as rapidly as yeasts and twenty times as fast as algae,
- b) their crude protein content may reach the 85 % range,
- c) the amino acid profile shows better balance of key essential amino acids such as methionine, lysine, and tryptophane than yeasts and algae,
- d) they are a good source of water-soluble vitamins, especially vitamin B₁₂,
- e) the sulfur amino acid content is higher than in soya meal,
- f) the mineral content is a function of the media and the process in general, calcium, magnesium and potassium content is midway between cow's milk and eggs, whereas the phosphorus content is higher than milk and egg (Waslien, 1975),
- g) the operating fermenting pH is near neutral.

There are also a number of concerns when bacteria are considered for food. Humans are unfamiliar with bacterial protein,

and do associate "bacteria" mainly with disease. Besides the adverse effect of the high nucleic acid content (discussed elsewhere), some non favorable characteristics, specific to bacteria, can be summarized as follows:

- a) bacteria are susceptible to bacteriophages, which decrease or even stop the biomass production,
- b) if gram-negative bacteria are used, they produce endotoxin, which must be removed subsequently,
- c) may be a possibility of hybrid formation with potential pathogenicity,
- d) they lack carotenoids and vitamins A and D; there are no reports on ascorbic acid level,
- e) the recovery of cells costs more for bacteria than for yeast because of their smaller size (0.5 to 2 μ).

Bacterial SCP has been considered and investigated by a great number of researchers. A summary of selected work on bacterial proteins is shown on Table 25. More detailed information can be found in reviews on the subject by Lipinsky et al. (1970), Waslien (1975) and Litchfield (1977).

Table 25 : Studies on bacterial SCP production

| Microorganism | Substrate | Reference |
|--|---|--|
| <u>Acinetobacter calcoaceticus</u> | ethanol | Laskin, 1975 |
| <u>Acinetobacter cerificans</u> | pure alkanes | -"- |
| <u>Aeromonas hydrophilia</u> | cheese whey | Butany & Ingledaur, 1973 |
| <u>Alcaligenes hydrogenophilus</u> sp. | H ₂ | Ohl et al., 1979 |
| <u>Bacillus megaterium</u> | collagen meat packing waste | Bough et al., 1972 |
| <u>Bacillus subtilis</u> | | Hackler et al. 1957 |
| <u>Brevibacterium</u> sp. | mesquite wood | Fu & Thayer, 1975 |
| <u>Cellulomonas</u> sp. | cellulosic waste sugar cane bagasse | Han et al., 1971 De Leon & Joson, 1980 |
| <u>Clostridium</u> sp. | jute-stick powder | Rahmatullah et al. 1980 |
| <u>Flavobacterium</u> | methanol | Hoechst, Germany |
| <u>Hydrogenomonas</u> ...tropa | H ₂ | Foster and Litchfield, 1964; Bongers, 1970 |
| <u>Lactobacillus casei</u> | cheese whey | Bernstein & Everson, 1973 |
| <u>Methanomonas methanica</u> | methanol | Hamer, 1968 |
| <u>Methylococcus capsulatus</u> | methane | Hamer, 1968 |
| <u>Methylomonas</u> sp. | methanol methanol, methanol/ formaldehyde | Goto et al., 1979 Papoutsakis et al., 1981 |
| <u>Methylomonas clara</u> | methanol | Faust et al., 1977 Hoechst/Udhe process |
| <u>Methylomonas methanolica</u> | methanol | Mogren, 1979; Nor protein |
| <u>Micrococcus cerificans</u> | alkanes | Esso-Nestlé process; Guenther and Perkins, 1968 |
| <u>Pseudomonas</u> sp. | ethanol or methanol methanol | Gow et al., 1975 Hoechst/Udhe process; Shell; Miura et al., 1980; Harrison et al, 1972 |
| | methanol and formaldehyde methane | Mateles et al., 1976 Harrison et al., 1972 |

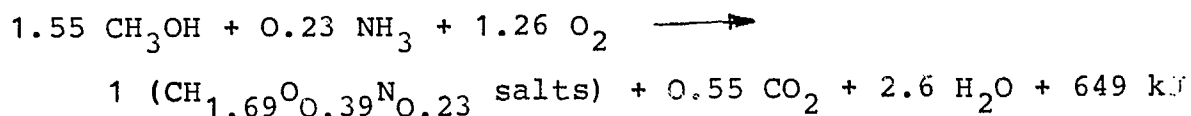
....table continued

| | | |
|------------------------------------|---|--|
| <u>Pseudomonas aeruginosa</u> | glucose, n-hexadecane, n-octadecane | Yamada et al., 1968 |
| <u>Pseudomonas butanovora</u> | n-C ₂ to C ₉ alkanes, C ₂ -C ₄ primary alcohols and carboxy acids, poly- valent C ₃ + C ₄ alcohols | Takahashi, 1980 |
| <u>Rhodopseudomonas gelatinosa</u> | agricultural by-product, "photosynthetic bacteria" | Shipman et al., 1975 |
| Mixed culture | activated sludge cellulolytic bacteria on sugar cane bagasse pith cellulomonas-Alcaligenes faecalia, on sugar cane bagasse | Hackler et al., 1957 Molina et al., 1980 De Leon & Joson, 1980 |
| | mixed bacteria on methane | BP process; Myers & Moran, 1980 |
| | mixed culture of methyl- omonas and Pseudomonas on methane | Shell Intern. 1975; Drozd et al. 1980 |
| | Methylomonas sp. on methanol | Kalunyants et al., 1977 |
| | Pseudomonas sp. on methanol | Kalunyants et al., 1977 |
| | mix of 7 bacteria on rice hulls | Chang et al., 1980 |

3.2.2 THE HOECHST/UHDE SCP PROCESS

At Hoechst in Germany, a continuous process for production of SCP has been developed in 1978 and a pilot plant constructed for production of 1000 metric tons of SCP/year.

The fermentation process uses methanol as a substrate for Methylomonas clara (Faust et al., 1977) according to the following conversion equation:



A detailed process flowsheet is presented in Figure 15. The inoculum is produced batchwise in a battery of breeding fermenters (50 l to 200 m³) under sterile and controlled conditions. Methanol is used as a carbon and energy source. Liquid ammonia is used as a nitrogen source. Other nutrient salts are also added to the medium such as phosphoric acid and K₂SO₄, MgSO₄, FeSO₄ and some trace elements.

It is important to closely control the addition and utilization of the medium during fermentation. Oxygen limitation as well as nitrogen limitation cause formation of a highly viscous polysaccharide-biopolymer which precipitates in the medium causing a rise in viscosity which lead to more oxygen limitation. An increase in the CO₂ partial pressure affects the amino acid content of the product while no changes at higher oxygen partial pressures were observed (Faust, et al., 1980). Also calcium-magnesium-, iron- and phosphate-salts can influence each other in a non balanced medium, which can lead to elemental limitations and unwanted precipitation of individual components.

Addition of methanol is also critical and it is controlled by measuring the methanol concentration in the off-gases. It is maintained at 50 ppm by uniform distribution of the added methanol throughout the fermenter so that development of localized high methanol concentrations in the fermenter is prevented.

The content of nucleic acids in the end product is directly proportional to the flow rate in the continuous culture. At higher dilution rates the total nitrogen content slightly increases but the real amino-acid-protein content decreases. The cell density of the culture increases at very low dilution rates but more lysis of old cells is also observed at this stage and with this the sensitivity of the culture to infection is consequently increased. These factors put an upper limit on the cell density. This upper limit depends also on the hydrodynamics of the fermenter whereby fermenters with a good mixing profile and controlled concentration gradients of the substrate components, do have an advantage. It is reported

Fig. 15: Hoechst/Uhde SCP-Process

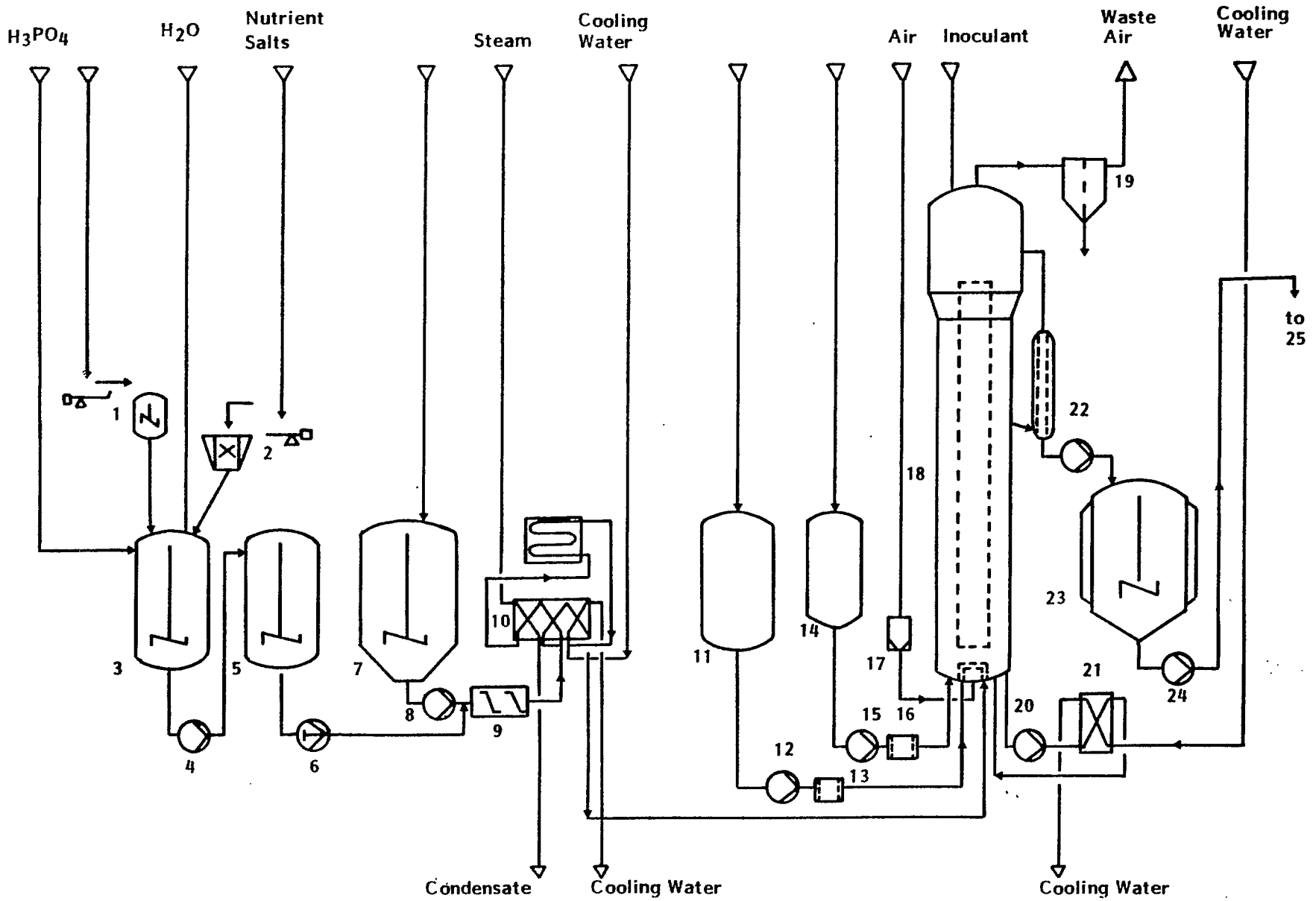


Fig. 15: (continued)

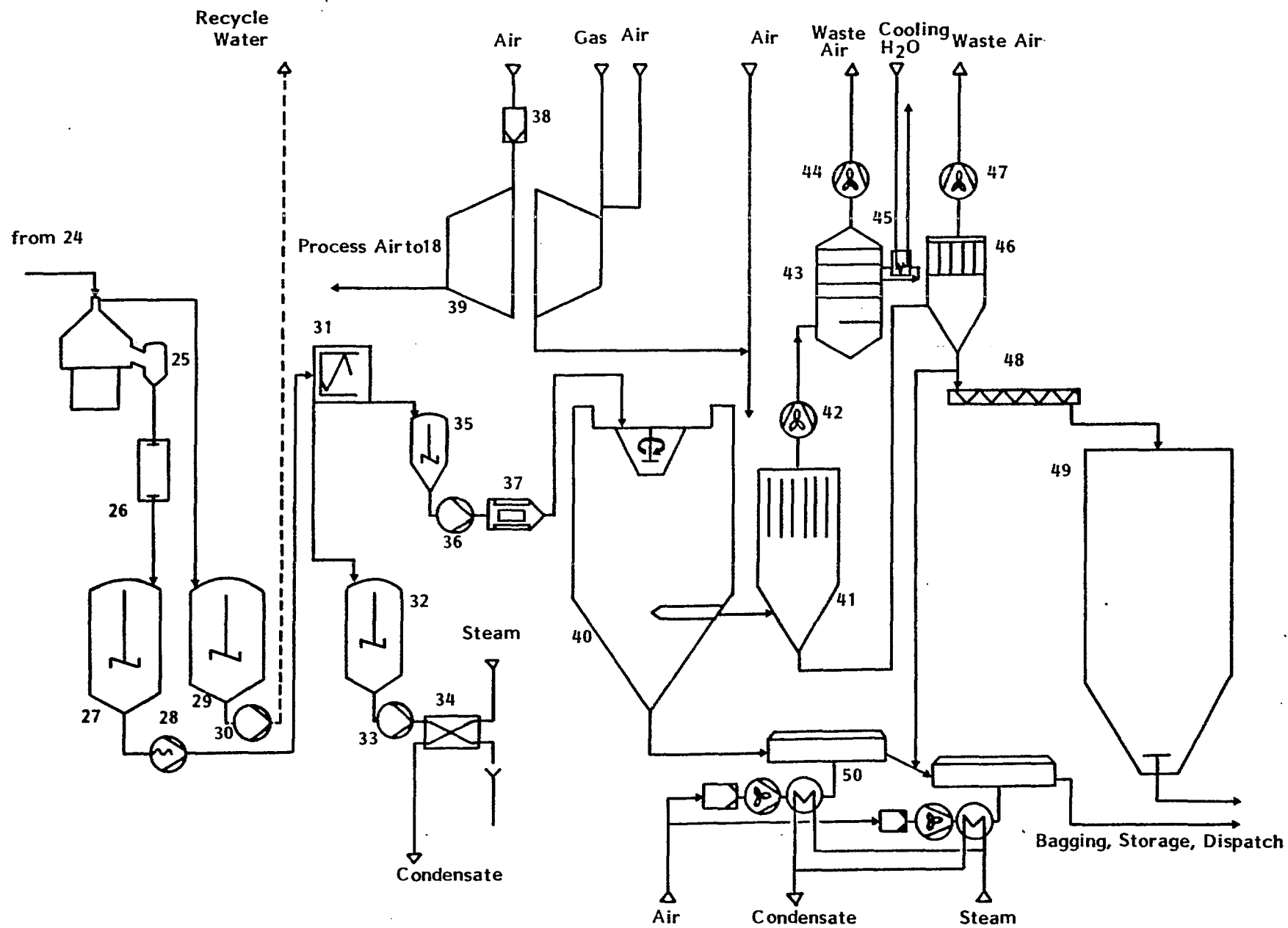


Figure legend

1. Mixing vessel for trace elements
2. Crushers
3. Mixing vessel for nutrient s...
4. Pump
5. Nutrient solution reservoir
6. Pump
7. Recycle water reservoir
8. Pump
9. Mixing device
10. Thermal sterilization section
11. Methanol reservoir
12. Pump
13. Sterilizing filter
14. NH_3 reservoir
15. Pump
16. Sterilizing filter
17. Sterilizing filter
18. Fermenter
19. Separator
20. Pump
21. Cooler
22. Pump
23. Harvest tank
24. Pump
25. Separator
26. Conditioning section
27. Concentrate vessel
28. Pump
29. Clear-liquid vessel
30. Pump
31. Decanter
32. Waste water tank
33. Pump
34. Waste water conditioning section
35. Concentrate vessel
36. Pump
37. Thermolysis facilities
38. Filter
39. Air compressor
40. Drier
41. Filter
42. Ventilator
43. Scrubber/cooler
44. Ventilator
45. Cooler
46. Solids separator
47. Ventilator
48. Screw conveyer
49. Silo
50. Granulating facilities

that the Hoechst/Uhde process is ideally controllable. All important parameters such as pH, temperature, aeration and flow rate are automatically controlled and kept at a constant value. The reaction heat generated during fermentation is dissipated continuously by external cooling cycles or by cooling elements installed in the fermenter.

The most important fermentation parameters are shown in Table 26.

Table 26 : Parameters for SCP production from methanol by the Hoechst/Uhde process

| | |
|--------------------------------|----------------------------|
| Temperature | 40°C |
| Average methanol concentration | 50 ppm |
| Doubling time | 3 h |
| Cell concentration | 15 g/l |
| Productivity | 5 g/lh |
| Yield | 0,5 kg biomass/kg methanol |
| Oxygen demand | 2,1-1,9 kg/kg protein |
| Fermentation heat | 30,000 kJ/kg protein |
| Dilution rate | 0.3-0.5 h ⁻¹ |

The cell suspension is continuously withdrawn after an average of 3 hours residence time and is further thickened in a two stage operation. In this process, the major quantity of water is mechanically separated and returned, after reesterilization to the fermenter as process water to which fresh nutrients are added. The cell mass, which concentration is increased at that point to 200-300 g/l, is then subjected to thermolysis. It is further dewatered in a spray drier or an air drier to a powder or granules. The carrying medium for drying is oil heated air. The dried product has about 5 % residual moisture.

The raw bioprotein so produced contains approximately 0 % crude protein. The firm, however, believes that this product

will not be cost competitive with soy protein until methanol feed costs 50% less than an equivalent amount of soybean meal. Therefore, the protein content is upgraded for human consumption also, to contain up to 90% protein (Zanetti, 1984). The improvement involves a purification process that breaks up bacterial cells in a nonaqueous solution. The lipids are dissolved and nucleic acids are extracted.

Table 27 shows a composition of the Hoechst/Uhde product in comparison with soy meal and fish-flour.

Table 27: Chemical composition of SCP products from the Hoechst/Uhde methanol process in comparison with soy meal and fish-flour

| Source | C-substrate | Use | a. acids | NPN ⁽¹⁾ | Fat | Ash | CH ₂ ⁽²⁾ | RM ⁽³⁾ |
|------------|--------------------------|------|-------------|--------------------|-----|-----|--------------------------------|-------------------|
| Bacteria | Methanol | food | 90 | 1 | 1 | 5 | - | 3 |
| Bacteria | Methanol | feed | 69 | 12 | 9 | 7 | - | 3 |
| Yeast | Molasses | feed | 41 | 5 | 6 | 8 | 33 | 8 |
| Soy meal | CO ₂ from air | feed | 41 | 5 | 1 | 6 | 36 | 11 |
| Fish-flour | Plankton | feed | 56 | 10 | 5 | 15 | 4 | 10 |

(1) non protein nitrogen (mainly nucleic acids)

(2) carbohydrates

(3) residual moisture

The possible processing of the SCP-biomass is shown in Figure 16.

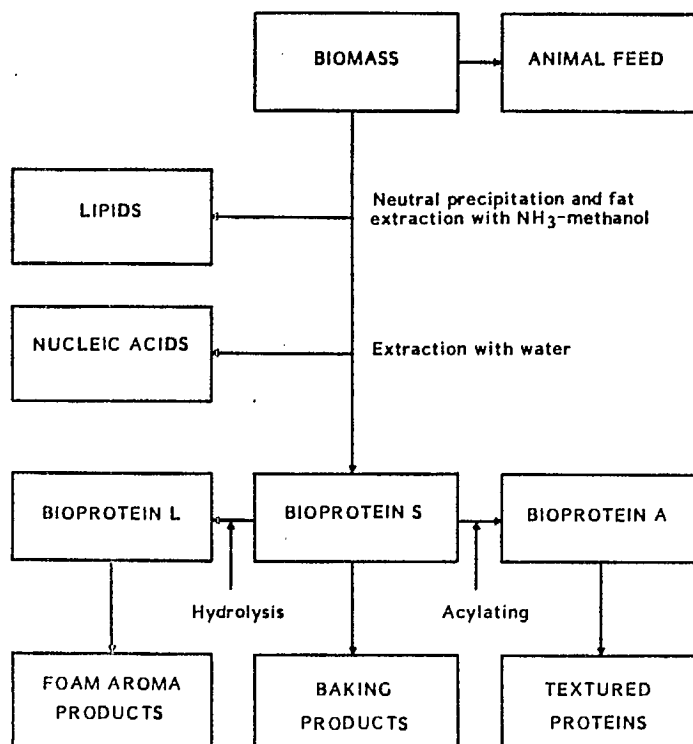


Fig. 16 : Processing of the SCP-biomass to various products.
(Source: Dimmling and Sambeth, 1981).

The above processing scheme allows not only the purification of the final product for direct human consumption but also recovery of products with increased value and therefore of commercial interest such as fat, nucleic acids and pure proteins.

By using $\text{NH}_3 / \text{CH}_3\text{OH}$ and $\text{NH}_3 / \text{C}_2\text{H}_5\text{OH}$ an excellent extraction medium for lipids was developed whereby also the mechanical form of the cell wall is completely destroyed (Schlingmann et al., 1980). With this method it was also possible to separate efficiently and mildly proteins from nucleic acids as well. This is accomplished in an aqueous environment at pH 7 - 7.5. This extraction procedure is accomplished at atmospheric pressure.

The separation of the components is accomplished through defatting of the cell dry mass with $\text{CH}_3\text{OH}/\text{NH}_3$ following extraction of the nucleic acids in an aqueous medium. For this purpose

the bioprotein (containing about 70% pure protein, 12% nucleic acids and 8% fat) is suspended in 5 times the volume of methanol. Following this step, 10-15 wt % (based on raw protein) of NH_3 (gas) is introduced and dissolved and the mixture is agitated for 15 minutes at 20-25°C. The separation of the solid/liquid phases is done through pressure filtration and twice wash with methanol.

The filtrate is concentrated in vacuum, ammonia and methanol are recycled and the fat concentrate is fractionated.

The next step involves the separation of nucleic acids and protein. Fat free bioprotein and water are mixed in proportion 1:10 at pH 7.5 and temperature of 60-70°C. Under these conditions, the nucleic acids go into solution and can be separated from the solid protein by decantation. The fat production contains about 24% of free fatty acids and about 45% phospholipids. A possible application is as an emulsifying agent in various industries.

The nucleic acid byproduct is also a raw material for a number of applications. The extract contains RNA and DNA in a ratio of 4:1. By further separation the DNA can be used as a raw material for D-desoxyribose while RNA can be used as a raw material for production of 5'-nucleotides: guanosine monophosphate (GMP) and inosine monophosphate (IMP) for use as flavour potentiators in foods.

The pure protein contains about 90-92% amino acids, 0.5-0.8% fat and 1-1.5% nucleic acids. This protein is of industrial interest as a functional protein. Properties such as emulsifying, swelling, water binding, foam generation, foam stability, extrusion properties, etc. are of interest in relationship to this product.

A particular feature of the Hoechst/Uhde SCP process is the development of an air-lift fermenter which allows a high oxygen transfer and a good mixing. This is possible also at large reactor volumes. The energy required for mixing and aeration is 25% lower in this way as compared to a stirred tank reactor.

In Table 28 are shown the requirements for operation of a plant producing 100,000 tons of SCP/year from methanol by the Hoechst/Uhde process (Dimmling and Sambeth, 1981).

Table 28: Requirements per hour for an SCP Hoechst/Uhde plant on methanol with a capacity of 100,000 tons/year

Raw materials

| | |
|---|-------|
| Methanol | 25 t |
| H ₃ PO ₄ (54% P ₂ O ₅) | 1.4 t |
| NH ₃ | 2 t |

Energy and utilities

| | |
|-----------------------------|--------------------------|
| Steam (8 bar) | 12.5 t |
| Cooling water (20°C) | 13,000 m ³ |
| Process water | 125 m ³ |
| Electricity (without silos) | 6.400 kw |
| Natural gas | 290 x 10 ⁸ kJ |
| Hot water | 800 m ³ |

The operating cost of this SCP production depends very much on the cost of the raw material. At a reasonable cost for methanol one can estimate that a ton SCP would cost between DM 1000-1200 (at present IC\$ = 2 DM). About DM 400-500 should be taken for the processing of the raw SCP. (These are 1981 prices). According to the company, SCP products, when used in specialized applications, can already today compete with comparable products on the market.

The capital costs for an SCP production plant of the above capacity depend very much from the location of the plant. For a 100,000 t/yr SCP plant in the Federal Republic of Germany the cost would amount to DM 140 million (1981). The cost for buildings and land is not included in the above figure. A required area for the above plant is about 220 x 180 meters.

3.3 ALGAL SCP

3.3.1 INTRODUCTION

The interest in the mass culture of microalgae was sparked by studies on dense suspensions of Chlorella in 1919 by Warburg. The United States, Japan, Israel and Italy began research in the large scale production of Chlorella, following the lead of German scientists in 1942. Commercial success was achieved by Japan and Taiwan with sales of Chlorella to health food markets in the Far East. In the 1960's, microalgae were evaluated for use as photosynthetic gas exchangers in the United States space program. The nutritional characterization of various species of microalgae began to be evaluated in the late 1960's with the view of introducing algae in food and feed. Current research is geared towards integrated systems for wastewater treatment, protein production and water reclamation.

Microalgae possess several distinct advantages over conventional protein sources. Perhaps most significant is the high protein content of the whole product. This is clearly evident from Table 29, which compares the composition of several species of microalgae with that of traditional proteinaceous foods.

Table 29: Gross Biochemical Composition of Various Food Sources

| Species | % Cell Dry Weight | | | |
|--------------------------------|-------------------|---------------|--------|---------------------|
| | Proteins | Carbohydrates | Lipids | Total Nucleic Acids |
| egg | 49 | 3 | 45 | - |
| meat muscle | 57 | 2 | 37 | - |
| fish | 55 | - | 38 | 1 |
| milk | 27 | 38 | 30 | - |
| corn | 10 | 85 | 4 | - |
| wheat | 14 | 84 | 2 | - |
| soy flour | 47 | 41 | 7 | - |
| microalgae: | | | | |
| <u>Spirulina maxima</u> | 65 | 20 | 2 | - |
| <u>Spirulina platensis</u> | 46-50 | 8-14 | 4-9 | 2-5 |
| <u>Chlorella pyrenoidosa</u> | 57 | 26 | 2 | - |
| <u>Chlorella vulgaris</u> | 51-58 | 12-17 | 14-22 | 4-5 |
| <u>Scenedesmus obliquus</u> | 49-55 | - | 5-10 | - |
| <u>Scenedesmus quadricauda</u> | 54 | - | 2 | - |

(Source: Aaronson, et al., 1980)

In addition, microalgae are more efficient than any other plant in terms of protein production per unit area. Their productivity has been shown to be 127 times that of soybean and 560 times that of rice (see Table 30).

Table 30: Annual Yield of Various Food Crops

| Crop | Annual Yield H (kg/ha) |
|---------|---------------------------|
| soybean | 645 |
| corn | 269 |
| wheat | 151 |
| rice | 146 |
| algae | 81,800 |

The fact that microalgae are primary producers is both an advantage and a disadvantage. This permits efficient bio-conversion with solar energy as the main energy input but also geographically restricts the operation to regions with high irradiance levels. For optimum growth, culture density must be limited to permit light penetration. Thus large shallow ponds are necessary. Land costs and capital investments are high. Pure cultures are not feasible with the open ponds, and weeds and herbivores can be a serious problem. Dilute cultures result in the need for processing large volumes of liquid.

The nutritional quality of algae as compared to casein is indicated in Table 31.

Table 31 : Net Protein Utilization (NPU), Digestibility Coefficient (DC), and Biological Value (BV) of different drum dried algae at 10 % protein level in the diet, compared with casein.

| Protein Source | NPU | BV | DC |
|--------------------|------|------|------|
| Casein | 83.4 | 87.8 | 95.1 |
| <u>Scenedesmus</u> | 65.8 | 71.6 | 81.4 |
| <u>Chlorella</u> | 57.1 | 76.0 | 79.7 |
| <u>Spirulina</u> | 57.0 | - | 85.0 |

(Source: Becker, 1981)

The amino acid compositions of Scenedesmus and Spirulina are compared with several conventional protein sources in Table 32.

Table 32 : Amino acid compositions of Scenedesmus 276-3a
(data from 4 different laboratories) in comparison
with Spirulina maxima, etc.

| Amino Acids | <u>Scenedesmus acutus</u> | | | | <u>Spiru- lina</u> | Soya meal (extr.) | Fish meal |
|---------------|---------------------------|-----|-----|-----|------------------------|-------------------------|--------------|
| | A | B | C | D | | | |
| Isoleucine | 4.4 | 3.2 | 4.2 | 3.7 | 6.0 | 4.6 | 5.4 |
| Leucine | 9.3 | 8.6 | 6.6 | 8.1 | 8.5 | 7.3 | 7.7 |
| Phenylalanine | 4.6 | 3.9 | 3.6 | 5.0 | 5.0 | 4.0 | 5.1 |
| Tyrosine | 3.6 | 2.8 | 3.0 | 3.4 | 4.0 | 2.9 | 2.7 |
| Threonine | 5.2 | 4.8 | 5.8 | 4.8 | 4.6 | 4.2 | 4.0 |
| Tryptophane | 1.4 | 1.4 | 1.2 | ? | 1.4 | 1.2 | 1.5 |
| Valine | 7.2 | 6.2 | 7.0 | 5.5 | 6.5 | 5.2 | 5.0 |
| Arginine | 5.6 | 5.8 | 6.3 | 5.3 | 6.5 | 5.0 | 7.7 |
| Histidine | 1.5 | 1.7 | 1.5 | 1.6 | 1.8 | 2.3 | 2.4 |
| Lysine | 5.7 | 5.3 | 5.0 | 5.6 | 4.6 | 7.0 | 6.5 |
| Cystine | 0.8 | 1.0 | 0.7 | 1.4 | 0.4 | 1.0 | 1.4 |
| Methionine | 1.4 | 1.4 | 1.2 | 2.1 | 1.4 | 2.6 | 1.4 |

(Source: Soeder, 1980)

Vitamin and mineral contents of algae are presented in Table 33..

Chlorella, Scenedesmus and Spirulina have been shown to be valuable protein sources in feeding rats, mice, poultry, pigs, sheep, cows, and humans. (Berend, 1980; Dugan, et al., 1970) Lipstein, 1980; Soeder, 1980; McGarry, 1971). Results of feeding tests are presented in Table 34.

Table 33 : Proximate composition, vitamin and mineral contents
of algae

| | RDA FOR ADULT MALES ¹ | ALGAE |
|-------------------------------|-------------------------------------|---------|
| Proximate Composition: | | |
| protein (g) | 56 | 51 |
| carbohydrates (g) | | 27 |
| fat (g) | | 7 |
| fiber (g) | | 6 |
| ash (g) | | 9 |
| Kcal/g | | 3.6 |
| Vitamins: | | |
| thiamine (mg) | 1.4 | 1.2 |
| riboflavin (mg) | 1.6 | 3.0 |
| niacin (mg) | 18 | 10.4 |
| pyridoxine (mg) | 2.0 | 0.2 |
| folacin (mg) | 0.4 | 3.4 |
| vitamin B ₁₂ (mg) | 3.0 | 25 |
| ascorbic acid (mg) | 45 | 40 |
| pantothenic acid (mg) | | 0.7 |
| biotin (mg) | | 26 |
| carotenoids (IU) | 5,000 | 156,000 |
| vitamin E (mg) | 15 | 2.6 |
| Minerals: | | |
| calcium (g) | 0.8 | 0.2 |
| phosphorus (g) | 0.8 | 1.8 |
| calcium phosphate (g) | 1.0 | 0.2 |
| magnesium (g) | 0.35 | 0.6 |
| sodium (g) | - | 0.1 |
| potassium (g) | - | 0.8 |
| sulfur (mg) | - | 187 |
| iron (mg) | 10 | 31 |

1. Recommended Dietary Allowances, 8th ed., Natl. Acad. Sci., 1974.
(Source: Waslien, 1975)

Table 34: Protein nutritive value of various sewage-grown algae - bacteria biomasses in comparison with casein and soybean meal. Total protein level equals 10%.

| Protein Source | 10-day Protein Efficiency Ratio | Relative Biological Value | 21-day Protein Efficiency Ratio | Relative Biological Value |
|--|---------------------------------|---------------------------|---------------------------------|---------------------------|
| Casein | 3.74 \pm 0.70 | 100 | 2.70 \pm 0.30 | 100 |
| Soybean meal | 3.56 \pm 0.40 | 95 | - | - |
| <u>Chlorella + Euglena</u> | 2.85 \pm 0.30 | 76 | 2.40 \pm 0.16 | 89 |
| (<u>Chlorella + Euglena</u>) + soybean meal (1:1) | 3.18 \pm 0.30 | 85 | 2.30 \pm 0.30 | 85 |
| <u>Micractinium</u> | 2.12 \pm 0.40 | 57 | 2.15 \pm 0.40 | 80 |
| <u>Micractinium</u> + soybean meal (1:1) | 2.96 \pm 0.2 | 79 | 2.48 \pm 0.27 | 89 |
| <u>Scenedesmus</u> | - | - | - | 89 |

(Source: Soeder, 1980)

Soeder (1980) states that Spirulina powder with a moisture content of as little as 4% acquires a bitter taste if stored in air, and that green algae meal turns rancid after only a few weeks. In contrast, McGarry (1971) found that three year-old sun dried algae was readily eaten by laying hens, with no apparent effect on egg laying performance.

Although no toxicity has been observed in acute, chronic and secondary toxicological trials, the potential of algae to accumulate heavy metals, polycyclic hydrocarbons, etc., warrants caution in determining what substrates are acceptable for mass culture of Photosynthetic Single Cell Protein.

If algae SCP is to compete with conventional protein materials, cheap nutrient sources must be utilized in the selected process. Thus, the successful operation would most likely attempt to use waste materials as substrates.

The year round availability of the substrate is an important consideration, therefore wastes from seasonal operations, such as those of agriculture processing plants (tomato, sugar, potatoe, etc.) are less desirable. Whenever possible, the waste material must possess all the nutrients essential for algal growth. Materials that require supplementation with expensive chemicals may not be acceptable.

Domestic sewage and animal manure may meet substrate selection criteria. The variable nature of the former and its possible contamination by toxic substances or infectious diseases, make it the least favourable of the two.

In the United States, livestock produce approximately 30×10^6 kg of manure per day. Roughly 45% of this is collectable (Lincoln and Hill, 1980)

A relatively small poultry operation (10,000 hens) will generate at least 2 tons of wet manure daily. Crude analysis of poultry manure reveals that 25.5% is protein (N x 6.25), 8.3% is fibre, 2.1% is oil and 22.1% is ash (Weller, 1977).

A detailed analysis of poultry manure is provided in Table 35.

Table 35 : Detailed analysis of poultry manure

| Manure | Contents | |
|---|----------|------------------------------|
| bulk output | 189.7 | g/chicken day |
| | 0.418 | lb/chicken day |
| volume | 177.3 | ml/chicken day |
| | 10.8 | in ³ /chicken day |
| total solids | 25.4 % | |
| | 48.2 | g/chicken day |
| volatile solids | 67.7 % | |
| | 32.6 | g/chicken day |
| energy | 136.7 | kcal/chicken day |
| specific gravity | 1.07 | |
| NH ₃ - Nitrogen | 838 | mg/kg |
| | 0.159 | g/chicken day |
| total unoxidized nitrogen | 14,860 | mg/kg |
| | 2.819 | g/chicken day |
| | 5.85 % | of TS |
| COD | 192,000 | mg/kg |
| | 36.4 | g/chicken day |
| BOD | 44,160 | mg/kg |
| | 8.38 | g/chicken day |
| | 0.0185 | lb/chicken day |
| BOD/COD ratio | 0.23 | |
| population equivalent (based on assumption of 0.08 kg BOD/capita) | 9.2 | |

(Source: Dugan, et al., 1970)

3.3.2 PRODUCTION AND PROCESSING TECHNIQUES

The three major components in the mass culturing of microalgae are growth, separation and drying. For each of these, a variety of options exist with great diversity in efficiency, cost and technical complexity.

Because microalgae are photosynthetic organisms, provision of sufficient sunlight for the culture is a primary consideration in the design of a suitable "reactor". Shallow culture depths and dilute media are therefore standard features of current technology. In contrast to the relatively deep (90-250 cm) oxidation ponds of the past, the modern "High Rate Algal (Oxidation) Ponds" (HRAP or HROP) are shallow (20 - 50 cm deep), mechanically mixed structures with short retention times (days as opposed to weeks or months).

HRAP's are generally constructed in the form of meandering channels separated by baffles (Fig. 17). Mixing is most often accomplished by paddlewheels. Pond mixing serves several purposes: it provides nutrient dispersion, resuspends settled algae and facilitates gas exchange between the pond and the ambient air. It also prevents the formation of a deep anaerobic sludge blanket that would result in pond failure.

The main carbon source for algae is in the form of carbon dioxide. In an efficiently operating HRAP, most of this CO_2 is generated by bacteria existing in a sludge layer at the bottom of the pond. A symbiotic relationship is thus established between the algae and bacteria. (Fig. 18). Organic matter introduced as sewage to the pond is oxidized by the bacteria to CO_2 and simple organics. Algae utilize the CO_2 photosynthetically to produce oxygen and biomass.

Carbon dioxide may also be added to the culture from external sources such as combustion gases and exhaust from fermentation processes, industrial nitrogen reduction, biogas production processes (Soeder, 1980), glutamic acid plants and scrubbed furnace gases (Rolz and Humphrey, 1982)

Harvesting techniques range from simple sedimentation and filtering to complex and energy intensive centrifugation

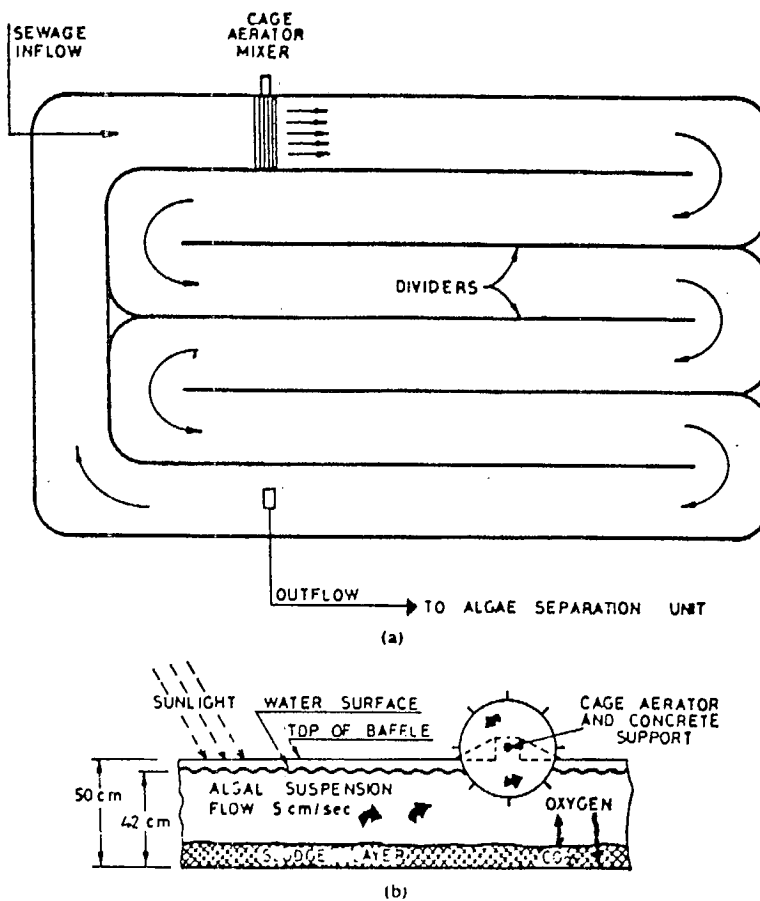


Fig. 17 : High-rate oxidation pond: (a) schematic plan :
(b) schematic cross section.

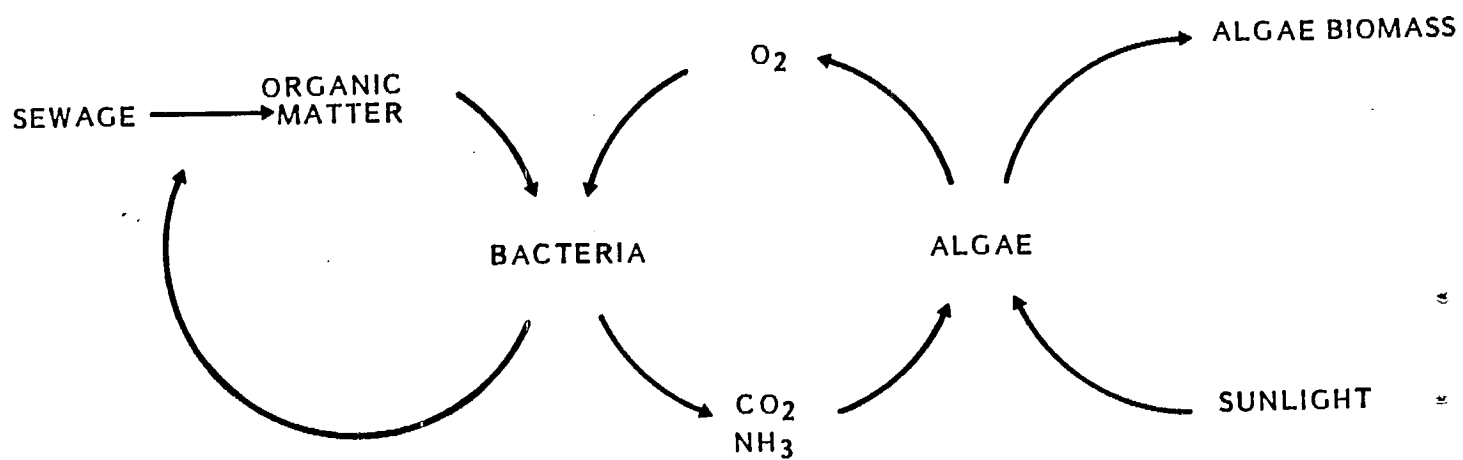


Fig. 18 : Algae - Bacteria Symbiosis

methods and electroflotation. A flow sheet of the various methods available is presented in Fig. 19.

The choice of harvesting technique depends on a number of factors, not the least of which is the species of microalgae to be removed.

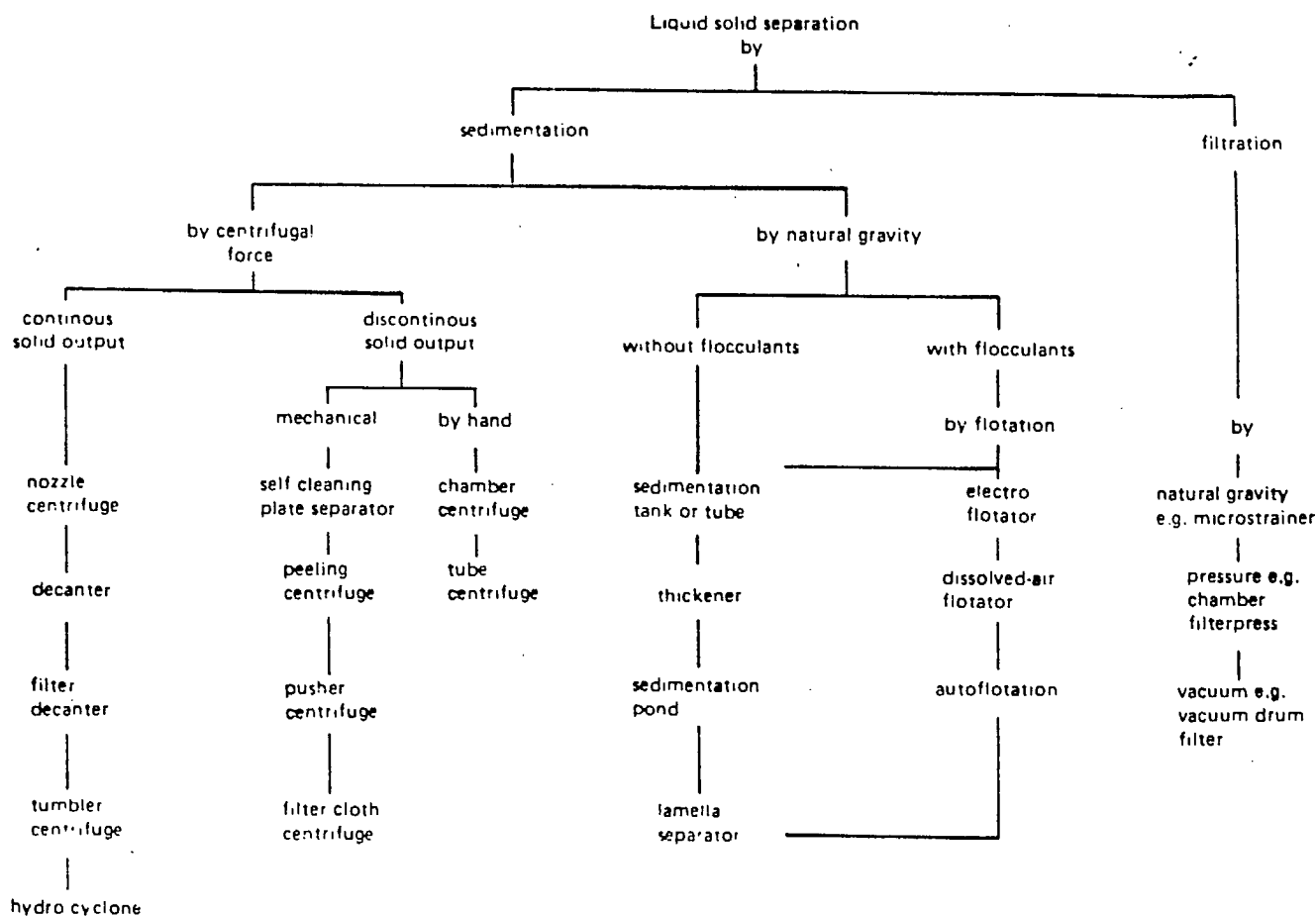


Fig. 19 : Separation Methods for Microalgae

Colonial spine-bearing species such as Microactinium and Scenedesmus are readily harvested by filtration with fine mesh fabrics, as are the filamentous blue-green algae Spirulina. Oscillatoria can be removed by microstraining. More advanced methods are required for separation of the small coccoid cells of Chlorella. Use of flocculating agents such as alum, lime and polycations is also recommended. For chemical flocculation of

the algae, the pond effluent is first brought to a pH of 6.5 by the addition of acid. Alum is then added at a dose of 150 mg/l (McGarry and Tangkasame, 1971b)

There is, however, a concern about the toxicological impact of the added alum (Soeder, 1980).

The dose of alum required may be reduced by treating the pond effluent with a combination of alum and polyelectrolytes. McGarry (1971b) showed that 60 mg of alum plus 3 mg of polycation (PEI 600, Purifloc c31, Purifloc c32) per liter was effective in floating algae solids.

At a cost of 5 - 15 dollars per million litres treated (Benemann, 1980), microstraining provides a workable method for harvesting algae from ponds dominated by Micractinium and Oscillatoria species. Unfortunately, poor separation is achieved with Scenedesmus and Chlorella. In this process, feed enters axially into a rotary drum covered by a straining fabric. The resulting matt, or "Schutzdecke", is dislodged by a backwash spray, the particles collecting in an axial trough.

"Pond isolation" has been tested by Benemann et al. (1980). This method involves a batch fill and draw operation in a secondary, deep (1.5 m) pond. Settling occurs over a period of approximately 2 weeks. The large surface area required and the unreliability of the process are serious drawbacks.

Among the methods used for dewatering the algal paste are sun-drying, spray-drying and drum-drying.

Of these, sun-drying is of course the least energy and cost intensive. Lincoln (1980) obtained a product with 15 % final moisture by filtering a slurry through a double layer of black sail cloth at loadings up to 13.3 kg solids per square meter. Optimum conditions for sun-drying of a 6 % initial solids slurry were found by McGarry (1971) to be a depth of 0.5 cm at solar radiation levels of 480 g cal/sq cm/day. Loading of up to 1270 kg/ha/day produced a final product with less than 10 % moisture after a single day.

Drum drying may be preferred in spite of its higher cost because it yields a sterilized product, with high nutritional

value and low water content (thus greater storage capacity).

The method of drying plays a significant role in determining the digestibility of the final product. In general, sundried algae is poorly digested by monogastric animals (pigs, poultry, etc.). Although this method may prove satisfactory if ruminants such as cows or sheep are to be the recipients, drumdrying is usually recommended. The high energy requirements of this cell-rupturing process can be offset by the methane generated as a byproduct of the wastewater treatment.

Table 36 illustrates the effect that drying can have on the nutritional quality of the end product.

Table 36 : Effect of drying method on protein efficiency ratio (PER)

| Alga | Drying Method | PER |
|--------------------|-----------------|------|
| Casein | - | 2.50 |
| <u>Scenedesmus</u> | drum dried | 1.99 |
| <u>Scenedesmus</u> | sun dried | 1.14 |
| <u>Scenedesmus</u> | cooked sundried | 1.20 |
| <u>Scenedesmus</u> | freeze dried | 1.12 |
| <u>Spirulina</u> | sun dried | 1.63 |
| <u>Spirulina</u> | drum dried | 1.80 |
| <u>Chlorella</u> | drum dried | 2.20 |
| <u>Chlorella</u> | raw | 0.84 |
| <u>Chlorella</u> | autoclaved | 1.31 |

(Source: Becker, 1981)

Rolz (1982) proposed a system featuring the thermophillic association of a unicellular blue-green algae with a gliding filamentous photosynthetic bacterium (Chloroflexus aurantiacus). High temperature wastewaters from agroindustrial operations were

suggested as nutrient sources. The filamentous nature of the microorganisms would facilitate harvesting, and the high temperature of the system would reduce contamination of the culture.

The use of marine microalgae is also being investigated by several groups. (Goldman, 1975, Ashare, 1978). Using mixtures of 50 % wastewater and 50 % seawater, Goldman achieved yields of up to 20 g dry wt. per m² per day. For locations where fresh water availability is low and/or prohibitively expensive, such a system could prove of great value.

The diversity of microalgae production systems can be illustrated in two examples.

The Sosa Texacoco Co. in Mexico produces approximately 5 tons dry weight per day of Spirulina for sale as health food. A flowsheet of the operation is given in Figure 20.

In contrast to this massive operation is the self-sufficient single family operation proposed by Golueke, (Fig. 21) in which the waste from 1 cow and 50 chickens is used to supply the needs of a family of four.

Berend (1980) has designed a complete facility to treat the waste generated by a small city in Israel (Nahariya: population 33,000). Sewage (8,200 m³/day, BoD₅ : 400 - 500 mg/l) is pumped into three HRAP's with a total surface area of 6.7 ha. Brush aerators drive the liquid through a maze of 15 m wide channels at a depth of 0.40 m in summer, 0.55 m in winter. Dissolved air flotators in combination with alum flocculation concentrate the slurry to 4 % solids and generate an effluent with 30 mg/l SS which after chlorination is utilized for crop irrigation. A battery of 6 secondary flotators increase the solids content of the algae slurry to 7 %. An automatic de-sludger disc centrifuge with clarifier bowl (20m³/hr capacity) yields a 15 % solids product which passes to a double drum sump dryer. The resulting algae chips are then air dried to a final moisture content of 10 %. Plant output is calculated to be 1,196 ton of algae chips per year with the treatment of 3.5×10^6 m³/year of raw sewage.

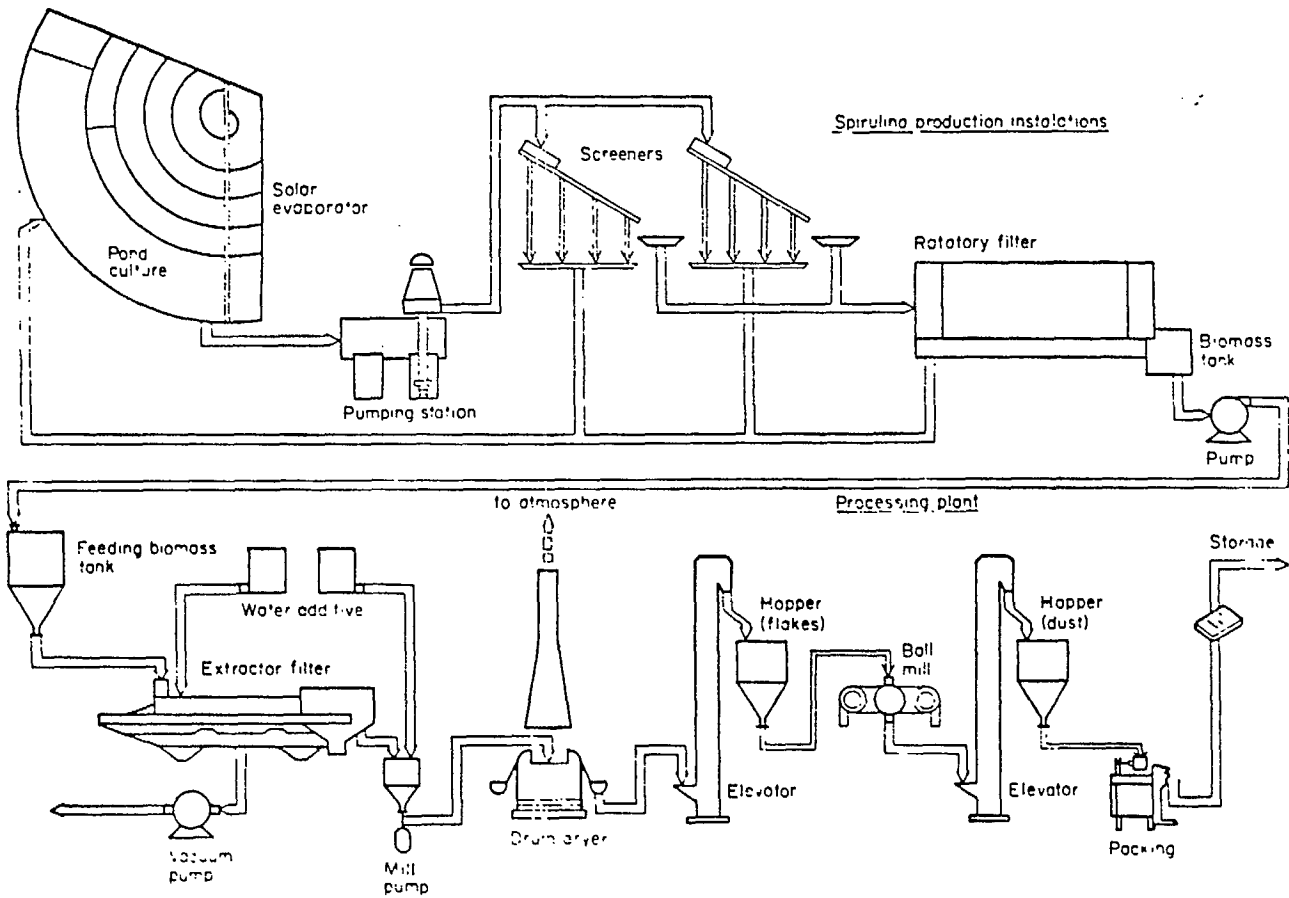


Fig. 20: Flow diagram of Sosa Texacoco Plant
(Benemann, 1979)

Further examples of microalgae production systems are given in Table 37.

Table 37: Small Scale Microalgae Operations

| Genus | Location | Type of System ^a | Duration Days | Yields ^b | | Theoretical Yield | |
|-------------------------|---------------------------|------------------------------|---------------|---|------------------------------------|---|--|
| | | | | a total dry wt per m ² per day | ton total dry wt per acre per year | m ton total dry wt per hectare per year | |
| CROALGAE | | | | | | | |
| <u>Chlorella</u> | Cambridge, Massachusetts | L(56 m ² /unit) | 52 | 2 | 3.3* | 7.3* | |
| <u>Chlorella</u> | Essen, Germany | L(6 m ² /unit) | ~ 30 | 4 | 6.5* | 14.6* | |
| <u>Chlorella</u> | Tokyo, Japan | L(15 m ² /unit) | 10 | 3.5 | 5.7* | 12.8* | |
| <u>Chlorella</u> | Tokyo, Japan | L(13.8m ² total) | 27 | 16 | 26.0* | 58.4* | |
| <u>Chlorella</u> | Jerusalem, Israel | L(4 m ² /unit) | 35 | 12 | 19.6* | 43.8* | |
| <u>Chlorella</u> | Jerusalem, Israel | L(300 m ² /unit) | 30 | 27 ^c | 44.0* ^c | 98.5* ^c | |
| <u>Chlorella</u> | Tokyo, Japan | L(147.8m ² total) | 365 | 8.6 | 14.0 | 31.4 | |
| <u>Scenedesmus</u> | " | " | " | " | " | " | |
| <u>Scenedesmus</u> | Dortmund, Germany | L(320 m ² total) | ? | 10 | 16.3* | 36.5* | |
| <u>Scenedesmus</u> | Trebon, Czechoslovakia | L(12 m ² /unit) | ? | 15 | 24.5* | 54.8 | |
| <u>Scenedesmus</u> | Trebon, Czechoslovakia | L(50 m ² /unit) | 65 | 16 | 26.0* | 58.4* | |
| <u>Scenedesmus</u> | Trebon, Czechoslovakia | L(900 m ² /unit) | 89 | 12 | 19.6* | 43.8* | |
| <u>Scenedesmus</u> | Tylicz, Poland | L(50 m ² /unit) | 71 | 12 | 19.6* | 43.8* | |
| <u>Scenedesmus</u> | Rupite, Romania | L(50 m ² /unit) | 62 | 23 | 37.5* | 84.0* | |
| <u>Scenedesmus</u> | Firebaugh, California | L(1000 m ² /unit) | 70 | 10 | 16.3* | 36.5* | |
| <u>Scenedesmus</u> | Bangkok, Thailand | L | ? | 13.4 | 21.8* | 48.9* | |
| <u>Scenedesmus</u> | Bangkok, Thailand | L(609 m ² total) | ? | 15 | 24.5* | 54.8* | |
| <u>Glyptothrix</u> | Tokyo, Japan | L(5 m ² /unit) | ? | 6.4 | 10.4* | 23.4* | |
| <u>Phaedactylum</u> | Plymouth, England | L(15.6 m ² total) | ? | ~ 10 | 16.3* | 36.5* | |
| <u>Spirulina</u> | Bangkok, Thailand | L(609 m ² total) | ? | 15 | 24.5* | 54.8* | |
| Green Algae | Haifa, Israel | L(270 m ² total) | 365 | 15 | 24.5 | 54.8* | |
| Diatoms | Woods Hole, Massachusetts | L(1.080m ² total) | 7 | 10 (max) | 16.3* | 36.5* | |
| Diatoms | Woods Hole, Massachusetts | L(8 m ² total) | 15 | 13 (max) | 21.2* | 47.4* | |
| Diatoms | Fort Pierce, Florida | L(15 m ² total) | 15 | 25 (max) | 40.8* | 91.3* | |
| <u>Micractinium</u> | Richmond, California | L(48 m ² total) | 30 | 23 (max) | 37.5* | 84.0* | |
| <u>Micractinium</u> | Richmond, California | L(2700 m ² /unit) | 31 | 7.3 (max) | 11.9* | 26.6* | |
| <u>Micractinium</u> | Richmond, California | L(12 m ² total) | ? | 11 (max) | 17.9* | 40.2* | |
| GREEN MACROALGAE | | | | | | | |
| <u>Gracilaria</u> | Fort Pierce, Florida | L | 365 | 35.0 | 57.1 | 127.8 | |
| <u>Gracilaria</u> | Fort Pierce, Florida | L | 365 | 20.0 | 32.6 | 73 | |
| <u>Gracilaria</u> | Fort Pierce, Florida | L | 150 | 16.9 | 27.5* | 61.7* | |
| <u>Gracilaria</u> | Woods Hole, Massachusetts | L | 166 | 16.9 | 27.5* | 61.7* | |
| <u>Hypnea</u> | Fort Pierce, Florida | L | 150 | 17.6 | 28.7* | 64.2* | |
| <u>Agardhiella</u> | Woods Hole, Massachusetts | L | 166 | 27.7 | 45.2* | 101.1* | |

* This value does not reflect the true annual yield, since the duration of the study was less than one year.

^a - land-based system.

^b Average yields, unless indicated otherwise.

^c Included non-algal solids from wastewater.

(Source: Ashare, 1978)

3.3.3 ALGAL SCP FROM POULTRY WASTE (AN ALTERNATIVE MODEL)

For livestock industries, such as poultry farming (broilers and layers), the reduction in available land generates a serious waste disposal problem. Insufficient acreage often exists for simple land disposal, and the marginal nature of agricultural economics make conventional waste treatment facilities impractical (Dugan, et al., 1969).

The typical layer operation, with 10,000 to more than 500,000 birds housed under one roof or a series of closely adjacent buildings, lends itself to convenient waste handling by HRAP technology. A benefit of this procedure would of course be the generation of a high protein feed supplement.

Feed trials with laying hens have revealed no change in weight, morbidity or mortality for hens with algae meal supplementing up to 15% of the diet. Egg production, flavour and acceptability were also not adversely affected (Dugan, 1972), as shown in Table 38.

Table 38: Feed Intake and Laying Performance of Hens Fed Algae Meal

| Feed | Daily Feed Intake (g) | Egg Production (%) |
|---------------|-----------------------|--------------------|
| standard mash | 130 | 71 |
| 5% algae | 102 | 69 |
| 10% algae | 110 | 72 |
| 15% algae | 113 | 61 |

(Source: Dugan, 1972)

In studies by Lipstein et al. (1980), no difference was observed in egg production rate, egg weight, feed conversion, body weight, feed intake, or egg shell quality for layers fed up to 12 % algae.

Based on the evidence that poultry manure is a satisfactory substrate for algae culturing, while the algae in turn is a suitable feedstock for chickens, it was decided to evaluate an algae process for this industry.

A medium-sized operation of 20,000 laying hens is considered. Much of the data used was extracted from the pilot operation developed by Dugan (1970).

The system proposed, while providing an efficient wastewater treatment function, will also generate algae in sufficient quantities to supplement the feed at the 7,3 % level. An added bonus will be the production of methane by anaerobic digestion for use in drying the algae product, heating the poultry enclosure, or for the generation of electricity.

A flow sheet of the entire operation is presented in Fig. 22.

3.3.3.1 Poultry Enclosure

The proposed system requires hydraulic handling of the poultry manure. Cages are suspended over troughs sufficiently large to catch all droppings. The troughs can be easily constructed from sheet metal and will serve to replace the barriers that must exist between tiers in multilevel operations. A dumping bucket at the head of each trough periodically flushes the manure into a sedimentation tank. The organization of the tiers of cages in a particular operation will determine the complexity of the trough network. Flushing water is supplied directly from the algal pond. From the flow sheet of the process, the total solids content of this element is 562 kg per day. At a concentration of 1 % TS, this represents a volume of 56,200 litres. If four dumping buckets are employed, each of a capacity of 150 l, then the dumping rate of each bucket will be 4 times per hour. Overflow of drinking water into the trough would serve as makeup water where re-

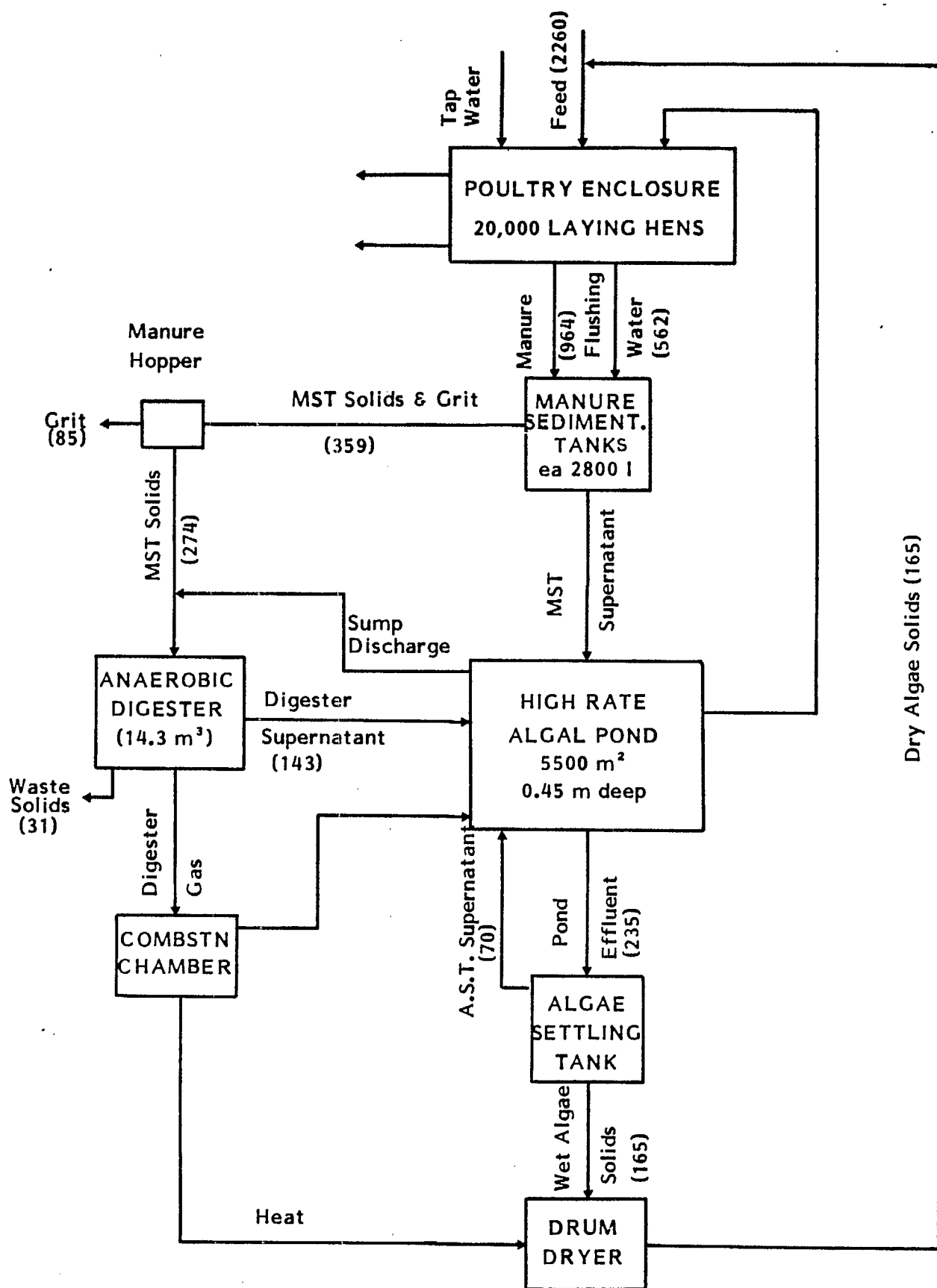


Fig. 22 : Flowsheet of the Algal SCP Process

(Note: Numbers in brackets indicate mass flow rate given as kg/day)

quired to compensate for losses due to evaporation or spillage. A plan of the pilot scale chicken enclosure and sedimentation tank components is given in Figures 23 and 24.

3.3.3.2 Manure Sedimentation Tank

Two tanks each of a capacity of 2,800 l operate in a batchwise manner. Each receives flushing water for a period of one hour. While flow is diverted to the other tank, a settling period of 30 minutes will be observed before the tank is emptied (the supernatant by a sump and the settled solids by a sludge pump). The total solids concentration in the tank as a result will be 2.6%, sufficiently low to achieve the desired settling in the allotted time.

Sludge from the manure sedimentation tank is pumped to an anaerobic digester via a manure hopper. Grit is removed from the solids at the hopper.

The supernatant is removed directly to the algal pond.

3.3.3.3 High Rate Anaerobic Digester

A single tank with a capacity of 14.3 m³ will be required. Approximately 91.7 kg of volatile solids are added each day, at the recommended loading rate of 6.4 kg/m³/day. Solids concentration is maintained at 4% in the digester through addition of sump discharge from the algal pond. At a conversion efficiency of 40%, 15 cubic meters of gas would be generated per day. This product would be 60% methane, with the rest being mostly carbon dioxide. A heating value of approximately 2.21×10^4 BTU/m³ is taken for the gas.

Temperature in the digester is maintained at 35-37°C by means of hot water flowing through coiled tubing within the tank. The water is heated using the methane produced.

Supernatant from the digester is pumped to the algal pond. Carbon dioxide remaining after combustion of the digester gas will also be added to the culture.

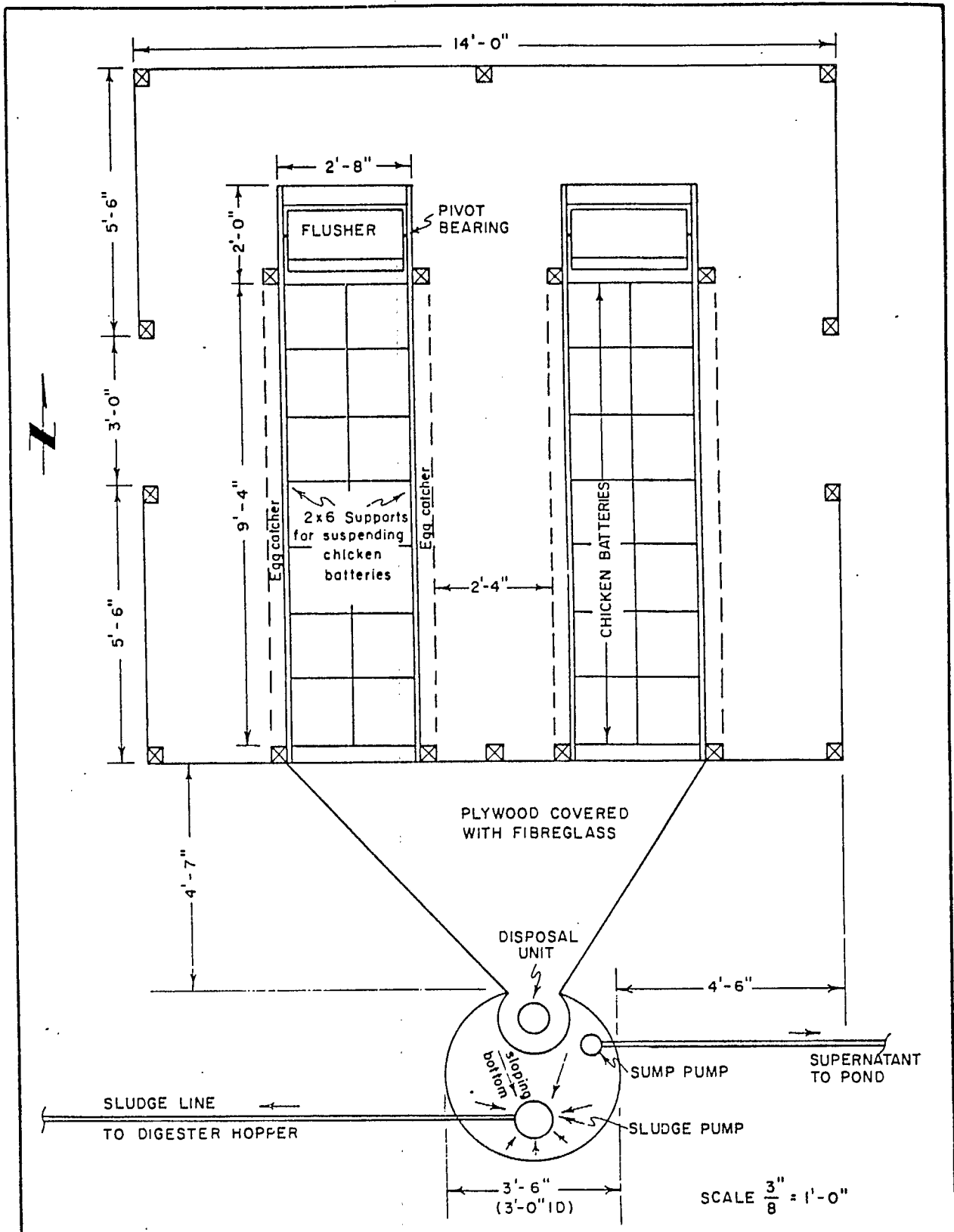


FIGURE 23 PLAN VIEW OF POULTRY ENCLOSURE AND MANURE COLLECTION FACILITIES
 (Source: Dugan, et al., 1969)

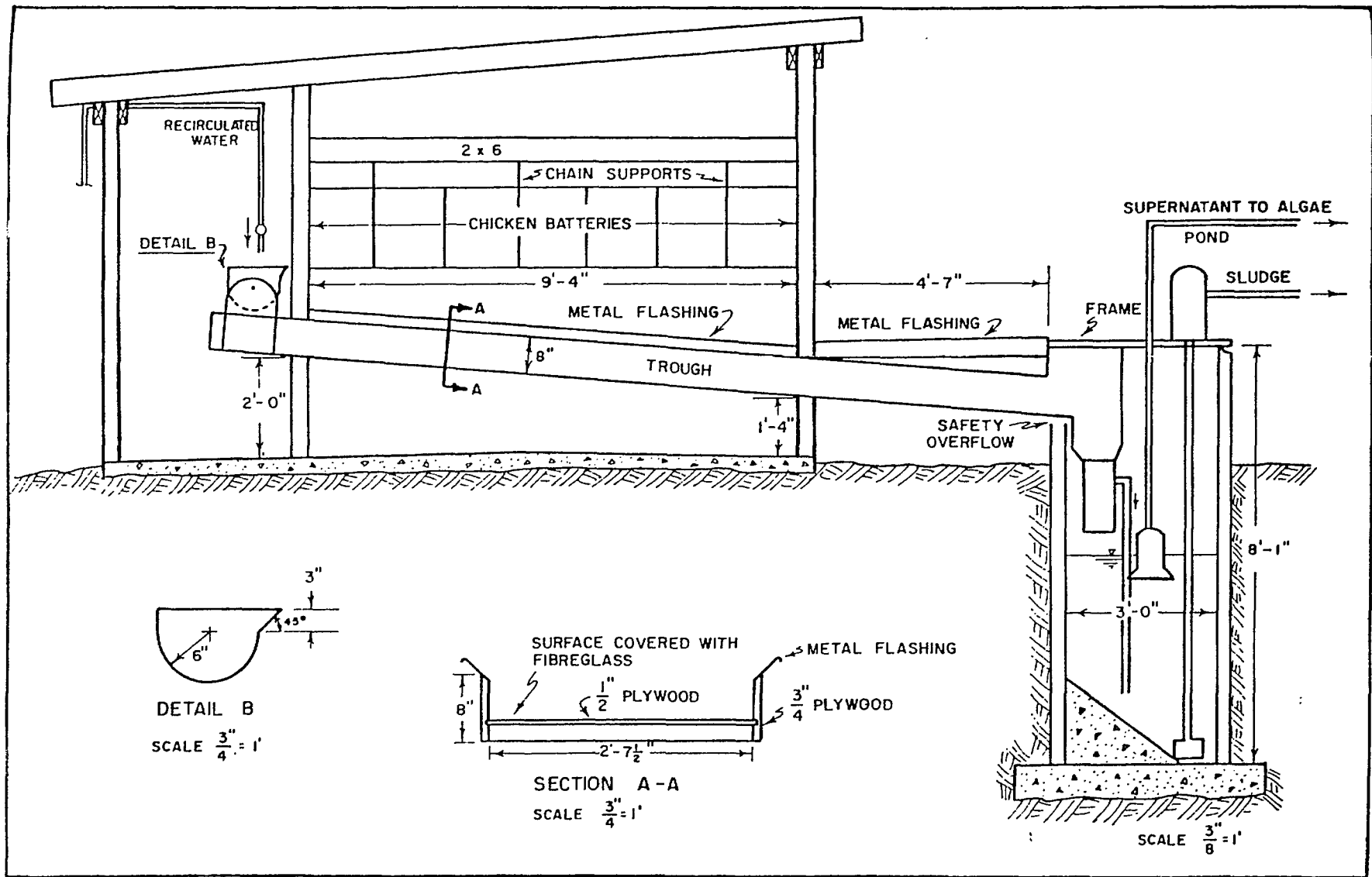


FIGURE 24. CROSS SECTION OF POULTRY ENCLOSURE AND MANURE COLLECTION FACILITIES'

(Source: Dugan, et al., 1969)

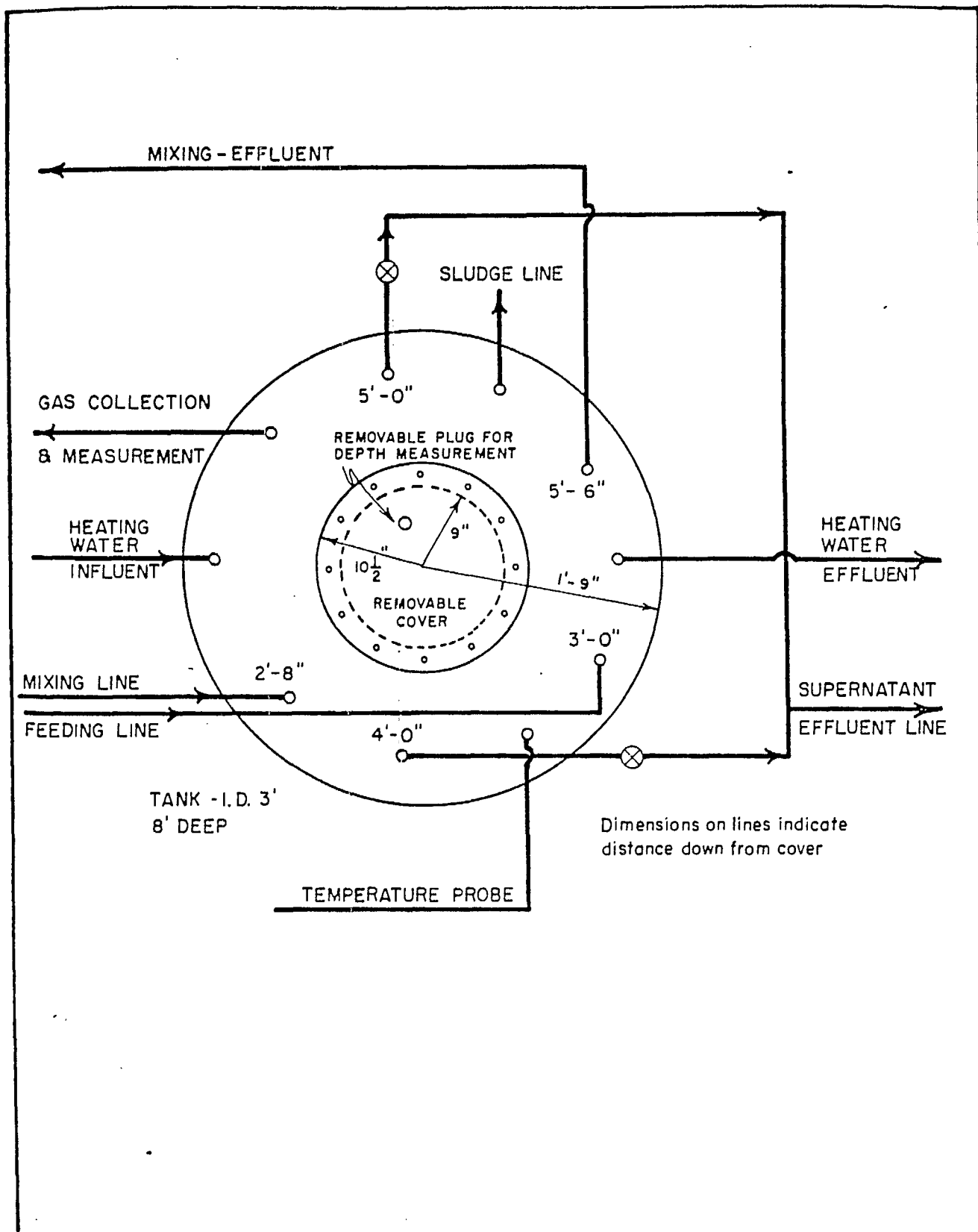


FIGURE 25. PLAN VIEW OF DIGESTER

(Source: Dugan, et al., 1969)

A plan for the pilot scale digester is shown in Figure 25.

3.3.3.4 High Rate Algal Pond

The pond to be utilized will have an overall surface area of 5500 m² (0.55 ha) and an operating depth of 0.45 m (this may be varied, especially in winter months to maximize pond efficiency). Mixing and aeration is accomplished by four paddle wheels. Pond loading occurs at a rate of 300-325 kg BoD/ha. Productivity is estimated to be 25 g dry wt. per m² per day. Channel width is to be 10 m.

The perimeter and channel dividers are to be constructed of concrete blocks and the base to be of hard packed gravel, except under the paddle wheels and pump inlets, which are to have concrete aprons.

Positioning the pond along a highly reflective wall of the poultry enclosure with a southwestern exposure, may help to maximize the incident radiation per square meter of pond surface.

A schematic of the pond is provided in Fig. 26..

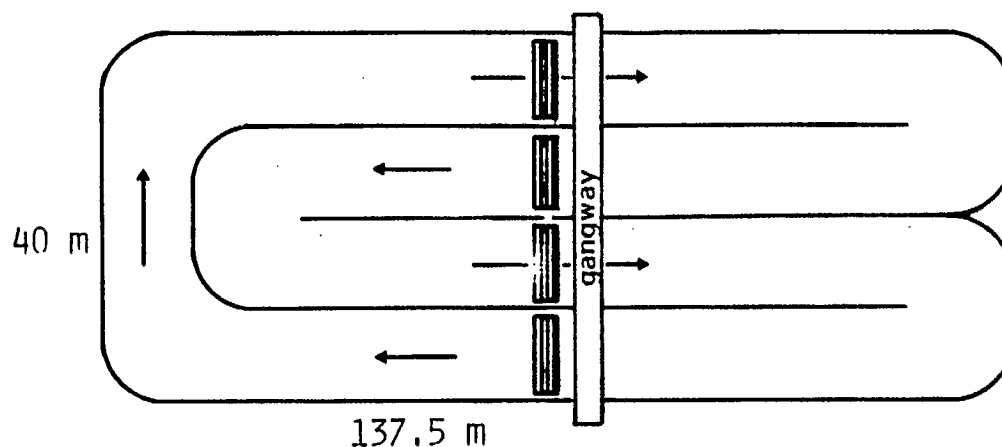


Fig. 26 : High Rate Algal Pond

Where the availability of land is limited, it may be feasible to construct the pond on the roof of the poultry enclosure.

The design of the enclosure would have to incorporate the added weight and impulse loads so generated. Advantages of such a system include facilitated manure flushing due to the head resulting from elevated culture, insulation of enclosure, high pond efficiency as a result of elevated temperature and decreased freezing period.

3.3.3.5 Algae Settling Tank

Effluent from the pond will be pumped into the settling tank for a period of approximately 16 minutes each hour. Seventy percent of the solids charged in this manner would settle within 15 minutes (Dugan, 1970) for a tank with a retention time of 38 hours. Algae harvested in this manner would total 165 kg/day.

3.3.3.6 Dryer

The extra cost of drum drying is felt to be compensated by the enhanced digestibility and storage capacity of algae feed produced by this method. The heat required for drying would be supplied from the methane generated in the anaerobic digester.

3.3.3.7 Economics of the considered system

In a standard poultry feed, 18 % (weight basis) may be soy meal. At a protein content of 40 %, this represents 7.2 kg protein per 100 kg feed. For the operation considered here, the protein contribution of soybean would thus be 162.7 kg per 2260 kg of feed. Addition of 165 kg of algae solids (55 % protein) could displace 90.8 kg of this protein or 227 kg of soy meal.

March 1982 prices for soy meal were \$ 182 (US) / ton or \$ 0.201 (US) / kg. Thus substitution of 165 kg of algae solids into the feed (7.3 %) would result in a daily saving of \$ 45.63 or \$ 16,653.86 / year.

In addition, the methane produced would be utilized to reduce heating cost for the poultry enclosure, or for generation of electricity for the operation. Any excess could be sold as a byproduct. The credit for wastewater treatment would be significant for situations where land spreading of the manure was not a feasible alternative.

In addition to the capital cost of the operation, certain factors (the economics of which are largely unknown at present) contribute to the operating cost. These include sampling and analysis of pond condition, production of inoculum, elimination of weeds, diseases and herbivores, land charges and water costs (Benemann, 1977).

Although soybean meal may be thought of as a competing product, soy producers may in fact welcome the introduction of algae production in that it would free more soybean for the more economically rewarding health food market.

In general the process would not require highly skilled labour and thus these costs would be minimized. The operation, as shown, requires no complicated technology.

In terms of efficiency of land utilization, Oswald (1980) has stated that algal biomass systems could match current production of animal feeds while using only 3 - 5 % of the land now used for animal feed production.

The material and energy balance for the proposed system is shown in Table 39.

Table 39 : Material and Energy Balance for Proposed System

| Component of System | Material Balance (kg/day) | Energy Balance (kcal/day) |
|------------------------------------|------------------------------|------------------------------|
| <u>Chickens</u> | | |
| Input: chicken feed | 2260 | 7.70×10^6 |
| tap water (25 l/100 hens) | - | - |
| flushing water | 562 | 1.48×10^6 |
| Output: Manure (48.2g/chicken.day) | 964 | 2.73×10^6 |
| (O.17731/c.day) * | | |
| eggs (13.85 g/c.day) | 277 | 1.38×10^6 |
| feathers (0.04 g/c.day) | 1 | 4.00×10^3 |
| flushing water | 562 | 1.48×10^6 |
| <u>Manure Sedimentation Tank</u> | | |
| Input: manure | 964 | 2.73×10^6 |
| flushing water | 562 | 1.48×10^6 |
| Output: | | |
| sedimentation solids | 274 | 0.94×10^6 |
| grit | 85 | - |
| sedimentation supernatant | 1166 | 2.86×10^6 |
| <u>Digester</u> | | |
| Input: | | |
| sedimentation solids | 274 | 0.94×10^6 |
| Output: | | |
| digester supernatant | 143 | 0.40×10^6 |
| digester gas | 31 | 0.07×10^6 |
| digester sludge | 100 | - |
| <u>Pond</u> | | |
| Input: | | |
| sedimentation tank supernatant | 1166 | 2.86×10^6 |
| digester supernatant | 143 | 0.40×10^6 |
| Output: | | |
| flushing water | 562 | 1.48×10^6 |
| algae solids | 165 | 0.38×10^6 |
| sump pump withdrawal | 171 | - |

(Based on Data from Dugan et al., 1970)

*chicken-day

3.4 SCP FROM LIGNOCELLULOSICS

3.4.1 MODEL PROCESS DEVELOPMENT

Application of the Waterloo Process to an Ontario Farming Operation.

3.4.1.1 Criteria of Choice

A set of reasonable criteria are applied by which the evaluation for the best option of an SCP process could be made. These criteria are outlined in Table 40. Although a number of these considerations may seem obvious to the reader, their significance can not be underestimated.

Defined conditions were set in which to operate the process. These were as follows:

- a) the process was to be confined to Canada and preferably Ontario. Though Canada, at present is not experiencing any lack of food resources, in the interest of national security for the future it was felt these processes should be developed. Ontario was chosen in particular for reasons of convenience.
- b) The process should utilize a waste product as substrate. This condition was set with regards to the economics and moral values in mind. Most markets for waste materials are not extensive. Since the substrate may account for upwards of 50 % of total product cost (Dimmling and Seipenbusch, 1978) it is important that the raw material be abundant and inexpensive.
- c) The process should be easily integrated into the existing operation which produces the raw material. This is such that a minimal amount of modification (and hence capital costs)

Table 40 : Criteria for process selection

- (1) Does the process yield a suitable end-product on both physiological and psychological levels?
- (2) What is the degree of technological risk involved?
 - risk inherent to scale-up of pilot processes
 - risk inherent for the operation to be adequately supplied with feedstock over an economically viable period.
- (3) What are the relative benefit/detractions for the process with regards to by-product formation and utilization?
- (4) What is the value of the product
 - nutritional value based on feeding trials
 - is the composition of product time-invariant
 - will the product pass government controls
 - does the product require further-treatment before consumption
- (5) What level of technological sophistication is necessary?
- (6) Are the potential economics sufficiently attractive to accept risks implementing the process?

(Source: Shuler, 1981)

will be induced during the development.

- d) The process should be relatively well developed in the literature. This was important to allow the author a source of reliable information so as to minimize the amount of assumptions which must be made in regards to specific application of the process.

Even with these stipulations, a number of process routes are still potentially feasible. The utilization of crop residues from Ontario farming operations was chosen for analysis. The process decided upon was that developed at the University of Waterloo Chemical Engineering Department by Dr. M. Moo-Young and associates (Moo-Young et al., 1979).

3.4.1.2 General Information

The considered farming operation is an existing farm in Woodsley, Ontario (approx. 20 miles east of Windsor) comprising of 125 acres of land with crop characteristics as summarized in Table 41. Crop management is based in chisel plow tillage in the spring with staggered planting dates depending on species of plant. Harvesting takes place in midsummer to late fall. Crop residues are presently collected and baled. A portion of the residues are mixed with cattle manure and plowed back into the soil. Surplus is used as bedding for the livestock.

Table 41 : General Characteristics of the Farming Operation

Total size: 125 acres

| Crop | Distribution | Rotation Order | Harvest |
|--------------|--------------|----------------|---------|
| corn | 50 A | 1 | October |
| summer wheat | 15 A | 2 | July |
| oats | 20 A | 3 | August |
| hay | 40 A | - | - |

Livestock on the site consist of 75 purebred Holstein cattle utilized in dairy production. Cattle are kept indoors. Manure collection is achieved by an inset floor conveyor which passes animal excreta to an outdoor holding pile.

3.4.1.3 Determination of Substrate Available to the Process

As discussed earlier, it is important that the amount of crop and cattle residues removed from the soil does not have a detrimental effect on future crop productivity. The portion of residue which may be removed must be calculated in terms of each beneficial effect in which these wastes play a role (i.e. erosion control, nutrient replacement, and soil structure stabilization). The basis of this analysis has been well outlined elsewhere (Posselius and Stout, 1980).

3.4.1.3.1 crop residues

a) *quantification of total arisings*

| | corn | oats | wheat |
|--------------------------|---------|---------|---------|
| Area (acres) | 50 | 20 | 15 |
| Yield of grain (bu/acre) | 150 | 80 | 80 |
| Total production (bu) | 7,500 | 1,600 | 1,200 |
| Mass per bushel (lb/bu) | 56 | 32 | 60 |
| Residue/Grain ratio | 1:1 | 2:1 | 1.7:1 |
| Total residue (lb) | 420,000 | 102,400 | 122,400 |

b) *residue requirements to limit water erosion*

In North America, it has been estimated (Wischmeier and Smith, 1978) that soil losses due to erosion are tolerable up to 5 tons/acre-yr. The amount of residue required to hold erosion due to water to that tolerance level may be estimated by the universal soil loss equation for water (USLE w).

$$A = R K L S C P \quad (i)$$

where; A = computed soil loss per unit area per year

R = rainfall and run-off factor (determined from Fig. 27)

K = soil erodibility factor (determined from Fig. 28)

LS = slope length and steepness factor
(determined from Fig. 29)

C = cover and management factor (related to amount of
residue left on the soil by Table 43)

P = support practice factor (determined from Table 42)

setting A = 5 tons / A-yr (tolerance level)

and using values for the remaining variables as determined in
Figure 27 - 29 and Table 42, it is possible to calculate the
cover and management factor (C).

$$5 = (75) (.34) (0.1) C (0.3)$$

$$\therefore C = 6.54$$

By Table 43 it can be seen that this value for 'C' may be ob-
tained by returning very little residues to the surface
(\ll 500 lb/acre).

c) residue requirements to limit wind erosion

Wind erosion parameters on soil loss involve a complicated
function of surface roughness, climate factors, field size, and
vegetative cover. As such, it is only possible to estimate the
quantity of residue required to be left on the ground to hold
wind erosion to 5 tons/acre-yr. This estimation is achieved
through Table 44. Assuming that oat and corn residues have
the same effect in wind erosion control as wheat straw, then
by Table 44, 1,600 lb/A of residues is required to hold wind
erosion to within tolerable limits.

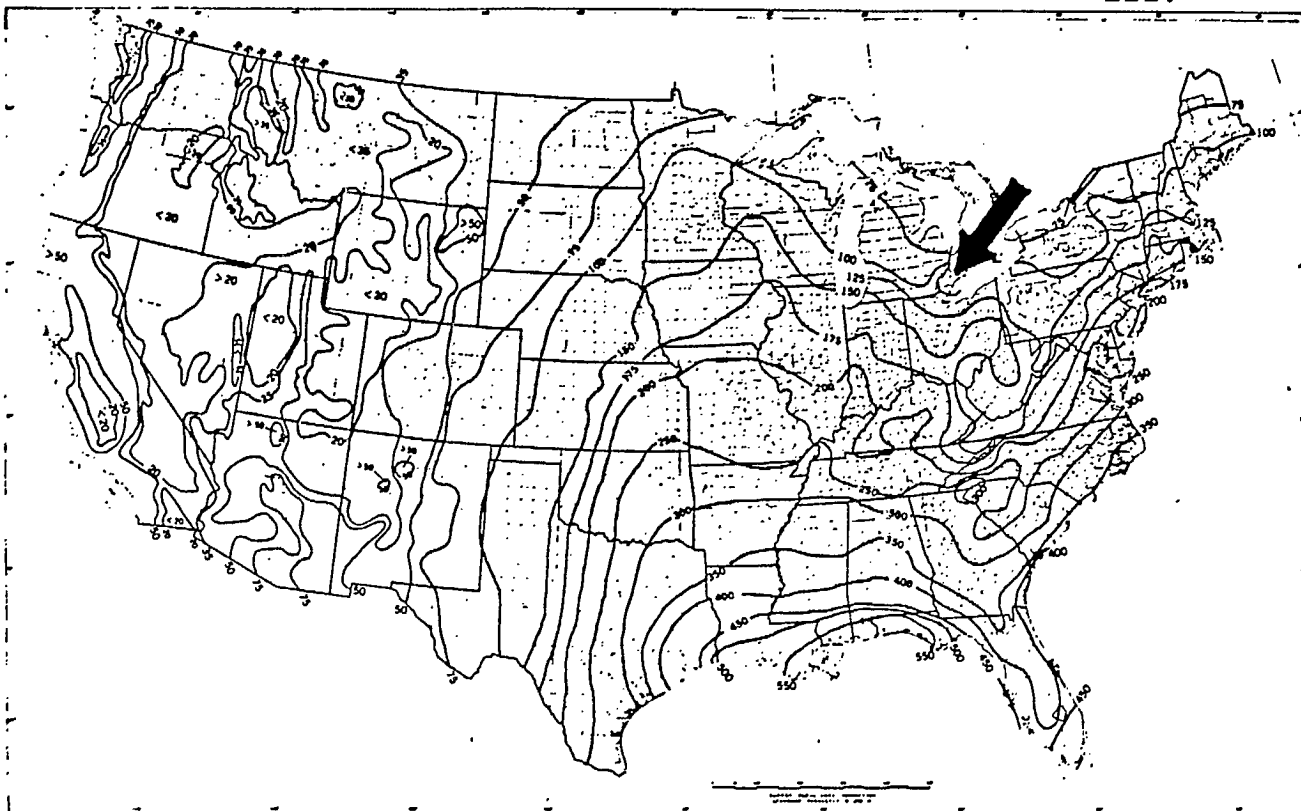


Fig. 27: Figure used to determine 'R' factor in USLWw
 (area marked by arrow) $R = 75$
 (Source: Posselius and Stout, 1980)

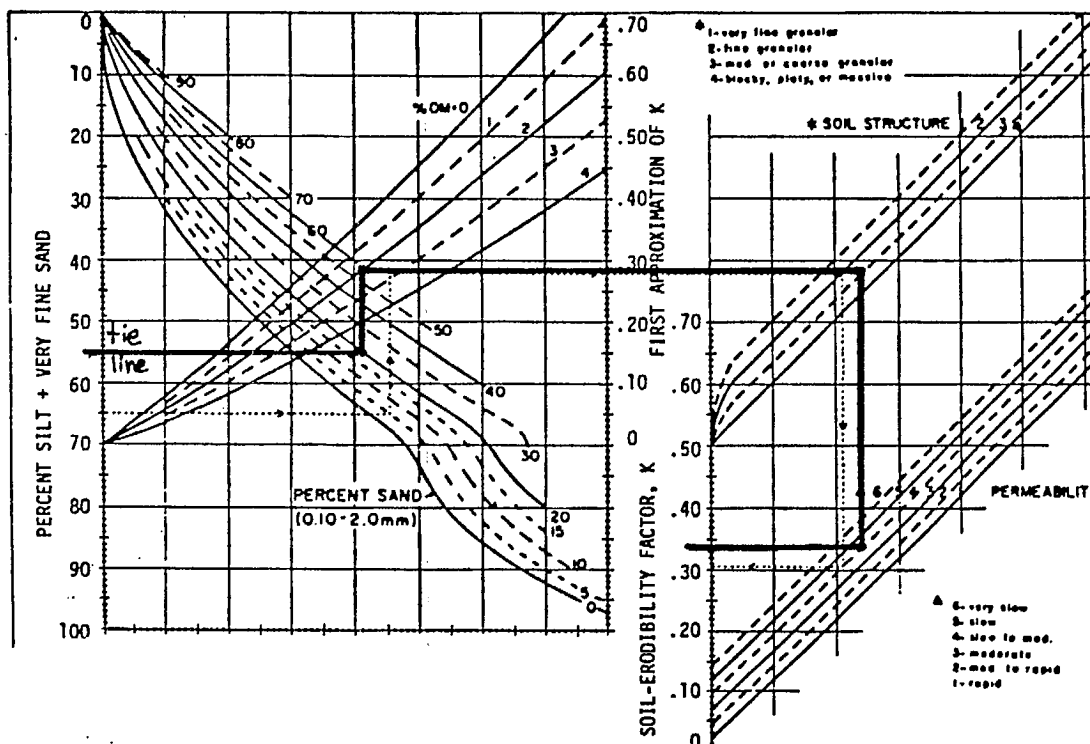


Fig. 28: Nomograph used in determination of 'K' factor.
 Characteristics of soil were estimated.
 (tie lines shown) $K = .34$
 (Source: Posselius and Stout, 1980)

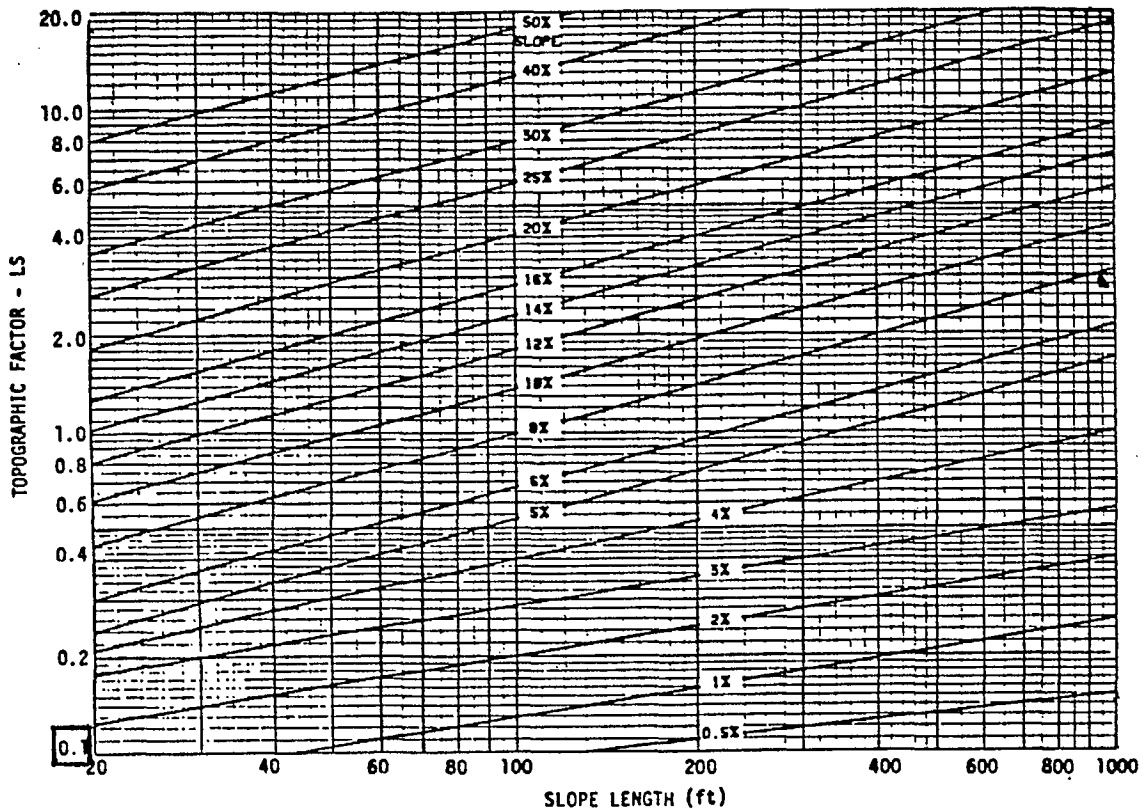


Fig. 29: Graph used in quantitation of 'LS' factors. Field contours are extremely flat. Therefore LS was taken at minimum. $LS = 0.1$.

(Source: Posselius and Stout, 1980).

| Percentage slope | P_c | P_{sc} | P_{tc} |
|----------------------------|------------|-----------------------------|--------------------------|
| | Contouring | Strip cropping ¹ | Terracing and contouring |
| Parallel to field boundary | 0.8 | - | - |
| 1.1- 2 | 0.6 | 0.30 | - |
| 2.1- 4 | 0.5 | 0.25 | 0.10 |
| 4.1- 7 | 0.5 | 0.25 | 0.10 |
| 7.1-12 | 0.6 | 0.30 | 0.12 |
| 12.1-18 | 0.8 | 0.40 | 0.16 |
| 18.1+ | 0.9 | 0.45 | - |

¹A system using 4-yr rotation of corn, small grain, meadow, meadow.

²for slope up to 12% only.

Table 42: Data used to determine 'P' factor. Strip cropping utilized with minimal slope. $P = 0.30$.

(Source: Posselius and Stout, 1980).

| | Till plant, chisel plow & rotary strip tillage ¹ | | | | | Zero tillage-no-till or slot planting ² | | | | |
|--|---|-----------|-----------|-----------|-------|--|-----------|-----------|-----------|-------|
| | lb corn residue on surface/A ³ | | | | | lb corn residue on surface/A ³ | | | | |
| | 1000-2000 | 2000-3000 | 3000-4000 | 4000-6000 | 6000+ | 1000-2000 | 2000-3000 | 3000-4000 | 4000-6000 | 6000+ |
| 1. Cont. corn ⁴ | .355 | .244 | .189 | .131 | .080 | .284 | .193 | .131 | .070 | .030 |
| 2. Cont. corn ⁴ RdR, cover crop RdL | .343 | .239 | .219 | .206 | .062 | .253 | .196 | .180 | .163 | .034 |
| 3. RRROx ⁴ | .185 | .136 | .111 | .085 | .056 | .150 | .097 | .080 | .052 | .036 |
| 4. RRROx ⁴ | .125 | .097 | .083 | .068 | .056 | .103 | .079 | .062 | .045 | .036 |
| 5. RRROM ⁴ | .149 | .109 | .086 | .069 | .051 | .122 | .088 | .065 | .043 | .028 |
| 6. RRROMH ⁴ | .125 | .092 | .072 | .058 | .043 | .102 | .074 | .055 | .036 | .024 |
| 7. RROM ⁴ | .094 | .074 | .063 | .052 | .043 | .079 | .060 | .047 | .035 | .028 |
| 8. RROMH ⁴ | .076 | .060 | .051 | .042 | .035 | .064 | .048 | .038 | .029 | .023 |
| 9. RROMHH ⁴ | .064 | .050 | .043 | .036 | .030 | .054 | .041 | .033 | .025 | .020 |
| 10. ROM | | .042 | | | | | .036 | | | |
| 11. ROMH | | .032 | | | | | .028 | | | |
| 12. ROMHM | | .027 | | | | | .023 | | | |
| 13. ROMHHM | | .023 | | | | | .020 | | | |
| 14. OHHHM | | | | | | | | | | |

- ¹Includes tillage systems which leave residues on 66% or more of the soil surface after planting.
- ²Includes tillage systems which leave residues on 90% or more of the soil surface after planting.
- ³One pound of residue from small grain, hay crops, and soybeans is equivalent to 2 lbs of corn residue.
- ⁴When soybeans are grown continuously - increase "C" factor by 20-25%; one-half of R crop - increase "C" factor by 15%; one-third of R crop - increase "C" factor by 10%. (When computing corn residue, assume that there will be 1 lb of stalks with each pound of grain produced. Corn (shelled) equals 56 lb/bu. Therefore, 110 bu of corn will yield 6160 lb of residue.

Table 43: Table used to determine amount of residue which must be returned to soil to hold water erosion to 5 tons/A/year. Given calculated value of 'C' = 6.54 it can be seen for rotary strip tillage that residues to be returned are much less than 1,000 lb/A for corn stover and 500 lb/A for other crop residues (since C >> 0.355). (Source: Posselius and Stout, 1980).

| Soil texture ¹ | Wheat residue | | Sorghum residue | | Growing wheat | |
|---------------------------|---------------|--------------|-----------------|-----------|---------------|------------------|
| | Standing | Flattened | Standing | Flattened | In furrow | On smooth ground |
| | lb/A | lb/A | lb/A | lb/A | lb/A | lb/A |
| Silts | 450 | 925 | 1,800 | 2,600 | 500 | 425 |
| Clay and silty clay | 800 | 1,600 | 3,300 | 4,750 | 975 | 825 |
| Loamy fine sands | 1,050 | 2,125 | 4,200 | 6,200 | 1,200 | 1,000 |

¹Silts with 50% nonerodible fractions greater than 0.84mm in diameter. Clay and silty clay with 25% nonerodible fractions. Loamy fine sand with 10% nonerodible fractions.

Table 44: Data used to estimate amount of residue which must be returned to the land to hold wind erosion to 5 tons/A/year. For characteristics of soil marked on the table, residue to be returned must be 1,600 lb/acre. (Source: Posselius and Stout, 1980)

d) *residue requirements to limit nutrient depletion
within the soil*

| | corn | oats | wheat |
|---------------------------------------|---------|---------|---------|
| residue available (lb.wt.wt.) | 420,000 | 102,400 | 122,400 |
| moisture content (%) | 15.5 | 14.0 | 14.0 |
| residue available (lb. dry wt.) | 355,000 | 88,000 | 105,300 |
| residue nutrient content (% dry wt.) | | | |
| N | .88 | .63 | .54 |
| P | .12 | .16 | .07 |
| K | 1.33 | 1.65 | .97 |
| residue nutrient content (lb.dry wt.) | | | |
| N | 3,124 | 555 | 621 |
| P | 426 | 141 | 74 |
| K | 4,722 | 1,453 | 1,021 |
| crop nutrient demand (lb dry wt.) | | | |
| N | 10,000 | 4,000 | 3,000 |
| P | 2,250 | 900 | 675 |
| K | 2,250 | 900 | 675 |

As can be seen above, the nutrient levels available in the residues do not meet the demand required for optimal crop growth. As well, the relative ratios of nitrogen, phosphorus and potassium in the residues are not proper for plant uptake. This would lead to variations in soil nutrient levels which may cause nitrogen deficiencies during rapid crop decay in spring and early summer (Allison, 1973).

To eliminate nutrient imbalance and deficiency, commercial fertilizers will be used to replace soil nutrients. Therefore, the effect of residue removal on nutrient loss to the soil will be considered to be negligible under these conditions.

e) *residue requirements to limit detrimental effects
of soil structure degradation*

The deleterious effect of residue removal on the physical structure of soil is difficult to quantify. At present, research is involved at the University of Guelph in this area

(Tanner, 1982). In some cases, loss of soil integrity (tilth) may be responsible for greater decreases in soil fertility than erosion.

In this study, it was assumed that residues returned to the soil for limitation of wind erosion were also sufficient to retain soil tilth. The validity of this assumption is admittedly questionable.

f) *net residue available for the process*

The most substantial effect of crop residue removal arises from wind erosion. Under the conditions described, 1,600 lb/acre of residue is required to hold soil loss to 5 tons/acre/yr. As a safety margin, 25 % is added to this figure to ensure that organic loading of the soil is sufficient. Therefore, a total of 2,000 lb/acre-yr of residues are required to retain soil fertility. Available residues for conversion to SCP are as follows:

| | corn | oats | wheat |
|---------------------------------|---------|---------|---------|
| total residue available (lb) | 420,000 | 102,400 | 122,400 |
| total residue returned (lb) | 100,000 | 40,000 | 30,000 |
| net residue for processing (lb) | 320,000 | 62,400 | 92,400 |

Converting to metric, this yields a total of 215.4 mt. of available residues per year.

3.4.1.3.2 Cattle manure

Manure from livestock is required in the Waterloo process for the production of methane as an energy credit and to provide N, P, K compounds as a nutrient source in the fermentation. The data shown in this determination has been outlined by Palz and Chartier (1980).

| | | |
|--|-----------|------------------|
| Number of cattle | | 75 |
| Manure production (kg(dry solids)/cow-day) | | 4.5 |
| Total manure produced (kg(d.s.)/year) | | 123,000 |
| Percentage mixed with crop residue and returned to soil (to conserve original ratio) | | 25 |
| Total net manure available for anaerobic digestion (kg(d.s.)/year) | | 92,400 |
| Organic dry matter content (%) | | 80 |
| Biogas yield (l/kg (o.d.m.)) | | 312 |
| Total biogas production (l/year) | | 23×10^6 |
| Nutrient composition (% dry matter) | | |
| | N | 3.0 |
| | P | 0.35 |
| | K | 2.0 |
| Nutrient composition | (kg/year) | |
| | N | 2,770 |
| | P | 323 |
| | K | 1,850 |

3.4.1.3.3 Summary of available substrate

The total mass of substrate which arises from this farming operation is shown in Table 45. . Also shown in this table is the substrate available on a daily basis of process operation (assuming 330 productive days per year).

It is apparent that a large scale SCP plant fed only from one farm is not likely to be economically viable due to the limited amounts of residues generated. Therefore it was decided this process should be centrally located and provided with residues arising from five similar operations, all with the characteristics of the farm just discussed.

Table 45: Mass summary of available residues for use in the Waterloo process

| | <u>Crop Residues</u> (fresh weights) | <u>Manure</u> |
|--|---|---------------|
| Availability per year (basis of one farm) | 215.4 mt | 660 mt |
| Availability per day of processing (basis of one farm) (330 productive days/year) | 0.65 mt | 2.0 mt |
| Availability per day of processing (basis of 5 similar farms) (330 productive days/year) | 3.3 mt | 10.0 mt |

3.4.1.4 Description of the Process

Slight modifications have been made to the Waterloo process which has been described in a number of publications (Moo-Young, et al., 1979a; Moo-Young, et al., 1980; Moo-Young, et al., 1980a; Moo-Young, et al., 1981; MacDonald, et al., 1981). See Figure 30 for a summary of the operation. It should be noted that the following is a single-case analysis used for example purposes. A number of scenarios have been outlined by Moo-Young and colleagues and information on these are available in the above references.

3.4.1.4.1 pretreatment

Two days worth of crop residues (6.6 mt) is kept in enclosed storage S-1. Working on a three shift (24 hour) basis, 0.14 mt of cellulose residues are passed through hammermill M-1 per hour. This step reduces the material to 0.7 mm linear dimensions. A conveyor system transports the ground residue to a continuous treatment tank (TT-1) which solubilizes the lignin and swells the cellulose crystallite in a 1:20 (solid/liquid) ratio of 4% NaOH (w/v) solution held at 100°C. The residence time of this reactor is one hour. This treatment also acts to sterilize the broth. The outflow from TT-1 passes through a shell and tube heat exchanger where it is cooled to a temperature of about 40°C and exits into holding tank, HT-1. The

holding tank is sized such that it is full to capacity every 4 hours. In HT-1, the pH is adjusted to 5.5 (from pH 12) with the addition of sufficient H_2SO_4 .

3.4.1.4.2 aerobic fermentation

Fermentation is carried out using Chaetomium cellulolyticum, a cellulolytic fungi with a high protein content (45 %) and rapid growth rate ($\mu = 0.24 \text{ hr}^{-1}$).

On a 4 hour cycle, media is discharged from HT-1 into one of 6 fermenters which operate in parallel. Fermentation is carried out in a batch and dump mode such that the process is continuous though fermentations are not. Fermenters are of the air-lift type to provide both aeration and agitation. After addition of the inoculum, fermentation proceeds for 22 hours at 37°C with a complete cycle of a fermenter finished in 24 hour. The remaining 2 hours is utilized in draining, cleaning, and recharging the vessel with fresh media.

3.4.1.4.3 product separation and concentration

After fermentation is completed, the broth is passed to a holding tank (HT-2). Biomass and unfermented solids are separated by a drum filter (DF-1) to 45 % solids and then further dried by means of a continuous drum drier to a final value of 80 % solids content.

3.4.1.4.4 anaerobic digestion

Little information was available on the aspects of the anaerobic digestion of cattle manure for use in the Waterloo process. A total of 10 mt/day (wet wt.) is available to the operation. The optimal size of digester for this load under semi-continuous conditions (12 day retention time) is 120,000 l (AD-1). Mesophilic temperatures (39°C) are used. This digestion step reduces the input solids concentration from 15 % to 9 % in the effluent. A proportion of the waste effluent is stored in tank HT-3 to be used as a nutrient source for the aerobic fermentation step. The amount of this effluent required was not

specified, however, it was assumed that enough would be produced to meet the needs of the fermentation. Surplus sludge is used as crop fertilizer.

Sludge gas containing methane is stored under low pressure to be used as an energy source for the production of steam at boiler, B-1. The steam is used to heat the treatment tank (TT-1).

Surplus methane may be sold as a by-product credit.

3.4.1.4.5 summary of the process

Figure 30 illustrates the entire process in schematic form. The mass balance (based on one full day of operation) is shown in Table 46.

3.4.1.5 Economic Evaluation of the Process

The economic characteristics of this process are difficult to determine due to the number of assumption that are required to be made.

Collection and transport costs have been set to zero. This is understandably not a realistic assumption, however, by locating the process central to the 5 farms involved in it would minimize transport costs. Collection systems are presently in operation (as described earlier) and therefore will not incur added cost to the process.

The nutrient demands of the aerobic fermentation are unknown to the author. It will be assumed that fortification with sludge from the anaerobic digester satisfies these requirements. By-product credit for surplus sludge will not be added to the economics since this material will be utilized by the farmers involved in the operation.

All of the following estimations have been derived from either Moo-Young et al. (1979a), local equipment manufacturers, or from formulae presented in Peters and Timmerhaus (1968).

Fig. 30: Schematic diagram of model process - SCP from lignocellulosics

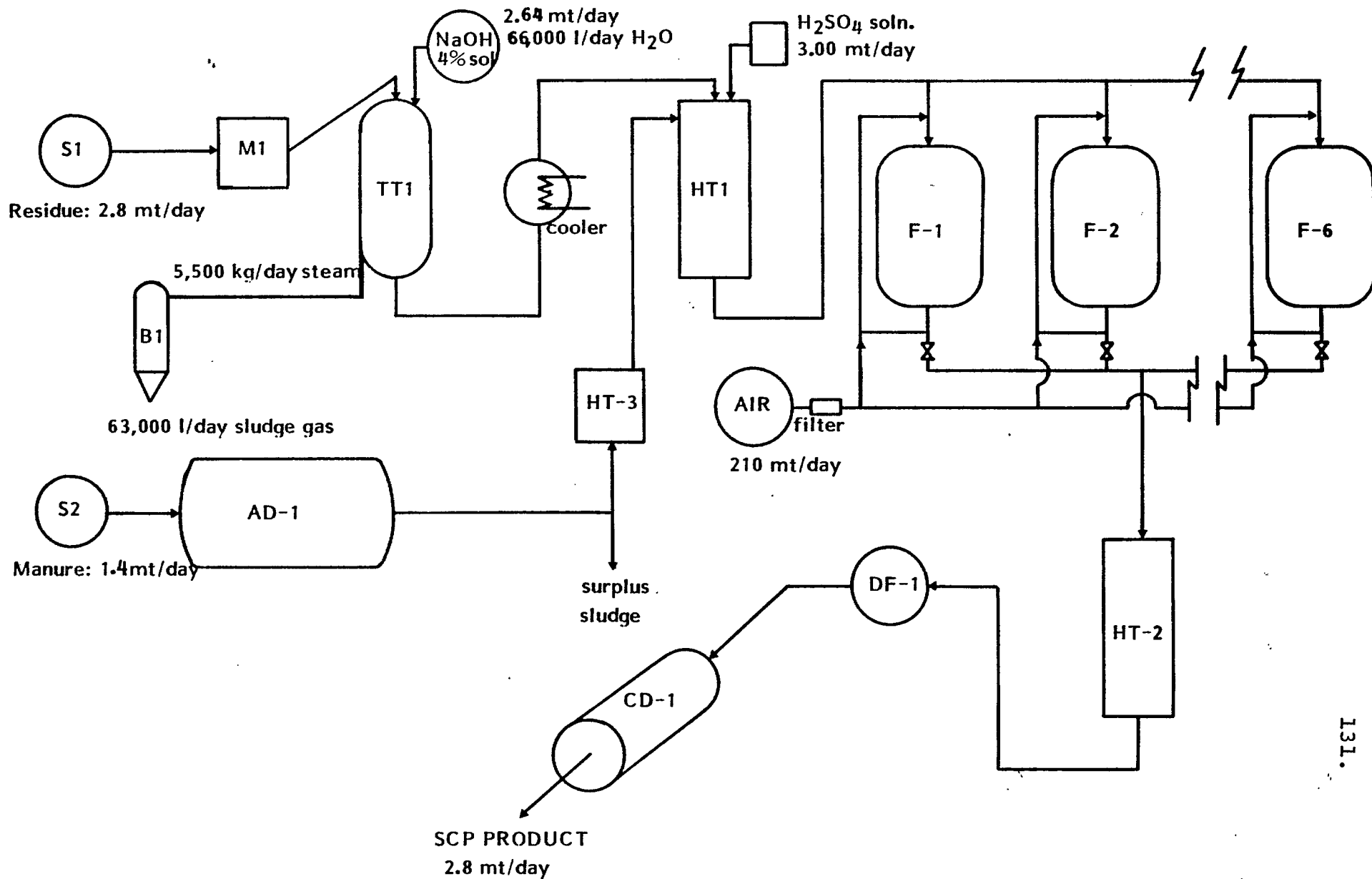


Table 46 : Mass Balance of the Process
(basis of one productive day)

Inputs

aerobic fermentation:

mt/day (dry wt.)

| | |
|-----------------------------------|------|
| 1. Crop residue (total) | 2.8 |
| cellulose (40%) | 1.1 |
| hemicellulose (27%) | 0.76 |
| lignin (14%) | 0.39 |
| protein (4%) | 0.11 |
| ash (8%) | 0.22 |
| other (7%) | 0.20 |
| 2. NaOH | 2.64 |
| 3. H ₂ SO ₄ | 3.00 |
| 4. O ₂ in air | 44 |
| 5. Water | 66 |

anaerobic digestion:

| | |
|--------------------|--------|
| 1. Manure (total) | 1.4 |
| carbohydrate (46%) | 0.64 |
| lignin (13%) | 0.18 |
| ash (9%) | 0.13 |
| inert (22%) | 0.31 |
| N (3.0%) | 0.042 |
| P (.35%) | 0.0049 |
| K (2.0%) | 0.028 |

Outputs

aerobic fermentation:

| | |
|------------------------|--------------------|
| 1. Total solid product | 2.8 |
| protein (33%) | 0.92 |
| carbohydrate (26%) | 0.73 |
| fat (7.3%) | 0.20 |
| nucleic acids (3.7%) | 0.10 |
| minerals (3.7%) | 0.10 |
| vitamins (high) | - |
| cellulose (4%) | 0.11 |
| lignin (12%) | 0.34 |
| ash (11%) | 0.31 |
| | biomass |
| | undigested residue |
| 2. Water | 66 |

.....continued Table

anaerobic digestion:

| | <u>1/day</u> |
|-----------------------|--------------|
| 1. Sludge gas (total) | 63,000 |
| methane (65%) | 41,000 |
| CO ₂ (35%) | 22,000 |

The final composition of sludge effluent is unknown.

3.4.1.5.1 major purchased equipment costs for the process

The characteristics of the major equipment and their costs is shown in Table 47. The sizing of tanks was increased 25 % over their required capacity as a safety margin.

3.4.1.5.2 determination of total costs for plant set-up

Steps in this determination is shown in Table 48. Bracketed inserts show the costs of each item as a percentage of the PEC.

3.4.1.5.3 estimation of fixed operating costs

See Table 49 for a summary of the fixed operating costs for this process. For an annual production rate of 920 mt. of high quality feed (33 % protein), the fixed operating costs account for \$ 0.29/kg.

3.4.1.5.4 estimation of direct operating costs

Costs which arise through the daily operation of the SCP plant are shown in Table 50. As can be seen, chemical expenses far outweigh any other costs to run the process.

Direct operating costs account for \$0.81 per kg of product.

3.4.1.5.5 determination of by-product credits

Since digester sludge is utilized in the process or returned to the land of farms involved in the operation, no by-product credit is assigned to it.

The total amount of energy available from the biogas (at 22 MJ/m³) is 1,400 MJ per day. This would provide 10% of the total steam requirements for the process by utilizing this methane in the boiler. Since the costs for steam on a daily basis are extremely low when compared to chemical costs, this credit will not change the economic characteristics to any great extent.

Table 47 : Purchased Equipment Costs (PEC)

| Equipment | Quantity | Characteristics | Cost (\$ can.) |
|--------------------|----------|--|-------------------------|
| Hammer mill | 1 | - 7.5 hp motor - with stand | 4,200 |
| Treatment vessel | 1 | - 4,000 l cap'y - jacketed - #316 st.st. - alkali resistant | 10,000 |
| Holding tanks | 2 | - 15,000 l cap'y - #304 st.st. | 18,250 |
| Holding tank | 1 | - 4,000 l cap'y - #304 st.st. | 3,000 |
| Aerobic fermenters | 6 | - 15,000 l cap'y - #304 st.st. - jacketed | 145,000 |
| Drum filter | 1 | - 1 1/2 hp motor - 150 kg/hr cap'y - 3.2 m ² filter | 85,000 |
| Continuous dryer | 1 | - rough estimate based on Moo-Young data | 55,000 |
| Anaerobic digester | 1 | - 120,000 l cap'y | 50,000 |
| Steam package | 1 | - rough estimate based on Moo-Young data | 55,000 |
| Total PEC | | | <u>426,000</u> ===== |

Table 48: Estimation of costs for plant set-up

| <u>Item</u> | <u>Cost (\$ Can.)</u> |
|-------------------------------|-------------------------|
| PEC | 426,000 |
| Pumps and pipes (30%) | 128,000 |
| Sub-total | 554,000 |
| Sole tax (7%) | 30,000 |
| Sub-total | 584,000 |
| Building requirements (14%) | 60,000 |
| Sub-total | 644,000 |
| Working capital (10%) | 43,000 |
| Total plant construction cost | <u><u>\$687,000</u></u> |

Table 49: Determination of fixed operating costs (yearly basis)

| <u>Item</u> | <u>Cost (\$ Can.)</u> |
|---|-----------------------|
| Maintenace and repairs (5% per year) | 34,000 |
| Labor (6 workers at \$12,000 per annum) | 72,000 |
| Tax and Insurance (3% per year) | 21,000 |
| Interest and total investment (20% per year) | <u>137,000</u> |
| Estimated fixed operating costs | \$264,000 |
| Fixed operating cost per kg product | 0.29/kg |

Table 50: Steps in the determination of direct operating costs

| <u>Item</u> | | <u>Costs</u> (\$ <u>Can./day</u>) |
|--------------------------------------|--|---------------------------------------|
| NaOH | 4% (w/w) sol ⁿ , 66,000 l/day Can. \$0.50/kg | 1,320 |
| H ₂ SO ₄ | to neutralize NaOH Can. \$0.12/kg | 360 |
| Water | 66,000 l/day; Can. \$0.44/m ³ | 30 |
| Power | 3.5 kwh/kg product Can. \$0.05/kwh | 490 |
| Steam | 5,500 kg/day Can. \$8.00 mt | 40 |
| Miscellaneous | 1% of total | 20 |
| Total direct operating costs | | \$2,260 |
| Direct operating cost per kg product | | 0.81/kg |

When the fixed and direct operating costs for this process are summed, it is found that the total price of the SCP product is \$1.10/kg. On a protein basis (final product: 33% protein) the cost is \$3.30/kg protein.

Recent data has placed the price of 1 kg protein (from distillers dried grains) at a value of 0.75 Can.\$ (Synfuels Week, 1982). Therefore, on a protein basis, this process is highly uneconomical. However, provided there is no toxicity associated with the product, the overall product is valuable as a balanced feed for livestock. It contains not only protein, but various other nutrients as well as lignin (important for roughage) and unhydrolyzed cellulose which may be digested by ruminants. Thus, comparison on a protein basis alone may not be valid. (Note: see section 3.4.1.6 for recent data on feeding trials of this material).

Scaling-up of the operation may also serve to bring down the cost of the product. However, Moo-Young, et al. (1979a) found that for a process similar to this the break-even point (i.e. return on investment = 0) was reached at a protein productivity of 0.69 mt/day which is 0.23 mt/day less than the process described here. Moo-Young and colleagues extensively utilized recycle systems for heated process streams and steam condensates. As well, large by-product credit was allotted to lignocellulosics which had been simultaneously degraded into forage-grade carbohydrate fodder during the pretreatment stages. All these factors have a great influence on the overall economics of the process. A thorough investigation with actual data from a pilot plant operation (which is not available to us in detail) will allow to develop reliable technological and economic parameters for scale-up and industrial production of this SCP product.

3.4.1.6 Addendum: Recent Information on Nutritional Value of Waterloo Process - SCP

Unfortunately, actual animal feeding trials of SCP product from the Waterloo Process (W-SCP) are limited in number and scope.

Initial feeding trials on rats, rabbits, and mice have been carried out at the University of Guelph (Mowat and Scrivastava, 1979; Buchanan-Smith, 1980; 1981). These studies indicated good animal performance on feed containing 10-20% of fungal protein and no teratogenic effects or abnormalities were observed with mice through four generations. Concern, however, was expressed over the low palatability exhibited for some samples and decreased utilization efficiency seen with a higher SCP content of others.

More recently, a study on the nutritive value of W-SCP grown on various feedstocks has been undertaken at the University of Guelph (Leeson, 1983) and results are rather inconclusive.

Chaetomium cellulolyticum grown on corn stover in a 1,000 l facility (located at the University of Waterloo) showed no overt toxicity when fed to broiler chickens. However, the crude protein content (CP) for oven-dried samples of this SCP were very low (CP = 23% w/w measured as total nitrogen) and the crude fibre content (CF) was much too high for the monogastric diet (CF = 24% w/w). Since the amino acid composition of these samples was only about 50% of the crude protein, it is postulated that non-protein nitrogen (probably arising from media nutrients) contributed to the overall CP value. The metabolizable energy

$$(\text{ME} = \frac{\text{total energy intake (kcal)} - \text{total energy excreted (kcal)}}{\text{total feed intake (kg)}})$$

of this material (fed to adult roosters) was determined to be 1125 kcal/kg. In comparison, rapeseed meal (a high fibre, low energy protein ingredient considered by feed manufacturers to be a limiting factor in diet incorporation) has an ME of 1900 kcal/kg or 170% that of W-SCP.

Net protein utilization (NPU) is also a useful measure of the biological availability of crude protein consumed by an organism. When broiler chicks were fed the Waterloo product, NPU was found to be 40% that calculated for soybean meal. Based on the above results, the final SCP product obtained from corn-stover cannot be recommended as a protein feedstuff.

Waterloo-SCP derived from pulp-mill sludge by this same process also exhibited these detrimental qualities (CP = 36%; CF = 25%; ME = 1353 kcal/kg).

When feeding trials (with adult roosters) were performed using this fungal product derived from pure glucose or cellulose medium, different results were obtained. In this case, the birds refused to accept W-SCP (from glucose) at all levels of inclusion in their diets. The trial was duplicated and the same results were observed. Cellulose based W-SCP was accepted but only on an erratic basis by the test animals. Reasons for this feed refusal were postulated as due to the presence of a mycotoxin and/or chemical contamination. In addition to the concern over feed refusal, crude fibre of the glucose derived W-SCP was found to be 21% of the dry product. This indicates that the fibre is of fungal origin and as such, severely limits the usefulness of W-SCP as a monogastric feed ingredient. This conclusion, however, could not be confirmed in a duplicate experiment using SCP grown under identical conditions but in an earlier fermentation run. In this case, no significant variation in nutritive value and palatability was observed between the SCP mixture and control protein (casein) using rats as the test organism.

To further confuse the issue, feeding trials were performed on samples derived from a glucose medium at a 10,000 l fermentation prototype facility constructed by Envirocon Ltd. (Vancouver, B.C.) under licence to Waterloo. Apart from an increase in scale, these two processes are identical. However, this product was noticeably different in colour and texture as compared to the product obtained from the Waterloo facility (i.e. a white cream mash compared to the green-black-brown granular material produced at Waterloo).

In addition, the Envirocon-SCP had an ME of 2233 kcal/kg (100% improvement over that of Waterloo) and at this level, would be acceptable for inclusion in monogastric diets.

It is not clear from these results whether the single cell protein produced by the Waterloo Process is a valuable ingredient in poultry and other monogastric diets. It would

seem that the largest problem associated with the product is unexplained variations in its nutritive quality as apparent from the investigations described here. Physical dissimilarities between the Waterloo material and Envirocon.- SCP indicates a major difference in product development. In addition, palatability and nutritional value of W-SCP grown under the same conditions but in different fermentation runs give markedly different results. Until better reproduction of product quality (as proven by more intensive feeding trials) can be demonstrated, the success in the marketing of SCP generated by the Waterloo Process is doubtful.

4. MARKETING AND ECONOMICS

4.1 MARKETING SINGLE CELL PROTEIN

The problems associated with selling single cell protein in the marketplace are essentially the problems of selling the product of a new industrial process to a consumer who is unfamiliar with the product. The key marketing decisions for the product of a biotechnology process are the same as those for the product of any industrial process. The correct time to commercialize the product, the suitable target market, pricing, method of distribution, message and target of an advertising effort - all are important elements of the marketing plan. However, the marketing of single cell protein does present problems which are unique to products of this general type of process. The most serious problem is consumer fears of bacterial contamination and toxic carryover which prevent acceptance of the product as a feedstuff for animal or human consumption.

4.1.1 THE PRODUCT

The product of a single cell protein fermentation is a fluid stream containing a low concentration of cells, is usually concentrated and then dried.

The final product is a granular meal which can be ground to reduce granule size or to produce a flour. It is possible to produce a protein product of highly consistent nutritional composition from a fermentation process, provided the inputs are consistent. This is easily accomplished with homogenous substrates but can be a problem for processes based on waste substrates where the material used may come from a variety of sources and may be of inconsistent composition.

The single cell protein product, as either a granular meal or a flour, could be used in any situation where other high protein flours and meals (such as soya bean flour and meal) are

utilized. Single cell protein products could be used as protein supplements in virtually all animal feeds requiring a protein supplement, or as a high protein meal or flour for human consumption. Many single cell protein products are palatable for both animals and humans and could be used in a variety of foods, commercially formulated animal feeds, and even domestic animal feeds.

Single cell protein products are generally of higher protein content than other protein sources so higher protein concentrations can be achieved with less volume than if other protein sources were used. Single cell protein products are also a well balanced source of amino acids, essential minerals, trace elements and vitamins.

One problem with single cell protein is the high concentration of nucleic acids. Unlike animals, humans are unable to metabolize nucleic acid and will develop gout and other conditions if nucleic acids accumulate in the body. Processes exist by which nucleic acids can be removed from the product as discussed earlier.

It is likely that the nutritive qualities of single cell products will be improved through laboratory research. Genetic engineering of the microorganisms used in these processes may be able to increase protein content and increase concentrations of other nutritive ingredients in the end product.

4.1.2 PROBLEMS IN MARKETING SINGLE CELL PROTEIN PRODUCTS

Specific problems associated with marketing a single cell protein product can be created by the type of process used and by the intended end use of the product. The major marketing problem created by this general type of process is the difficulty in selling a cellular protein product to a consumer who psychologically associates bacteria, fungi, and to some extent, yeast, with disease. The consumer in this case could be a farmer intending to feed a single cell protein containing feed to his

livestock or the intended end user of a single cell protein product meant for human consumption. A second source of consumer resistance is the fear of toxic carry-over from the substrate used for the fermentation. This is especially a problem with hydrocarbon-based processes but can also be a factor in carbohydrate and cellulose-based processes where the substrate is in a toxic form initially (i.e. pulp mill effluent) or where toxic materials are required to treat the substrate during or prior to fermentation (i.e. pre-treatment of cellulose). Concern for the possible toxic nature of single cell protein products resulted in a large protest by consumer groups in Japan. This action effectively killed Japanese plans to market a single cell protein product for human use.

The marketing problems presented by consumer resistance to a unicellular product and by consumer concern over the possible toxic nature of the product may or may not create a serious barrier to the successful commercialization of the product, depending on the intended end use of the product. There is certain to be more resistance to a single cell protein product intended for human consumption than to a similar product marketed as a protein supplement for animal feed. The reluctance of consumers to eat a single cell protein product, especially one derived from a toxic substrate, is likely to be so great that single cell protein for human consumption is likely to be used and accepted only in situations where protein shortages are sufficiently severe to demand that alternate and novel protein sources are used to prevent starvation. For this reason, this discussion of single cell protein marketing will consider only the use of such a product as a protein supplement in animal feeds.

At present (1984) two large-scale commercial operations which market their product in the 'free enterprise' environment of the Western World are the Imperial Chemical Industries (ICI) 'Pruteen' operation at Billingham, England, and the Attisholz

SCP from waste sulfite liquors. ICI currently produces between 40,000 and 50,000 tonnes of single cell protein product, Pruteen, each year. Pruteen has been officially approved for sale in thirteen European countries. However, it is generally acknowledged that ICI is losing money on the sale of Pruteen because the selling price cannot be set high enough to cover costs. ICI has spent more than 100 million pounds developing and commercializing Pruteen and hopes to recover some of this investment by selling entire operations to countries with both a protein shortage and an excess of the substrate (methanol from waste hydrocarbons). There are several large scale single cell protein production ventures in Communist countries. Very little is known about the economics of these operations and it is not likely that they are required to operate at a profit, as the government is more concerned with reducing dependence on foreign sources of conventional proteins for animal feeds. It is likely that governments insist on the use of the single cell protein and, with a captive market, the producers would not be required to implement what would ordinarily be an extensive marketing program.

In Canada, Envirocon , a Vancouver-based company, with support from the Federal Department of Industry, Trade, and Commerce and Regional Economic Expansion, has recently opened a plant designed to convert pulp mill waste into a fungal protein at the rate of one tonne of sludge per day (Feed and Farm Supply Dealer, 1983). The plant design was based on a process patented by the University of Waterloo, and has been designed and built by Envirocon to demonstrate the technical and economic viability of the process for commercial scale operations. The company intends to market the product of the operation as a protein supplement for animal feed.

ICI was faced with the first marketing of a single cell product on a large scale. The emphasis of the marketing program was the nutritional advantages of the product and the success experienced in large numbers of feeding experiments. Because

of the success of the ICI program, subsequent marketing efforts by other producers of single cell protein products should be less difficult. Likewise, the Envirocon operation in British Columbia will require the first marketing of a single cell protein product in Canada. Observation of the strategy employed by Envirocon and the success of the Envirocon product in the Canadian feed market will provide valuable information for future marketing programs by other companies.

4.1.3 THE MARKET FOR ANIMAL FEED PROTEIN SUPPLEMENTS

A single cell protein product marketed as a protein supplement for animal feeds would have to compete in the market with several other materials currently used as protein sources in feed formulations. These include soya bean meal, rapeseed meal, meat meal, feather meal, corn gluten, linseed, and others. Each available protein source has different nutrient levels and different physical characteristics which may limit the amount used in a feed.

The market into which a single cell protein product for animal feeds would be introduced is the feed formulation industry. Feed formulators produce complete feeds for a variety of livestock which are sold to farmers, either directly or through farm supply companies. Protein supplements are one of the key ingredients in most feeds. The various protein sources are obtained from 'processors' who process the raw materials into usable feed ingredients. The processors generally maintain a stock of finished product so that the formulator need only call in an order to obtain the required ingredients for the formulation. The processors are often independent but several of the large feed formulators have vertically integrated and now control processing operations to ensure a supply of feed ingredients.

In 1980 there were 609 feed companies in Canada (excluding small business operations). Ontario and Quebec comprise a large

percentage of the total Canadian operations with 196 and 226 feed operations, respectively. The total value of shipments of own manufacture from feed companies nationally was \$ 2,433 million in 1981, and \$ 2,281 million in 1982, with Ontario and Quebec together accounting for \$ 1,631 million of the 180 total (Statistics Canada 1981, 1982).

The cost of ingredients is important to the formulator, as shown by a breakdown of production costs. Statistics Canada data show that 78 % of production costs in the feed industry were comprised of raw material cost (Statistics Canada, 1980). The total cost to the feed industry of raw materials and supplies in 1980 totalled \$ 1,844,283,000, \$ 1,316,575,000 of which represents costs to feed companies in Ontario and Quebec. Of the national total for raw materials more than \$ 375 million is accounted for by soya bean flour, cake, and meal, gluten meal, meat meal, feather meal, and rapeseed meal, all of which are commonly used protein sources. Most of the materials used as protein supplements are imported in an unprocessed form and processed by Canadian processors. Most of the material imported comes from the United States. In the case of soya bean products, the largest single source of protein used in the industry, 1982 imports of unprocessed soya beans totalled 461,784 tonnes with a value of \$ 128 million, while imports of processed forms of soya bean totalled 387,681 tonnes with a value of \$ 108 million (Statistics Canada, 1982). Feed is generally formulated in and for domestic markets. Import and export figures for feeds as opposed to feed ingredients show imports of total value \$ 15 million and exports of total value \$ 17 million.

The decision to use a particular protein source in a particular meal is a complex one. The formulator must meet nutrient content standards set by the government and is constrained by the physical nature of the available materials (i.e. consistency), palatability, presence of growth-depressing and growth-promoting factors, availability of the protein source, and must do the formulation at least possible cost. To devise the most economical formulation which will satisfy the myriad of con-

straints placed on the formulator, the formulation is done on a computer using a linear program 'least cost' formulation. When all the constraints and the objective of the exercise (minimize cost) have been expressed as equations, the linear program will find the solution to all the simultaneous equations which optimizes the value of the objective. The values assigned to variables in the equations represent the amounts of each feed ingredient the formulator should use to do the formulation for that particular feed at lowest possible cost.

4.1.4 A MARKETING PLAN FOR SINGLE CELL PROTEIN AS ANIMAL FEED SUPPLEMENT

4.1.4.1 Product Strategy

The strategic plan for this product should be that for any new product and new technology. A decision to proceed with commercialization of a single cell protein product will require that significant investment be made, possibly with little or no return in the early stages of the product life cycle. The strategy is to make the needed investment to improve the product and the technology so that satisfactory product performance is achieved and maintained, and so that refined process technology allows low production cost. It may be necessary to absorb losses for some time as efforts are made to penetrate the market and to establish a level of demand sufficient to allow production facilities to operate under continuous conditions at or near maximum capacity to derive economies of scale of production. Because of the financial requirements of a new technology of this scale, investment will likely be needed from a variety of sources to reduce the risk to any individual investor inherent in this type of project. Government development programs, existing companies, venture capitalists, and individual investors may provide part of the capital required. Because of the risk inherent in new technology, it is likely that existing organizations with expertise in this area will be the driving force behind the commercialization of single cell protein technology.

These organizations may be the feed formulators and processors, with a specific interest in this product, or perhaps the breweries, with experience in fermentation and expertise in yeast genetics.

4.1.4.2 Product Development and Testing

A superior product must be developed at the primary research level before efforts are made to begin commercialization. However, even when a product and process have been developed, it will be necessary to do extensive testing at the pilot plant level. This must be done to ensure that problems associated with scaling up the process to full scale operation are discovered and solved, and to ensure that the process economics in practice are consistent with the theoretical estimates. Useful information may be forthcoming from the Envirocon operation in British Columbia, as this process will be the first such operation in Canada. It is unlikely that Envirocon will release specific information on the economics, but continued operation of the facility and subsequent investment in new operations would indicate the viability of the process.

Product produced at the pilot plant level will be used in large scale feeding tests to establish that the product is safe for animal use, that no toxic carryover exists and that the product is palatable to the animals. Feeding experiments would also indicate any growth enhancing or growth suppressing factors which might affect product performance. ICI, before commercializing the product, 'Pruteen', spent six years doing animal production trials on over 300,000 animals of six different species. Extensive toxicological testing was needed because of the toxic nature of the process substrate. Because of the work done by ICI, it is likely that a less extensive testing effort would be required for a similar process to be established in Canada. Also, processes based on non-toxic substrates would require less extensive testing.

Feeding tests, as well as other analyses, are required to obtain licensing from the federal regulatory body responsible for regulation of the industry. In Canada the Feeds and Fertilizer Division of Agriculture Canada is responsible for the licensing of feed ingredients and establishes regulations governing the formulation of animal feeds and the disclosure by feed formulators of feed composition. Agriculture Canada also establishes minimum requirements for animal nutrition which must be met by complete animal feeds. The regulations and guidelines governing the feed industry are currently under review and so no valid information is available on exactly what standards will apply to the licensing of a new feed ingredient or to the disclosure of specific feed ingredients. Complete disclosure regulations could have a negative impact on the consumer acceptance of a single cell product if livestock farmers react negatively to the inclusion of microorganisms in animal feed. Knowledge of the applicable regulations will be essential to plan an effective testing program so that the product will be accepted by both formulators and farmers and so that the product marketed will satisfy government regulations.

4.1.4.3 Product Launch

There is a sufficiently large market for protein supplements, and therefore a sufficiently large potential market for a single cell product in Canada to establish a commercial venture. Because transportation cost could add significantly to the cost of the product, the first commercial venture should be established in a region where there is a high concentration of feed formulators. For this reason the venture should be established in either the Ontario or the Quebec market. Acceptance of the product in the marketplace and profitability of the operation would perhaps make operations in other regions of the country economically attractive. Declining production costs could make transportation of the product to other regions possible while maintaining a competitive and profitable price level.

The target market for the product launch should be the large, quality feed formulators. A large formulator may realize more benefits from any price advantage available from a single cell protein product. A 'price advantage' may be evident in the actual cost of the material on a percent protein basis or may be derived from cost savings accruing to the formulator resulting from reduced requirements for expensive mineral and amino acid supplements if these substances are part of the nutritional composition of the single cell protein product in sufficient amounts. These key ingredients include the sulfur-containing amino acids lysine, methionine, and cysteine, and B vitamins, biotin, riboflavin, folic acid and pantothenic acid.

If there is reluctance in the industry to use the product in spite of successful feeding test results, this will likely disappear as soon as one formulator uses the product and gains an advantage over competitors in terms of materials cost and product quality. The key thrust of the product launch, then, should be to gain that first acceptance of the product by a formulator. The formulator may be persuaded to use the novel protein on several different accounts. The nutritional balance and high protein concentration would allow greater formulation flexibility. Also, single cell protein products genetically engineered for high protein, vitamin and mineral content would show a high performance effect, raising the overall productivity and efficiency of animal production.

To be the first to offer a superior product by using the single cell protein, the formulator takes the risk that farmers will reject the product because of fears of toxic carryover and bacterial contamination. The regulations governing disclosure of feed ingredients will have a major impact in this area. It may be possible and desirable to keep the true nature of the protein ingredients a secret if consumer surveys indicate that cellular nature of the product will be a major obstacle to acceptance in the animal production industry.

If farmers are aware of the origin of the protein supplement, it would be necessary to 'sell' the product to the farmers as well. If public concern arises over the possibility of toxic accumulations in livestock fed a single cell protein product, it would be necessary to convince consumers of animal products of the safety of the single cell protein.

4.1.4.4 Timing

The selling task cannot begin until large scale production is achieved and a steady supply of consistent quality can be assured. Because a variety of protein sources are available, and since several of these may be suitable for a specific feed, unavailability of the product when ordered will mean loss of revenue. All tests required for the marketing effort must be completed before marketing can begin but the marketing cannot begin until large scale production is started. This is because product quality and consistency cannot be guaranteed until the final production facility is in place.

For this reason it is important that construction and start-up of a large scale plant begin as soon as results from pilot plant operations indicate that the product is satisfactory and that the process is economically viable. Immediate construction of a large scale plant is necessary but will mean that the facility will probably operate at less than capacity until there is sufficient demand.

4.1.4.5 Distribution

As formulators generally order protein ingredients directly from processors, and since a dried and ground single cell protein product needs no further processing, there is no need for the producer to sell through an intermediary. If packaging facilities are included in the plant design, the product could be shipped directly to the feed formulators.

4.1.4.6 Advertising

It is unlikely that much advertising would be required, as the selling task would be primarily accomplished through personal selling. A small sales force would be needed to contact the formulation managers at feed formulators and to convince these managers to include the single cell protein product in their formulation programs. Any print advertising would be in the form of informational brochures distributed to feed formulators, other potential customers, and farmers interested in the product. The emphasis of this advertising should be the nutritional benefits of the product over other protein sources, the safety of the product for use in all feeds, and specifically for the formulators, some discussion of the potential cost savings. The informational brochures from ICI emphasize the extensive production and toxicological testing done on the product and give a nutritional and chemical breakdown of the product. The word 'bacteria' is not used in this literature. Rather, the bacterium used is described as 'a tiny living organism' and emphasis is placed on the similarities between 'Pruteen' and conventional protein sources - both are products of the basic building blocks, carbon, nitrogen, and oxygen. This advertising strategy would avoid potentially difficult problems in marketing the product. The best advertising message would be a discussion of superior results in feeding tests.

4.1.4.7 Pricing

Pricing, for this type of product, a commodity, is probably the most important element of the marketing plan. Subject to the nutritional and other constraints of the formulation program, the formulation is done on a least cost basis. This means that the nutritional composition of the product will have a direct impact on the price that a formulator is willing to pay. A nutritionally superior product will have an advantage over even lower-priced protein sources in many instances. It is not imperative, therefore, that a single cell

protein product be price competitive with other protein sources on a percent protein basis if there are nutritional advantages to using the single cell product from growth-promoting factors and vitamins, minerals, etc..

However, these comparative advantages possessed by a nutritionally superior product may not in every case be available to the formulator. This is because of an increasing trend in the industry to the use of 'micro mixes' to supply key mineral and amino acids for animal feeds. Micro mixes are pre-mixed in specific concentrations and are added in set proportions to obtain a required level of key minerals, sulfur-containing amino acids, and vitamins not ordinarily provided in sufficient quantities by conventional protein supplements and other feed ingredients.

If other materials included in the feed contain high levels of these ingredients the level of these ingredients in the feed may exceed the minimum requirements. This is not detrimental to the quality of the feed but means that the formulator is likely to choose a nutritionally simple protein source that contains little of these other substances and which is available at a lower price. It is possible that if a nutritionally superior protein source was available and was suitable for inclusion in nearly all feeds, that a manufacturer of micro mixes may take this into account and alter the composition of the micro mixes to reflect the higher levels of specific ingredients supplied by the protein source.

A useful concept for pricing a single cell protein product would be the estimated value to the consumer. Ideally the product would be priced at what the consumer would be willing to pay for it, less a certain amount to induce the consumer to buy. Hopefully this price, when calculated, covers costs and provides a suitable profit margin. If it doesn't, then it is unlikely that the product can be marketed unless costs of production are decreased or costs of competing products increase so that

the value of the product to the consumer is greater.

However, it is not easy to estimate the value of a protein source to a feed formulator. Because formulation requirements for each feed are different, the value to the formulator of a specific protein source will be different for each different feed. The best method to set a price is to include the nutritional data for a product in the formulation linear program of a feed formulator, specify a price, and run to the program to see if the program uses the protein source of interest. It is also possible to run a parametric analysis which would indicate the amount of the protein source which would be included in the formulation at all different price levels. The optimal price level is the highest price at which the protein product is still included in formulations in sufficient amounts to generate enough demand for the product.

4.2 ECONOMIC ANALYSIS

A detailed economic analysis for SCP production was performed to:

- a) demonstrate economics of the production of a microbial protein product by using best estimates available data and a newly developed computer program for this purpose.
- b) assess the cost and selling price of the final product for large scale industrial operation.
- c) demonstrate the methodology for arriving at the final price of the product in question.

Very limited operating and process data was available to the authors to perform this economic analysis and assessment. First of all, there are a very limited number of plants operating and secondly limited data was accessible for us from the operating plants to make a reasonable assessment.

The process, for which most data was available (through personal contacts and visits by the author) is the process developed by the Czechoslovak Academy of Sciences in Prague and presently under construction in connection with a pulp and paper mill in Paskov Czechoslovakia (see section 3.1 of this report). In the following analysis, a thorough economic assessment of this process (with available data) was made. The following is considered:

- a) the worst case
- b) the best case for a 20 year horizon
- c) consideration of waste disposal credit
- d) reduction of labour by 50 %
- e) shipping product in bulk rather than packaging
- f) reduction of capital investment by 50 %

A process developed at the Academy of Science, Prague, uses spent sulphite liquor from a pulp mill as feedstock to a fermentation yielding a dried yeast product containing 45 - 50 % protein. This yeast product has been tested in Czechoslovakia and found suitable for cattle feed at a concentration of 4 - 10 % of the complete animal diet.

This process addresses a germane Canadian problem, that of disposal of pulp and paper mill waste liquors, and an investigation of the economics of a plant in Canada was carried out. The proposed plant would have to be sited close to a pulp mill, but the economic analysis was not specific to a single site. Rather a general economic/financial model was constructed which includes an allowance for site-specific costs such as materials, transportation, utilities, etc..

4.2.1 THE PROCESS

Spent sulphite liquor containing 13 - 15 % solids and 2 - 3 % reducing sugars is the basic feedstock for the SCP plant. The assumed volume is 200,000 tonnes annually which would be available from an unbleached pulp mill with an annual capacity of 200,000 tonnes of unbleached pulp (roughly equivalent to 2 million tons of wood).

The feedstock has pH 1.8 - 2.5 and is at a temperature of approximately 80 degrees C. After stripping to remove sulphite and cooling to 35 degrees C. nutrients are added together with caustic potash and phosphoric acid, and the treated sulphite liquor is fermented with yeast (*Candida utilis*) in one of three parallel CHEPOS fermentors. The fermented medium is then separated, washed, evaporated and dried to produce a 92 % dry matter solid containing 45 - 50 % protein which is then packed in 25 kg bags for distribution. The separated liquor is returned to the pulpmill for disposal. The output rate of the plant is 25,000 - 28,000 tonnes of product annually.

4.2.2 THE SCP PRODUCT

The SCP product is a 92 % dry matter meal of 45 - 50 % protein that includes lysine, methionine, calcium, sodium, and phosphorous. The actual market value of such a product depends on detailed calculations of the impact of this new formula on the animal feed mixing problem. Various components of the SCP will have value in different types of feed - for example, lysine is an important component in poultry feed but less important in beef or dairy cattle feeds.

The component of the SCP product that is most easily valued is its protein content. Soyameal with a market price of \$ 330/tonne (May, 1984) has a protein content of 48.4 %, providing an estimate of the value of protein at \$ 688/tonne. The SCP product therefore substitutes for protein in soyameal at a price of \$ 310 - 344/tonne (45 % - 50 % protein). The additional ingredients may increase this price slightly, but at the same time a price discount may be necessary to gain entry with an unfamiliar product into a well established feed-mixing business.

4.2.3 THE SITE

The plant requires a site of approximately 8 hectares of serviced industrial land adjacent to a pulp or paper mill. It is anticipated that an Ontario municipality, attracted by the construction jobs plus the 42 permanent jobs that the plant offers, and a paper company, attracted by the opportunity to partially solve a difficult industrial waste problem will cooperate to provide a site. The availability of local incentives and grants suggests that the cost of this site is likely to be quite moderate and is assumed at \$ 10,000.

Property taxes on the site plus the capital equipment investment, also provide further opportunities to benefit the local community. It is thought that a five year property tax waiver is a reasonable local development incentive, with a

property tax rate of 2 1/2 % of the market value of the plant plus site beginning in year six.

4.2.4 PLANT CONSTRUCTION

The construction cost of the Czechoslovakian plant was estimated at \$ 21 million. An attempt was made to source the equipment and estimate the site preparation costs for Canadian construction, and this estimate seems reasonable. Little is known about the cost of the three CHEPOS fermenters required for the plant or of any royalty payments due to the owners of the design.

For the purpose of the analysis, the construction cost was assumed to be \$ 21 million which is assumed to include equipment, installation, site preparation, utility hookups, transportation costs for equipment and licence fees or royalty payments.

Plant construction is assumed to take 18 months with a 50 % progress payment due in the first year and the balance in the second year.

4.2.5 UTILITIES

Requirements for utilities and cost estimates are:

| | |
|------------------------|--|
| <u>Water:</u> | Cooling water: 500-800t/t product at \$ 06/t. Process water: 7 - 10t/t product at \$ 75/t. |
| <u>Steam:</u> | 1.2 - 1.5t/t product at \$ 8.00/t (assumed 500 psig, produced from natural gas). |
| <u>Electric Power:</u> | 1.0 - 1.3 Mwatt/t of product. Direct customer of Ontario Hydro, industrial rate, 90 % load factor, <115 Kw supply: \$ 0.0287/Kwh. |

Natural Gas: 0.15 - 0.2t/t product,
at 1400m³/t is 210 - 280 m³/t product
at \$ 0.16/m³

UTILITY COSTS ESTIMATES/T PRODUCT

| | <u>Minimum</u> | <u>Maximum</u> |
|----------------|----------------|----------------|
| Cooling Water | 30.00 | 48.00 |
| Process Water | 5.25 | 7.50 |
| Steam | 9.60 | 12.00 |
| Electric Power | 28.70 | 37.31 |
| Natural Gas | 33.60 | 44.80 |
| | <u>107.15</u> | <u>149.61</u> |

Labour

Labour requirements for the Czechoslovakian plant are 42 persons as follows:

| | |
|--------------------|-----------------|
| 34 Operators | (3 shifts of 8) |
| 4 Packers | |
| 1 Lab Chief | |
| 1 Technologist | 1st shift |
| 1 Chemicals Supply | |
| 1 Supervisor | |

Estimated labour costs are:

| | |
|-----------------|------------------------------------|
| Operator/Packer | \$ 7.50/hr + 25 % Fringe Benefits |
| Technical | \$ 12.00/hr + 25 % Fringe Benefits |
| Supervisor | \$ 15.00/hr + 25 % Fringe Benefits |

Total labour cost estimate:

| | |
|-----------------|--------------------------------|
| Direct Labour | \$ 25.14 - 28.16/tonne product |
| Indirect Labour | \$ 125,970 p.a. |

Maintenance

A maintenance allowance of 5 % of the market value of the plant is assumed.

Materials

Variable materials costs per tonne of product are estimated as follows:

| <u>Material</u> | Rqmt/t product | Cost/t material | Cost/t product |
|-----------------------|----------------|-----------------|----------------|
| Caustic Potash (45%) | .039 - .044 t | \$ 360.00 (1) | \$ 14.04-15.84 |
| Phosphoric Acid (75%) | .068 - .075 | 846.50 (2) | 57.56-63.48 |
| Nitric Acid (56%) | .005 - .015 | 200.00 (3) | 1.00- 3.00 |
| Caustic Soda (50%) | .005 - .015 | 420.00 | 2.10 -6.30 |
| Antifoam Oil | .002 - .005 | 300.00 | 0.60 -1.50 |
| Nutrients | .03 - .05 | negligible | negligible |

Packing (multiwall 25 kg paper (polyethylene film) bags)

| | | |
|-----------|-------------|----------|
| 40 bags/t | \$ 0.50/bag | \$ 20.00 |
|-----------|-------------|----------|

Total variable materials cost/tonne product \$ 95.30-110.12

(1) FOB Cornwall, Ontario (CIL)

(2) FOB Port Maitland, Ontario (ERCO)

(3) FOB Sarnia, Ontario (CIL)

Transportation: For a central Ontario location, transportation is estimated at 1,660,000/tonne miles annually. It is estimated that this can be contracted to a private trucker, or operated internally at a cost of \$ 200,000 p.a.

4.2.6 CONTRIBUTION ANALYSIS

The contribution margin for a product is the excess of unit price over variable production cost. At a minimum, the contribution margin must be positive since this contribution must cover fixed (time dependent) costs as well as covering investment costs and generating a shareholder return.

Unit price (or net revenue per tonne product) is \$ 310-344/tonne.

Variable cost/tonne is made up of:

| | |
|----------------------|--------------------|
| Utilities cost/tonne | \$ 107.15 - 149.61 |
| Labour cost/tonne | 25.14 - 28.16 |
| Materials cost/tonne | 95.30 - 110.12 |

Variable cost/tonne range \$ 227.59 - 287.89

Contribution margin/tonne at \$ 310/tonne \$ 22.11 - 82.41

Contribution margin/tonne at \$ 330/tonne \$ 42.11 -102.41

The worst case: production of 25,000 tonnes p.a. at \$ 310/tonne.
Contribution/tonne at \$ 22.11 Total contribution \$ 552,750.

The best case: production of 28,000 tonnes p.a. at \$ 330/tonne.
Contribution/tonne at \$ 102.41 Total contribution \$ 2,867,480.

Fixed costs

From the total contribution must be subtracted indirect labour (\$ 125,970), transportation (\$ 200,000), and a monthly fixed utility cost that results from the block structure of utility rates. This fixed utility cost is estimated at \$ 1,500/month or \$ 18,000 p.a..

Net contribution is therefore in the range \$ 352,750 to \$ 2,523,510. This net contribution must also cover maintenance, property taxes, interest payments and profit. Assuming main-

tenance at 5 % of capital investment and interest payments at 13 % of capital investment, property tax at zero (initially) and zero profit, \$ 352,750 allows an investment of \$ 1,959,722 while \$ 2,523,510 allows an investment of \$ 14,019,500 under the same conditions. That is, if the plant plus land requires an investment of \$ 14,019,500 at an interest rate of 13 % with maintenance at 5 % then at a net contribution of \$ 2,523,510 profits are exactly zero.

Best case - Summary

| | |
|--|--------------|
| Total revenue from 28,000 t at \$ 330/t..... | \$ 9,240,000 |
| Less variable costs at \$ 227.59/t..... | - 6,372,520 |
| Less annual fixed costs (\$ 125,970+200,000+18,000) .. | - 343,970 |
| Less maintenance at 5 % of \$ 14,019,500..... | - 700,975 |
| Less interest at 13 % of \$ 14,019,500..... | - 1,822,535 |
| Leaves a profit of..... | 0 |

The contribution margin analysis therefore reveals that if the plant can be built for less than \$ 14 million, then there is a chance of a profitable operation under a 'best case' scenario. If the needed capital investment exceeds \$ 14 million, then a profitable operation will require some other means of support, either from government incentive payments or, perhaps more realistically, from waste disposal credits from the adjoining paper or pulp mill.

In order to investigate these other options a financial/economic model was developed.

4.2.7 AN FINANCIAL/ECONOMIC MODEL OF AN SCP PLANT

A general financial/aconomic model of a commercial-scale SCP operation provides information on the economic feasibility of different types and size of SCP processes. The specific process must be developed and analyzed so that data are

available as inputs for the general model. The model generates measures of the economic feasibility of the process. These could include return on investment (ROI), net present value (NPV) of after-tax cash flows, payback period, internal rate of return. The most acceptable of these as a measure of the financial desirability of a project is the net present value of after tax cash flows.

The difference between the simple contribution margin analysis performed earlier and the financial/economic model approach, is that the model looks at the process parameters as they evolve over time, while the simple contribution analysis examines only a single year. Clearly, it is possible that an investment that is attractive over a twenty year period can make a loss in a single year.

The model is available as a computer program. This enables rapid generation of results, easy manipulation of the data, and easy modification of the model and inputs to allow a thorough sensitivity analysis.

Inputs Required for Model

Because minor changes in the SCP production process can have pronounced effects on the costs involved, it is important that the process to be commercialized is defined as specifically as possible, and that scale-up effects from going from pilot plant to full-scale operation have been taken into account. As with any model, the outputs are only as reliable as the inputs.

1. Yield - defined as kg of product produced per kg of substrate. This conversion factor defines the quantity of substrate required to produce the annual capacity of product.
2. Percent protein in product.
3. Substrate price per tonne.
4. Waste disposal credit per tonne of substrate.
5. Selling price of product.
6. Tons of product sold per annum.
7. Chemicals cost per tonne of product.
8. Direct utilities cost per tonne of product - i.e. electricity, natural gas, process water, cooling water, steam.
9. Direct labour cost per tonne of product.
10. Period costs - R and D, administration and selling expenses, indirect utilities cost, maintenance, insurance, depreciation. Many of these may be defined as a percentage of revenue, or of total investment.
11. Tax rate.
12. Capital cost allowance rates for equipment.
13. Additional tax and other government credits available.
14. Expected financing - re. size of initial debt and schedule for payments of principal.
15. Interest rate.
16. Inflation.
17. Cost of capital.
18. Capital investment for site plus plant and annual capital expenditures.
19. Working capital requirements to finance operations.

The model has been written in the form of a computer program using the Interactive Financial Planning System (IFPS) and is listed in Appendix 1.

Key inputs for the model

While all inputs help to determine the economics of the process being simulated and therefore should be estimated as accurately as possible, there are several variables which have an especially large impact on the economics of a single-cell production process.

(1) CAPITAL INVESTMENT

The capital investment required for a commercial-scale SCP operation is large. Recent estimates of investment cost for a 100,000 tonnes per year SCP-from-methanol facility range from 60-216 million US\$ (mid-1978 prices), depending on location (Ericsson et al., 1981).

Investment cost is largely determined by the size of the facility, the location, and the specific process used. Different substrate/organism combinations will have different requirements for pretreatment of substrate, heat removed during fermentation, cell recovery, etc., which result in requirements for different process design and different equipment. Investment cost breakdown will be different for each process but will always include equipment costs for raw material storage, media preparation, utilities, fermentation, cell recovery, drying, and product storage. It is important to remember that there are many costs not directly associated with the production process (i.e. working capital, land, roads, sewer systems) which also must be included. For a good checklist of fermentation plant design and construction see 'Economics of Fermentation Processes' (Bartholomew and Reisman, 1979).

(2) SUBSTRATE COST

Substrate cost can vary widely, from negative values for SCP from waste processes, to more than 50 % of annual operation costs for SCP from hydrocarbon processes. Substrates for SCP processes must be chosen after careful consideration of acceptability of product in the market, process-related costs and yields, and of the long-term economics of the substrate. It is unlikely that SCP from hydrocarbons can be economical in the long run since these processes require more energy than processes based on carbohydrates, and since the substrate is an important, non-renewable energy source. SCP from carbohydrates and cellulose holds more promise, if technical improvements can be achieved to make these processes economical (Edwardson et al., 1981; Lewis, 1976).

(3) PRODUCT SELLING PRICE

Selling price for SCP as a protein supplement will be largely determined by prices of competing protein sources such as soybeans. Most selling prices for SCP are computed on a price per unit protein basis, but it is possible that SCP could eventually command a higher price in the market if the product offers other nutritional advantages over conventional protein sources.

(4) ADDITIONAL TAX CREDITS

The Canadian government has recently committed itself to promote biotechnology in Canada. As part of this program, more funds are available for programs administered by the Department of Industry, Trade, and Commerce (ITC) to help start industrial biotechnology ventures. ITC is currently reorganizing its Enterprise Development Program into a new Regional Industrial Development Program. No information is yet available on this new program. The report of the Task Force on Biotechnology to the Minister of State for Science and Technology (MOSST) recommended that the 100 percent write-off for R and D expenses be replaced by a 150 percent write-off of all biotechnology

industrial R and D, and that 'investors in biotechnological industries be permitted a 100 percent write-off of investment expense against income from any source, as well as a 66 2/3 percent incremental investment write-off similar to that formerly allowed for frontier oil and gas exploration investments'. It is not clear that these recommendations will be implemented.

It is expected that the government would be willing to provide incentives to a Canadian SCP venture, as nearly all the systems used for feed supplements in Canada are imported from the USA.

4.2.8 FINANCIAL/ECONOMIC ANALYSIS OF SCP FROM WASTE SULPHITE LIQUOR

The contribution margin analysis suggested that the Prague-SCP from waste sulphite liquor process was borderline from an economic viewpoint. This analysis, therefore, begins with a 'best case' scenario.

Assumptions:

Yield: 28,000 tonnes p.a. SCP from 200,000 tonnes of sulphite liquor.

Percent protein in product: 50 %

Substrate price per tonne: \$ 0.

Waste disposal credit per tonne: \$ 0.

Selling price of product: \$ 688 per tonne protein.

Tonnes of product sold per annum: 28,000

Chemicals cost per tonne of product: \$ 95.30 (includes packing).

Direct utilities cost per tonne of product: \$ 107.15.

Direct labour cost per tonne of product: \$ 25.14

Period costs: Indirect labour \$ 125,970
Indirect utilities \$ 18,000,
Transportation \$ 200,000,
Maintenance 5 % of market value of plant,
Property tax 2.5 % of market value of plane
after 5 years.

(Note that there is no allowance for physical depreciation of the plant - it is assumed that maintenance at 5 % p.a. of market value can offset physical depreciation.)

Tax rate: Ontario tax structure.

Capital cost allowance rate for equipment:.2.

Additional taxes and credits: None.

Expected financing: 100 % loan for land, capital equipment and working capital at 13 % fixed rate repayable over 20 years.

Inflation: Inflation rate 6 % in perpetuity. All costs and prices inflate at this rate.

Cost of capital: Assumed 9 % for present value calculations.

Capital investment: Assumed at construction cost of plant plus land inflating at inflation rate, plus changes to working capital. The capital investment is therefore an approximation of the market value of the SCP operation assuming that maintenance charges are sufficient to offset physical depreciation.

Working capital to finance operations: Assumed at the market value of one month's production of SCP.

Plant Startup: It is assumed that the plant is built in 18 months. First year production is zero, second year production is assumed at 14,000 tons. The land cost plus half the construction cost is borrowed in the first year, the balance of the loan in the second year. Period costs are zero in the first year and half annual rates in the second year.

RESULTS OF THE ECONOMIC ANALYSIS

The financial/economic model was first run to show 'best case' economic analysis for a twenty year horizon. The model output is listed below.

? SOLVE

MODEL CZ VERSION OF 05/18/84 13:08 — 20 COLUMNS 56 VARIABLES

ENVIER SOLVE OPTIONS

? ALL

| | YEAR1 | YEAR2 | YEAR3 | YEAR4 | YEAR5 | YEAR6 |
|--|-------|-------|-------|-------|-------|-------|
|--|-------|-------|-------|-------|-------|-------|

INFLATION AND MARKET DATA

| | | | | | | |
|------------------------|-------|--------|--------|--------|--------|--------|
| COST OF CAPITAL | .0900 | .0900 | .0900 | .0900 | .0900 | .0900 |
| INFLATION | .0600 | .0600 | .0600 | .0600 | .0600 | .0600 |
| INTEREST RATE | .1300 | .1300 | .1300 | .1300 | .1300 | .1300 |
| SUBSTRATE PRICE PER T | 0 | 0 | 0 | 0 | 0 | 0 |
| WASTE CREDIT PER TONNE | 0 | 0 | 0 | 0 | 0 | 0 |
| WASTE DISPOSAL CREDIT | 0 | 0 | 0 | 0 | 0 | 0 |
| PRICE PER T PROTEIN | 688 | 729.3 | 773.0 | 819.4 | 868.6 | 920.7 |
| PRODUCT SELLING PRICE | 344 | 364.6 | 386.5 | 409.7 | 434.3 | 460.3 |
| TONNES SOLD PER ANNUM | 0 | 14000 | 28000 | 28000 | 28000 | 28000 |
| SUBSTRATE FLOW | 0 | 100000 | 200000 | 200000 | 200000 | 200000 |

PROCESS PARAMETERS

| | | | | | | |
|------------------------|-------|-------|-------|-------|-------|-------|
| YIELD | 0 | 7.143 | 7.143 | 7.143 | 7.143 | 7.143 |
| PERCENT PROTEIN | .5000 | .5000 | .5000 | .5000 | .5000 | .5000 |
| CHEMICALS COST PER T P | 75.30 | 79.82 | 84.61 | 89.68 | 95.06 | 100.8 |
| UTILITIES COST PER T O | 107.2 | 113.6 | 120.4 | 127.6 | 135.3 | 143.4 |
| DIRECT LABOUR COST PER | 25.14 | 26.65 | 28.25 | 29.94 | 31.74 | 33.64 |
| UNIT PACKAGE COST | 20 | 21.20 | 22.47 | 23.82 | 25.25 | 26.76 |

TAX SECTION

| | | | | | | |
|------------------------|-------|-------|-------|-------|-------|-------|
| TAX RATE | .4400 | .4400 | .4400 | .4400 | .4400 | .4400 |
| CCA RATE | .2000 | .2000 | .2000 | .2000 | .2000 | .2000 |
| ADDITIONAL TAX CREDITS | 0 | 0 | 0 | 0 | 0 | 0 |

CALCULATION OF VARIOUS TAX DEDUCTIONS:

| | | | | | | |
|----------|----------|----------|----------|----------|----------|----------|
| CCA POOL | 10700000 | 21625413 | 22101876 | 22155989 | 22213348 | 22274149 |
|----------|----------|----------|----------|----------|----------|----------|

| | | | | | | |
|-------------------|---------|---------|---------|---------|---------|---------|
| CCA SHIELD | 0 | 0 | 0 | 0 | 0 | 0 |
| CCA ALLOWABLE | 2140000 | 4325083 | 4420375 | 4431198 | 4442670 | 4454830 |
| CUM LOSS DED | 0 | 0 | 0 | 0 | 47582 | 0 |
| CUM LOSS POOL | 1366300 | 3656364 | 4099336 | 4270720 | 4270720 | 4490178 |
| CUM LOSS ADDITION | 1366300 | 2290064 | 442972 | 171384 | 0 | 267040 |

NOTE: TAX DEDUCTIONS ARE TAKEN SEQUENTIALLY AND ONLY WHEN NEEDED

| | | | | | | |
|------|---------|----------|---------|--------|-------|---------|
| TAX1 | -601172 | -1007628 | -194908 | -75409 | 47582 | -117498 |
| TAX2 | 0 | 0 | 0 | 0 | 0 | 0 |
| TAX3 | 0 | 0 | 0 | 0 | 0 | 0 |

BREAKDOWN OF INITIAL INVESTMENT COST

| | | | | | | |
|--------------------|----------|----------|----------|----------|----------|----------|
| LAND | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 |
| CAPITAL EQUIPMENT | 10500000 | 21000000 | 21000000 | 21000000 | 21000000 | 21000000 |
| WORKING CAPITAL | 0 | 425413 | 901876 | 955989 | 1013348 | 1074149 |
| INITIAL INVESTMENT | 10510000 | 21435413 | 21911876 | 21965989 | 22023348 | 22084149 |
| TAXABLE PROPERTY | 10700000 | 21010000 | 22270600 | 23606836 | 25023246 | 26524641 |

TO GENERATE PROJECTED ECONOMICS SOLVE FOR LINES 850 THRU 1360

INCOME STATEMENT

| | | | | | | |
|--------------------------------|---------|---------|----------|----------|----------|----------|
| REVENUE | 0 | 5104960 | 10822515 | 11471866 | 12160178 | 12889789 |
| DIRECT MANUFACTURING EXPENSES: | | | | | | |
| SUBSTRATE COST | 0 | 0 | 0 | 0 | 0 | 0 |
| VARIABLE CHEMICALS COS | 0 | 1117452 | 2368998 | 2511138 | 2661806 | 2821515 |
| DIRECT LABOUR | 0 | 373078 | 790925 | 838380 | 888683 | 942004 |
| UTILITIES COST | 0 | 1590106 | 3371025 | 3573286 | 3787683 | 4014944 |
| PACKAGING COST | 0 | 296800 | 629216 | 666969 | 706987 | 749406 |
| OTHER EXPENSES: | | | | | | |
| BLOCK UTILITIES | 0 | 18000 | 19080 | 20225 | 21438 | 22725 |
| INDIRECT LABOUR | 0 | 62985 | 125970 | 133528 | 141540 | 150032 |
| TRANSPORTATION | 0 | 100000 | 200000 | 212000 | 224720 | 238203 |
| PROPERTY TAX | 0 | 0 | 0 | 0 | 0 | 663116 |
| MAINTENANCE | 0 | 1050000 | 1113000 | 1179780 | 1250567 | 1325601 |
| INTEREST PAYMENTS | 1366300 | 2786604 | 2647274 | 2507943 | 2368613 | 2229283 |

| | | | | | | |
|-------------------|----------|----------|----------|----------|----------|----------|
| TOTAL EXPENSES | 1366300 | 7395024 | 11265487 | 11643250 | 12052038 | 13156829 |
| PROFIT BEFORE TAX | -1366300 | -2290064 | -442972 | -171384 | 108140 | -267040 |
| TAX | 0 | 0 | 0 | 0 | 0 | 0 |
| PROFIT AFTER TAX | -1366300 | -2290064 | -442972 | -171384 | 108140 | -267040 |

CASH FLOW INFORMATION

| | | | | | | |
|----------------------|----------|----------|----------|----------|----------|----------|
| CAPITAL EXPENDITURES | 10510000 | 10925413 | 476463 | 54113 | 57359 | 60801 |
| LOAN BALANCE | 10510000 | 21435413 | 20363643 | 19291872 | 18220101 | 17148331 |
| PRINCIPAL PAYMENTS | 0 | 0 | 1071771 | 1071771 | 1071771 | 1071771 |
| INITIAL LOAN | 10510000 | 21435413 | 21435413 | 21435413 | 21435413 | 21435413 |

SUMMARY STATISTICS

| | | | | | | |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 10700000 | 21435413 | 23172476 | 24562825 | 26036594 | 27598790 |
| ROI | -.1277 | -.1068 | -.0191 | -.0070 | .0042 | -.0097 |
| ATCF | -11876300 | -13215478 | -1991205 | -1297267 | -1020990 | -1399612 |
| PRESENT VALUE ATCF | -10895688 | -22018891 | -23556467 | -24475484 | -25139057 | -25973600 |
| ***** | | | | | | |
| ***** | | | | | | |

YEAR7 YEAR8 YEAR9 YEAR10 YEAR11 YEAR12

INFLATION AND MARKET DATA

| | | | | | | |
|------------------------|--------|--------|--------|--------|--------|--------|
| COST OF CAPITAL | .0900 | .0900 | .0900 | .0900 | .0900 | .0900 |
| INFLATION | .0600 | .0600 | .0600 | .0600 | .0600 | .0600 |
| INTEREST RATE | .1300 | .1300 | .1300 | .1300 | .1300 | .1300 |
| SUBSTRATE PRICE PER T | 0 | 0 | 0 | 0 | 0 | 0 |
| WASTE CREDIT PER TONNE | 0 | 0 | 0 | 0 | 0 | 0 |
| WASTE DISPOSAL CREDIT | 0 | 0 | 0 | 0 | 0 | 0 |
| PRICE PER T PROTEIN | 975.9 | 1034 | 1097 | 1162 | 1232 | 1306 |
| PRODUCT SELLING PRICE | 488.0 | 517.2 | 548.3 | 581.2 | 616.1 | 653.0 |
| TONNES SOLD PER ANNUM | 28000 | 28000 | 28000 | 28000 | 28000 | 28000 |
| SUBSTRATE FLOW | 200000 | 200000 | 200000 | 200000 | 200000 | 200000 |

PROCESS PARAMETERS

| | | | | | | |
|------------------------|-------|-------|-------|-------|-------|-------|
| YIELD | 7.143 | 7.143 | 7.143 | 7.143 | 7.143 | 7.143 |
| PERCENT PROTEIN | .5000 | .5000 | .5000 | .5000 | .5000 | .5000 |
| CHEMICALS COST PER T P | 106.8 | 113.2 | 120.0 | 127.2 | 134.9 | 142.9 |
| UTILITIES COST PER T O | 152.0 | 161.1 | 170.8 | 181.0 | 191.9 | 203.4 |
| DIRECT LABOUR COST PER | 35.66 | 37.80 | 40.07 | 42.47 | 45.02 | 47.72 |
| UNIT PACKAGE COST | 28.37 | 30.07 | 31.88 | 33.79 | 35.82 | 37.97 |

TAX SECTION

| | | | | | | |
|------------------------|-------|-------|-------|-------|-------|-------|
| TAX RATE | .4400 | .4400 | .4400 | .4400 | .4400 | .4400 |
| CCA RATE | .2000 | .2000 | .2000 | .2000 | .2000 | .2000 |
| ADDITIONAL TAX CREDITS | 0 | 0 | 0 | 0 | 0 | 0 |

CALCULATION OF VARIOUS TAX DEDUCTIONS:

| | | | | | | |
|---------------|----------|----------|----------|----------|----------|----------|
| CCA POOL | 22338598 | 22406914 | 22479329 | 22556088 | 22637454 | 22723701 |
| CCA SHIELD | 0 | 0 | 0 | 0 | 0 | 0 |
| CCA ALLOWABLE | 4467720 | 4481383 | 4495866 | 4511218 | 4527491 | 4544740 |
| CUM LOSS DED | 0 | 111827 | 231339 | 354342 | 481048 | 611678 |

| | | | | | | |
|-------------------|---------|---------|---------|---------|---------|---------|
| CUM LOSS POOL | 4500154 | 4500154 | 4388327 | 4156988 | 3802645 | 3321597 |
| CUM LOSS ADDITION | 9976 | 0 | 0 | 0 | 0 | 0 |

NOTE: TAX DEDUCTIONS ARE TAKEN SEQUENTIALLY AND ONLY WHEN NEEDED

| | | | | | | |
|------|-------|--------|--------|--------|--------|--------|
| TAX1 | -4389 | 111827 | 231339 | 354342 | 481048 | 611678 |
| TAX2 | 0 | 0 | 0 | 0 | 0 | 0 |
| TAX3 | 0 | 0 | 0 | 0 | 0 | 0 |

BREAKDOWN OF INITIAL INVESTMENT COST

| | | | | | | |
|--------------------|----------|----------|----------|----------|----------|----------|
| LAND | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 |
| CAPITAL EQUIPMENT | 21000000 | 21000000 | 21000000 | 21000000 | 21000000 | 21000000 |
| WORKING CAPITAL | 1138598 | 1206914 | 1279329 | 1356088 | 1437454 | 1523701 |
| INITIAL INVESTMENT | 22148598 | 22216914 | 22289329 | 22366088 | 22447454 | 22533701 |
| TAXABLE PROPERTY | 28116119 | 29803087 | 31591272 | 33486748 | 35495953 | 37625710 |

TO GENERATE PROJECTED ECONOMICS SOLVE FOR LINES 850 THRU 1360

INCOME STATEMENT

| | | | | | | |
|--------------------------------|----------|----------|----------|----------|----------|----------|
| REVENUE | 13663176 | 14482967 | 15351945 | 16273061 | 17249445 | 18284412 |
| DIRECT MANUFACTURING EXPENSES: | | | | | | |
| SUBSTRATE COST | 0 | 0 | 0 | 0 | 0 | 0 |
| VARIABLE CHEMICALS COS | 2990806 | 3170254 | 3360469 | 3562097 | 3775823 | 4002373 |
| DIRECT LABOUR | 998524 | 1058435 | 1121942 | 1189258 | 1260614 | 1336250 |
| UTILITIES COST | 4255841 | 4511192 | 4781863 | 5068775 | 5372901 | 5695275 |
| PACKAGING COST | 794371 | 842033 | 892555 | 946108 | 1002875 | 1063047 |
| OTHER EXPENSES: | | | | | | |
| BLOCK UTILITIES | 24088 | 25533 | 27065 | 28689 | 30411 | 32235 |
| INDIRECT LABOUR | 159034 | 168576 | 178691 | 189412 | 200777 | 212824 |
| TRANSPORTATION | 252495 | 267645 | 283704 | 300726 | 318770 | 337896 |
| PROPERTY TAX | 702903 | 745077 | 789782 | 837169 | 887399 | 940643 |
| MAINTENANCE | 1405137 | 1489445 | 1578812 | 1673540 | 1773953 | 1880390 |
| INTEREST PAYMENTS | 2089953 | 1950623 | 1811292 | 1671962 | 1532632 | 1393302 |
| TOTAL EXPENSES | 13673152 | 14228813 | 14826175 | 15467737 | 16156154 | 16894235 |
| PROFIT BEFORE TAX | -9976 | 254153 | 525770 | 805324 | 1093291 | 1390177 |
| TAX | 0 | 0 | 0 | 0 | 0 | 0 |

PROFIT AFTER TAX -9976 254153 525770 805324 1093291 1390177

CASH FLOW INFORMATION

| | | | | | | |
|----------------------|----------|----------|----------|----------|----------|----------|
| CAPITAL EXPENDITURES | 64449 | 68316 | 72415 | 76760 | 81365 | 86247 |
| LOAN BALANCE | 16076560 | 15004789 | 13933019 | 12861248 | 11789477 | 10717707 |
| PRINCIPAL PAYMENTS | 1071771 | 1071771 | 1071771 | 1071771 | 1071771 | 1071771 |
| INITIAL LOAN | 21435413 | 21435413 | 21435413 | 21435413 | 21435413 | 21435413 |

SUMMARY STATISTICS

| | | | | | | |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 29254717 | 31010000 | 32870600 | 34842836 | 36933407 | 39149411 |
| ROI | -.0003 | .0082 | .0160 | .0231 | .0296 | .0355 |
| ATCF | -1146195 | -885933 | -618416 | -343207 | -59845 | 232159 |
| PRESENT VALUE ATCF | -26600608 | -27045228 | -27329964 | -27474938 | -27498130 | -27415589 |

YEAR13 YEAR14 YEAR15 YEAR16 YEAR17 YEAR18

INFLATION AND MARKET DATA

| | | | | | | |
|------------------------|--------|--------|--------|--------|--------|--------|
| COST OF CAPITAL | .0900 | .0900 | .0900 | .0900 | .0900 | .0900 |
| INFLATION | .0600 | .0600 | .0600 | .0600 | .0600 | .0600 |
| INTEREST RATE | .1300 | .1300 | .1300 | .1300 | .1300 | .1300 |
| SUBSTRATE PRICE PER T | 0 | 0 | 0 | 0 | 0 | 0 |
| WASTE CREDIT PER TONNE | 0 | 0 | 0 | 0 | 0 | 0 |
| WASTE DISPOSAL CREDIT | 0 | 0 | 0 | 0 | 0 | 0 |
| PRICE PER T PROTEIN | 1384 | 1467 | 1556 | 1649 | 1748 | 1853 |
| PRODUCT SELLING PRICE | 692.2 | 733.7 | 777.8 | 824.4 | 873.9 | 926.3 |
| TONNES SOLD PER ANNUM | 28000 | 28000 | 28000 | 28000 | 28000 | 28000 |
| SUBSTRATE FLOW | 200000 | 200000 | 200000 | 200000 | 200000 | 200000 |

PROCESS PARAMETERS

| | | | | | | |
|------------------------|-------|-------|-------|-------|-------|-------|
| YIELD | 7.143 | 7.143 | 7.143 | 7.143 | 7.143 | 7.143 |
| PERCENT PROTEIN | .5000 | .5000 | .5000 | .5000 | .5000 | .5000 |
| CHEMICALS COST PER T P | 151.5 | 160.6 | 170.2 | 180.5 | 191.3 | 202.8 |
| UTILITIES COST PER T O | 215.6 | 228.5 | 242.3 | 256.8 | 272.2 | 288.5 |
| DIRECT LABOUR COST PER | 50.59 | 53.62 | 56.84 | 60.25 | 63.86 | 67.70 |
| UNIT PACKAGE COST | 40.24 | 42.66 | 45.22 | 47.93 | 50.81 | 53.86 |

TAX SECTION

| | | | | | | |
|------------------------|-------|-------|-------|-------|-------|-------|
| TAX RATE | .4400 | .4400 | .4400 | .4400 | .4400 | .4400 |
| CCA RATE | .2000 | .2000 | .2000 | .2000 | .2000 | .2000 |
| ADDITIONAL TAX CREDITS | 0 | 0 | 0 | 0 | 0 | 0 |

CALCULATION OF VARIOUS TAX DEDUCTIONS:

| | | | | | | |
|---------------|----------|----------|----------|----------|----------|----------|
| CCA POOL | 22815123 | 22912030 | 23014752 | 23123637 | 22108944 | 20898848 |
| CCA SHIELD | 0 | 0 | 0 | 1130112 | 1332439 | 1492083 |
| CCA ALLOWABLE | 4563025 | 4582406 | 4602950 | 4624727 | 4421789 | 4179770 |
| CUM LOSS DED | 746467 | 885665 | 1029537 | 48251 | 0 | 0 |

| | | | | | | |
|-------------------|---------|---------|---------|-------|---|---|
| CUM LOSS POOL | 2709919 | 1963453 | 1077788 | 48251 | 0 | 0 |
| CUM LOSS ADDITION | 0 | 0 | 0 | 0 | 0 | 0 |

NOTE: TAX DEDUCTIONS ARE TAKEN SEQUENTIALLY AND ONLY WHEN NEEDED

| | | | | | | |
|------|--------|--------|---------|---------|---------|---------|
| TAX1 | 746467 | 885665 | 1029537 | 1178362 | 1332439 | 1492083 |
| TAX2 | 0 | 0 | 0 | 1130112 | 1332439 | 1492083 |
| TAX3 | 0 | 0 | 0 | 0 | 0 | 0 |

BREAKDOWN OF INITIAL INVESTMENT COST

| | | | | | | |
|--------------------|----------|----------|----------|----------|----------|----------|
| LAND | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 |
| CAPITAL EQUIPMENT | 21000000 | 21000000 | 21000000 | 21000000 | 21000000 | 21000000 |
| WORKING CAPITAL | 1615123 | 1712030 | 1814752 | 1923637 | 2039056 | 2161399 |
| INITIAL INVESTMENT | 22625123 | 22722030 | 22824752 | 22933637 | 23049056 | 23171399 |
| TAXABLE PROPERTY | 39883253 | 42276248 | 44812823 | 47501592 | 50351688 | 53372789 |

TO GENERATE PROJECTED ECONOMICS SOLVE FOR LINES 850 THRU 1360

INCOME STATEMENT

| | | | | | | |
|--------------------------------|----------|----------|----------|----------|----------|----------|
| REVENUE | 19381476 | 20544365 | 21777027 | 23083649 | 24468667 | 25936787 |
| DIRECT MANUFACTURING EXPENSES: | | | | | | |
| SUBSTRATE COST | 0 | 0 | 0 | 0 | 0 | 0 |
| VARIABLE CHEMICALS COS | 4242515 | 4497066 | 4766890 | 5052903 | 5356077 | 5677442 |
| DIRECT LABOUR | 1416425 | 1501411 | 1591496 | 1686985 | 1788204 | 1895497 |
| UTILITIES COST | 6036992 | 6399211 | 6783164 | 7190154 | 7621563 | 8078857 |
| PACKAGING COST | 1126830 | 1194440 | 1266106 | 1342073 | 1422597 | 1507953 |
| OTHER EXPENSES: | | | | | | |
| BLOCK UTILITIES | 34169 | 36220 | 38393 | 40696 | 43138 | 45726 |
| INDIRECT LABOUR | 225593 | 239129 | 253476 | 268685 | 284806 | 301894 |
| TRANSPORTATION | 358170 | 379660 | 402439 | 426586 | 452181 | 479312 |
| PROPERTY TAX | 997081 | 1056906 | 1120321 | 1187540 | 1258792 | 1334320 |
| MAINTENANCE | 1993213 | 2112806 | 2239575 | 2373949 | 2516386 | 2667369 |
| INTEREST PAYMENTS | 1253972 | 1114641 | 975311 | 835981 | 696651 | 557321 |
| TOTAL EXPENSES : | 17684961 | 18531490 | 19437171 | 20405552 | 21440396 | 22545691 |
| PROFIT BEFORE TAX | 1696516 | 2012875 | 2339856 | 2678097 | 3028271 | 3391097 |
| TAX | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | |
|------------------|---------|---------|---------|---------|---------|---------|
| PROFIT AFTER TAX | 1696516 | 2012875 | 2339856 | 2678097 | 3028271 | 3391097 |
|------------------|---------|---------|---------|---------|---------|---------|

CASH FLOW INFORMATION

| | | | | | | |
|----------------------|----------|----------|----------|----------|----------|----------|
| CAPITAL EXPENDITURES | 91422 | 96907 | 102722 | 108885 | 115418 | 122343 |
| LOAN BALANCE | 9645936 | 8574165 | 7502395 | 6430624 | 5358853 | 4287083 |
| PRINCIPAL PAYMENTS | 1071771 | 1071771 | 1071771 | 1071771 | 1071771 | 1071771 |
| INITIAL LOAN | 21435413 | 21435413 | 21435413 | 21435413 | 21435413 | 21435413 |

SUMMARY STATISTICS

| | | | | | | |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 41498376 | 43988278 | 46627575 | 49425229 | 52390743 | 55534188 |
| ROI | .0409 | .0458 | .0502 | .0542 | .0578 | .0611 |
| ATCF | 533323 | 844197 | 1165364 | 1497441 | 1841082 | 2196983 |
| PRESENT VALUE ATCF | -27241631 | -26989008 | -26669071 | -26291911 | -25866486 | -25400739 |

YEAR19 YEAR20

INFLATION AND MARKET DATA

| | | |
|------------------------|--------|--------|
| COST OF CAPITAL | .0900 | .0900 |
| INFLATION | .0600 | .0600 |
| INTEREST RATE | .1300 | .1300 |
| SUBSTRATE PRICE PER T | 0 | 0 |
| WASTE CREDIT PER TONNE | 0 | 0 |
| WASTE DISPOSAL CREDIT | 0 | 0 |
| PRICE PER T PROTEIN | 1964 | 2082 |
| PRODUCT SELLING PRICE | 981.9 | 1041 |
| TONNES SOLD PER ANNUM | 28000 | 28000 |
| SUBSTRATE FLOW | 200000 | 200000 |

PROCESS PARAMETERS

| | | |
|------------------------|-------|-------|
| YIELD | 7.143 | 7.143 |
| PERCENT PROTEIN | .5000 | .5000 |
| CHEMICALS COST PER T P | 214.9 | 227.8 |
| UTILITIES COST PER T O | 305.8 | 324.2 |
| DIRECT LABOUR COST PER | 71.76 | 76.06 |
| UNIT PACKAGE COST | 57.09 | 60.51 |

TAX SECTION

| | | |
|------------------------|-------|-------|
| TAX RATE | .4400 | .4400 |
| CCA RATE | .2000 | .2000 |
| ADDITIONAL TAX CREDITS | 0 | 0 |

CALCULATION OF VARIOUS TAX DEDUCTIONS:

| | | |
|---------------|----------|----------|
| CCA POOL | 19536449 | 18016288 |
| CCA SHIELD | 1657626 | 1829424 |
| CCA ALLOWABLE | 3907290 | 3603258 |
| CUM LOSS DED | 0 | 0 |

| | | |
|-------------------|---|---|
| CUM LOSS POOL | 0 | 0 |
| CUM LOSS ADDITION | 0 | 0 |

NOTE: TAX DEDUCTIONS ARE TAKEN SEQUENTIALLY AND ONLY WHEN NEEDED

| | | |
|------|---------|---------|
| TAX1 | 1657626 | 1829424 |
| TAX2 | 1657626 | 1829424 |
| TAX3 | 0 | 0 |

BREAKDOWN OF INITIAL INVESTMENT COST

| | | |
|--------------------|----------|----------|
| LAND | 10000 | 10000 |
| CAPITAL EQUIPMENT | 21000000 | 21000000 |
| WORKING CAPITAL | 2291083 | 2428548 |
| INITIAL INVESTMENT | 23301083 | 23438548 |
| TAXABLE PROPERTY | 56575156 | 59969666 |

TO GENERATE PROJECTED ECONOMICS SOLVE FOR LINES 850 THRU 1360

INCOME STATEMENT

| | | |
|--------------------------------|----------|----------|
| REVENUE | 27492995 | 29142574 |
| DIRECT MANUFACTURING EXPENSES: | | |
| SUBSTRATE COST | 0 | 0 |
| VARIABLE CHEMICALS COS | 6018089 | 6379174 |
| DIRECT LABOUR | 2009226 | 2129780 |
| UTILITIES COST | 8563588 | 9077404 |
| PACKAGING COST | 1598430 | 1694336 |
| OTHER EXPENSES: | | |
| BLOCK UTILITIES | 48470 | 51378 |
| INDIRECT LABOUR | 320008 | 339209 |
| TRANSPORTATION | 508070 | 538555 |
| PROPERTY TAX | 1414379 | 1499242 |
| MAINTENANCE | 2827411 | 2997056 |
| INTEREST PAYMENTS | 417991 | 278660 |
| TOTAL EXPENSES | 23725663 | 24984793 |
| PROFIT BEFORE TAX | 3767332 | 4157782 |
| TAX | 0 | 0 |

| | | |
|------------------|---------|---------|
| PROFIT AFTER TAX | 3767332 | 4157782 |
|------------------|---------|---------|

CASH FLOW INFORMATION

| | | |
|----------------------|----------|----------|
| CAPITAL EXPENDITURES | 129684 | 137465 |
| LOAN BALANCE | 3215312 | 2143541 |
| PRINCIPAL PAYMENTS | 1071771 | 1071771 |
| INITIAL LOAN | 21435413 | 21435413 |

SUMMARY STATISTICS

| | | |
|--------------------|-----------|-----------|
| TOTAL INVESTMENT | 58866239 | 62398213 |
| ROI | .0640 | .0666 |
| ATCF | 2565878 | 2948546 |
| PRESENT VALUE ATCF | -24901703 | -24375591 |

The results from this run are quite discouraging. The after tax cash flow (ATCF) is negative through year 11, and the net present value of after tax cash flows is negative \$24.4 million over twenty years (recall this is a 'best case' analysis). In thinking how this situation might be changed, one line of attack is through some form of waste disposal credit. Existing paper or pulp mills already have elaborate waste treatment facilities, but if the SCP plant were attached to a new mill, then the investment in the SCP plant would offset otherwise needed investment in waste treatment facilities. Under these conditions, the mill owner would provide a credit to the SCP plant for waste disposal. A waste disposal credit of about \$10 per tonne of sulphite liquor feedstock is sufficient to generate a more attractive economic situation. This can be seen using a 'WHAT IF' command:

ENTER SOLVE OPTIONS
 ? WHAT IF
 WHAT IF CASE 1
 ENTER STATEMENTS
 ? WASTE CREDIT PER TONNE = 0,10,PREVIOUS*(1.00+INFLATION)
 ? SOLVE
 ENTER SOLVE OPTIONS
 ? L 1310 THRU L 1360

***** WHAT IF CASE 1 *****
 1 WHAT IF STATEMENT PROCESSED

| | YEAR1 | YEAR2 | YEAR3 | YEAR4 | YEARS5 | YEAR6 |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 10700000 | 21435413 | 23172476 | 24562825 | 26036594 | 27598790 |
| ROI | -.1277 | -.0602 | .0724 | .0845 | .0956 | .0818 |
| ATCF | -11876300 | -12215478 | 128795 | 949933 | 1361042 | 1125342 |
| PRESENT VALUE ATCF | -10895688 | -21177211 | -21077758 | -20404802 | -19520217 | -18849213 |
| | YEAR7 | YEAR8 | YEAR9 | YEAR10 | YEAR11 | YEAR12 |
| TOTAL INVESTMENT | 29254717 | 31010000 | 32870600 | 34842836 | 36933407 | 39149411 |
| ROI | .0911 | .0997 | .1075 | .1146 | .1211 | .1270 |
| ATCF | 1530256 | 1951105 | 2388845 | 2844490 | 3319113 | 3813854 |
| PRESENT VALUE ATCF | -18012110 | -17032917 | -15933026 | -14731483 | -13445218 | -12089260 |
| | YEAR13 | YEAR14 | YEAR15 | YEAR16 | YEAR17 | YEAR18 |
| TOTAL INVESTMENT | 41498376 | 43988278 | 46627575 | 49425229 | 52390743 | 55534188 |
| ROI | .1307 | .1200 | .1123 | .1069 | .1032 | .1006 |
| ATCF | 4259116 | 4108506 | 4062302 | 4103144 | 4217355 | 4394216 |
| PRESENT VALUE ATCF | -10700027 | -9470571 | -8355315 | -7321857 | -6347339 | -5415793 |
| | YEAR19 | YEAR20 | | | | |
| TOTAL INVESTMENT | 58866239 | 62398213 | | | | |
| ROI | .0990 | .0980 | | | | |
| ATCF | 4625391 | 4904462 | | | | |
| PRESENT VALUE ATCF | -4516202 | -3641095 | | | | |

After tax cash flow is now positive in the first full year of production and the twenty year horizon yeilds a net present value (of ATCF) of - \$3.6 million.

Annual rates of return peak at over 13% in year 11.

At \$10 per tonne sulphite liquor, the pulp or paper mill is spending \$2 million (\$10 x 200,000 tonnes) annually on waste treatment. This is considerably more than would usually be the case, but an amount slightly above this is needed to make the SCP plant attractive.

The simple contribution analysis suggests that the fundamental problem is that the variable costs are too high and hence the contribution uneconomically low. It seems unlikely that utilities or chemicals costs can be reduced very much, but it may be possible to reduce labour costs. If direct labour can be reduced by 50%, then the following numbers emerge:

```

ENTER SOLVE OPTIONS
? WHAT IF
WHAT IF CASE 2
ENTER STATEMENTS
? DIRECT LABOUR COST PER T OF PRODUCT =12.57,PREVIOUS*(1.00+INFLATION)
? SOLVE
ENTER SOLVE OPTIONS
? L 1310 THRU L 1360

```

```

***** WHAT IF CASE 2 *****
1 WHAT IF STATEMENT PROCESSED

```

| | YEAR1 | YEAR2 | YEAR3 | YEAR4 | YEARS5 | YEAR6 |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 10700000 | 21435413 | 23172476 | 24562825 | 26036594 | 27598790 |
| ROI | -.1277 | -.0981 | -.0021 | .0101 | .0212 | .0074 |
| ATCF | -11876300 | -13028939 | -1595743 | -878077 | -576648 | -928610 |
| PRESENT VALUE ATCF | -10895688 | -21861885 | -23094092 | -23716143 | -24090925 | -24644625 |

| | YEAR7 | YEAR8 | YEAR9 | YEAR10 | YEAR11 | YEAR12 |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 29254717 | 31010000 | 32870600 | 34842836 | 36933407 | 39149411 |
| ROI | .0167 | .0253 | .0331 | .0402 | .0467 | .0526 |
| ATCF | -646933 | -356716 | -57445 | 251422 | 570462 | 900284 |
| PRESENT VALUE ATCF | -24998520 | -25177543 | -25203993 | -25097789 | -24876716 | -24556634 |

| | YEAR13 | YEAR14 | YEAR15 | YEAR16 | YEAR17 | YEAR18 |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 41498376 | 43988278 | 46627575 | 49425229 | 52390743 | 55534188 |
| ROI | .0579 | .0628 | .0672 | .0713 | .0749 | .0781 |
| ATCF | 1241536 | 1594902 | 1961112 | 2340933 | 2735185 | 3144731 |
| PRESENT VALUE ATCF | -24151672 | -23674403 | -23136003 | -22546393 | -21914365 | -21247702 |

| | YEAR19 | YEAR20 |
|--------------------|-----------|-----------|
| TOTAL INVESTMENT | 58866239 | 62398213 |
| ROI | .0811 | .0837 |
| ATCF | 3570491 | 4013436 |
| PRESENT VALUE ATCF | -20553278 | -19837157 |

The situation has been improved slightly. One further economy is to ship the product in bulk, avoiding the packaging cost. Considering this on top of the labour cost reduction results in the following results:

```

ENTER SOLVE OPTIONS
? WHAT IF CONTINUE
WHAT IF CASE 3
ENTER STATEMENTS
? UNIT PACKAGE COST = 0
? SOLVE
ENTER SOLVE OPTIONS
? L 1310 THRU L 1360

```

```

***** WHAT IF CASE 3 *****
2 WHAT IF STATEMENTS PROCESSED

```


| | YEAR1 | YEAR2 | YEAR3 | YEAR4 | YEARS | YEAR6 |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 10700000 | 21435413 | 23172476 | 24562825 | 26036594 | 27598790 |
| ROI | -.1277 | -.0843 | .0251 | .0372 | .0484 | .0345 |
| ATCF | -11876300 | -12732139 | -966527 | -211108 | 130339 | -179204 |
| PRESENT VALUE ATCF | -10895688 | -21612075 | -22358411 | -22507965 | -22423254 | -22530107 |
| | YEAR7 | YEAR8 | YEAR9 | YEAR10 | YEAR11 | YEAR12 |
| TOTAL INVESTMENT | 29254717 | 31010000 | 32870600 | 34842836 | 36933407 | 39149411 |
| ROI | .0439 | .0524 | .0602 | .0673 | .0738 | .0797 |
| ATCF | 147437 | 485317 | 835110 | 1197531 | 1573337 | 1963331 |
| PRESENT VALUE ATCF | -22449454 | -22205889 | -21821382 | -21315532 | -20705812 | -20007780 |
| | YEAR13 | YEAR14 | YEAR15 | YEAR16 | YEAR17 | YEAR18 |
| TOTAL INVESTMENT | 41498376 | 43988278 | 46627575 | 49425229 | 52390743 | 55534188 |
| ROI | .0851 | .0900 | .0944 | .0984 | .1016 | .0930 |
| ATCF | 2368366 | 2789342 | 3227218 | 3683006 | 4136318 | 3968820 |
| PRESENT VALUE ATCF | -19235269 | -18400568 | -17514574 | -16586937 | -15631144 | -14789779 |
| | YEAR19 | YEAR20 | | | | |
| TOTAL INVESTMENT | 58866239 | 62398213 | | | | |
| ROI | .0867 | .0822 | | | | |
| ATCF | 3902874 | 3921317 | | | | |
| PRESENT VALUE ATCF | -14030711 | -13331027 | | | | |

If, on top of both these economies, the waste disposal credit of \$10/tonne is added:

ENTER SOLVE OPTIONS
 ? WHAT IF CONTINUE
 WHAT IF CASE 4
 ENTER STATEMENTS
 ? WASTE CREDIT PER TONNE = 0,10, PREVIOUS*(1.00+INFLATION)
 ? SOLVE
 ENTER SOLVE OPTIONS
 ? L 1310 THRU L 1360

***** WHAT IF CASE 4 *****
 3 WHAT IF STATEMENTS PROCESSED

| | YEAR1 | YEAR2 | YEAR3 | YEAR4 | YEARS | YEAR6 |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 10700000 | 21435413 | 23172476 | 24562825 | 26036594 | 27598790 |
| ROI | -.1277 | -.0376 | .1166 | .1287 | .1399 | .1260 |
| ATCF | -11876300 | -11732139 | 1153473 | 2036092 | 2512371 | 2345750 |
| PRESENT VALUE ATCF | -10895688 | -20770395 | -19879702 | -18437283 | -16804414 | -15405720 |

| | YEAR7 | YEAR8 | YEAR9 | YEAR10 | YEAR11 | YEAR12 |
|--------------------|-----------|-----------|-----------|----------|----------|----------|
| TOTAL INVESTMENT | 29254717 | 31010000 | 32870600 | 34842836 | 36933407 | 39149411 |
| ROI | .1354 | .1439 | .1517 | .1588 | .1509 | .1403 |
| ATCF | 2823889 | 3322356 | 3842371 | 4385227 | 4418481 | 4334997 |
| PRESENT VALUE ATCF | -13860956 | -12193578 | -10424444 | -8572077 | -6859770 | -5318529 |
| | YEAR13 | YEAR14 | YEAR15 | YEAR16 | YEAR17 | YEAR18 |
| TOTAL INVESTMENT | 41498376 | 43988278 | 46627575 | 49425229 | 52390743 | 55534188 |
| ROI | .1329 | .1277 | .1242 | .1220 | .1206 | .1199 |
| ATCF | 4350307 | 4448641 | 4617623 | 4847607 | 5131143 | 5462558 |
| PRESENT VALUE ATCF | -3899551 | -2568311 | -1300598 | -79633 | 1106037 | 2264065 |
| | YEAR19 | YEAR20 | | | | |
| TOTAL INVESTMENT | 58866239 | 62398213 | | | | |
| ROI | .1196 | .1196 | | | | |
| ATCF | 5837615 | 6253244 | | | | |
| PRESENT VALUE ATCF | 3399421 | 4515193 | | | | |

This investment now looks quite attractive. The return on investment reaches a maximum of close to 16% and the twenty year net present value is \$4.5 million.

If the waste disposal credit is cut to \$5/tonne, however, the economics changes markedly and the plant looks unattractive.

ENTER SOLVE OPTIONS

? WHAT IF CONTINUE

WHAT IF CASE 5

ENTER STATEMENTS

? WASTE CREDIT PER TONNE = 0,5,PREVIOUS*(1.00+INFLATION)

? SOLVE

ENTER SOLVE OPTIONS

? L 1310 THRU L 1360

***** WHAT IF CASE 5 *****

4 WHAT IF STATEMENTS PROCESSED

| | YEAR1 | YEAR2 | YEAR3 | YEAR4 | YEARS5 | YEAR6 |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 10700000 | 21435413 | 23172476 | 24562825 | 26036594 | 27598790 |
| ROI | -.1277 | -.0610 | .0708 | .0830 | .0941 | .0803 |
| ATCF | -11876300 | -12232139 | 93473 | 912492 | 1321355 | 1083273 |
| PRESENT VALUE ATCF | -10895688 | -21191235 | -21119056 | -20472624 | -19613834 | -18967914 |

| | YEAR7 | YEAR8 | YEAR9 | YEAR10 | YEAR11 | YEAR12 |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 29254717 | 31010000 | 32870600 | 34842836 | 36933407 | 39149411 |
| ROI | .0896 | .0982 | .1060 | .1131 | .1196 | .1255 |
| ATCF | 1485663 | 1903836 | 2338740 | 2791379 | 3262816 | 3754179 |
| PRESENT VALUE ATCF | -18155205 | -17199734 | -16122913 | -14943804 | -13679356 | -12344615 |
| | YEAR13 | YEAR14 | YEAR15 | YEAR16 | YEAR17 | YEAR18 |
| TOTAL INVESTMENT | 41498376 | 43988278 | 46627575 | 49425229 | 52390743 | 55534188 |
| ROI | .1308 | .1199 | .1121 | .1065 | .1027 | .1000 |
| ATCF | 4266664 | 4106666 | 4051067 | 4083808 | 4190916 | 4361438 |
| PRESENT VALUE ATCF | -10952920 | -9724015 | -8611843 | -7583255 | -6614847 | -5690250 |
| | YEAR19 | YEAR20 | | | | |
| TOTAL INVESTMENT | 58866239 | 62398213 | | | | |
| ROI | .0983 | .0973 | | | | |
| ATCF | 4586843 | 4860559 | | | | |
| PRESENT VALUE ATCF | -4798156 | -3930882 | | | | |

A second approach to improving the economic picture is to try to reduce the capital investment required. To check the impact of this, a reduction of the capital cost by 50% is examined:

ENTER SOLVE OPTIONS
 ? WHAT IF
 WHAT IF CASE 6
 ENTER STATEMENTS
 ? CAPITAL EQUIPMENT = 5000000,10000000
 ? SOLVE
 ENTER SOLVE OPTIONS
 ? L 1310 THRU L 1360

***** WHAT IF CASE 6 *****
 1 WHAT IF STATEMENT PROCESSED

| | YEAR1 | YEAR2 | YEAR3 | YEAR4 | YEAR5 | YEAR6 |
|--------------------|----------|-----------|----------|----------|----------|----------|
| TOTAL INVESTMENT | 10700000 | 10435413 | 11512476 | 12203225 | 12935418 | 13711543 |
| ROI | -.0609 | -.0297 | .1302 | .1421 | .1530 | .1399 |
| ATCF | -5661300 | -5735478 | 500295 | 1157713 | 1399569 | 1335932 |
| PRESENT VALUE ATCF | -5193853 | -10021290 | -9634971 | -8814818 | -7905194 | -7108621 |

| | YEAR7 | YEAR8 | YEAR9 | YEAR10 | YEAR11 | YEAR12 |
|--------------------|----------|----------|----------|----------|----------|----------|
| TOTAL INVESTMENT | 14534236 | 15406290 | 16330668 | 17310508 | 18349138 | 19450086 |
| ROI | .1491 | .1574 | .1651 | .1720 | .1784 | .1842 |
| ATCF | 1580341 | 1835345 | 2101579 | 2379718 | 2670475 | 2974608 |
| PRESENT VALUE ATCF | -6244121 | -5323023 | -4355398 | -3350179 | -2315282 | -1257705 |
| | YEAR13 | YEAR14 | YEAR15 | YEAR16 | YEAR17 | YEAR18 |
| TOTAL INVESTMENT | 20617092 | 21854117 | 23165364 | 24555286 | 26028603 | 27590319 |
| ROI | .1799 | .1653 | .1548 | .1472 | .1418 | .1380 |
| ATCF | 3095389 | 2994796 | 2961111 | 2983306 | 3052697 | 3162486 |
| PRESENT VALUE ATCF | -248056 | 648126 | 1461064 | 2212469 | 2917865 | 3588292 |
| | YEAR19 | YEAR20 | | | | |
| TOTAL INVESTMENT | 29245738 | 31000483 | | | | |
| ROI | .1354 | .1336 | | | | |
| ATCF | 3307396 | 3483371 | | | | |
| PRESENT VALUE ATCF | 4231547 | 4853088 | | | | |

The present value of after tax cash flows is \$4.8 million indicating that the cash flow can support the reduced level of investment. At an investment level of \$15 million, however, the present value is still negative

ENTER SOLVE OPTIONS
 ? WHAT IF
 WHAT IF CASE 7
 ENTER STATEMENTS
 ? CAPITAL EQUIPMENT = 7500000,15000000
 ? SOLVE
 ENTER SOLVE OPTIONS
 ? L 1310 THRU L 1360

***** WHAT IF CASE 7 *****
 1 WHAT IF STATEMENT PROCESSED

| | YEAR1 | YEAR2 | YEAR3 | YEAR4 | YEAR5 | YEAR6 |
|--------------------|----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 10700000 | 15435413 | 16812476 | 17821225 | 18890498 | 20023928 |
| ROI | -.0912 | -.0784 | .0366 | .0487 | .0597 | .0462 |
| ATCF | -8486300 | -9135478 | -632205 | 41813 | 299315 | 92503 |
| PRESENT VALUE ATCF | -7785596 | -15474745 | -15962924 | -15933302 | -15738768 | -15683612 |

| | YEAR7 | YEAR8 | YEAR9 | YEAR10 | YEAR11 | YEAR12 |
|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| TOTAL INVESTMENT | 21225364 | 22498886 | 23848819 | 25279748 | 26796533 | 28404325 |
| ROI | .0555 | .0639 | .0717 | .0787 | .0852 | .0910 |
| ATCF | 341006 | 598400 | 865218 | 1142025 | 1429421 | 1728040 |
| PRESENT VALUE ATCF | -15497069 | -15196753 | -14798382 | -14315978 | -13762031 | -13147653 |
| | YEAR13 | YEAR14 | YEAR15 | YEAR16 | YEAR17 | YEAR18 |
| TOTAL INVESTMENT | 30108584 | 31915099 | 33830005 | 35859806 | 38011394 | 40292078 |
| ROI | .0964 | .1012 | .1056 | .1096 | .1132 | .1058 |
| ATCF | 2038557 | 2361685 | 2698182 | 3048848 | 3414534 | 3368118 |
| PRESENT VALUE ATCF | -12482719 | -11775993 | -11035239 | -10267327 | -9478320 | -8764300 |
| | YEAR19 | YEAR20 | | | | |
| TOTAL INVESTMENT | 42709602 | 45272179 | | | | |
| ROI | .0981 | .0925 | | | | |
| ATCF | 3286838 | 3277179 | | | | |
| PRESENT VALUE ATCF | -8125044 | -7540294 | | | | |

Conclusions

It does not appear that the Prague, SCP from waste sulphite liquors process can be built and operated economically in Canada, unless either, the cost of the plant can be heavily subsidized by grants or equipment allowances, or the plant can be subsidized by a paper or pulp mill operator.

The needed capital grant is of the order of \$8-10 million, while the needed operating subsidy is of the order of \$2 million p.a. under most favourable circumstances. Even with this level of subsidy, there is no guarantee of profitability of the plant which will depend on the achieved output level of SCP, the percent protein in the SCP product, and actual cost realizations.

APPENDIX 1

4.2.9 COMPUTER PROGRAM OF THE BASIC ECONOMIC MODEL

The following is the computer program of the basic economic model. The model was written using the Interactive Financial Planning System (IFPS), a versatile, user-friendly package.

```

MODEL CZ VERSION OF 05/18/84 13:08
10 COLUMNS YEAR1, YEAR2, YEAR3, YEAR4, YEAR5, YEAR6, YEAR7, YEAR8, YEAR9, '
20 YEAR10, YEAR11, YEAR12, YEAR13, YEAR14, YEAR15, YEAR16, YEAR17, YEAR18, '
30 YEAR19, YEAR20
40 SIMULTANEOUS AUTO
50 *
60 *
70 *
80 *
90 *
100 * INFLATION AND MARKET DATA
110 * _____
120 *
130 COST OF CAPITAL = 9%
140 INFLATION = 6%
150 INTEREST RATE = 13%
160 SUBSTRATE PRICE PER T = 0, PREVIOUS*(1.00 + INFLATION)
170 WASTE CREDIT PER TONNE = 0
180 WASTE DISPOSAL CREDIT = SUBSTRATE FLOW*WASTE CREDIT PER TONNE
190 PRICE PER T PROTEIN = 688, PREVIOUS*(1.00 + INFLATION)
200 PRODUCT SELLING PRICE PER T = PRICE PER T PROTEIN*PERCENT PROTEIN
210 TONNES SOLD PER ANNUM = 0, 14000, 28000
220 SUBSTRATE FLOW = 0, 100000, 200000
230 *
240 *
250 * PROCESS PARAMETERS
260 * _____
270 *
280 YIELD = SUBSTRATE FLOW/TONNES SOLD PER ANNUM
290 PERCENT PROTEIN = .5
300 CHEMICALS COST PER T PROD = 75.30, PREVIOUS*(1.00 + INFLATION)
310 UTILITIES COST PER T OF PRODUCT = 107.15, PREVIOUS*(1.00 + INFLATION)
320 DIRECT LABOUR COST PER T OF PRODUCT = 25.14, PREVIOUS*(1.00 + INFLATION)

```

330 UNIT PACKAGE COST = 20.0,PREVIOUS*(1.00+INFLATION)
 340 *
 350 *
 360 * TAX SECTION
 370 *
 380 *
 390 TAX RATE = 0.44
 400 CCA RATE = 0.2
 410 ADDITIONAL TAX CREDITS = 0
 420 *
 430 *
 440 * CALCULATION OF VARIOUS TAX DEDUCTIONS:
 450 *
 460 CCA POOL = TOTAL INVESTMENT,PREVIOUS-PREVIOUS CCA SHIELD + '
 470 CAPITAL EXPENDITURES
 480 *
 490 CCA SHIELD = IF TAX2 .LT. CCA ALLOWABLE THEN TAX2 ELSE CCA ALLOWABLE
 500 *
 510 CCA ALLOWABLE = CCA RATE * CCA POOL
 520 *
 530 CUM LOSS DED = IF TAX1.GT.0.AND. TAX1 .LT. CUM LOSS POOL THEN'
 540 TAX1 ELSE IF TAX1.GT.0 .AND. TAX1 '
 550 .GE. CUM LOSS POOL THEN CUM LOSS POOL ELSE 0
 560 *
 570 CUM LOSS POOL = CUM LOSS ADDITION, PREVIOUS+CUM LOSS ADDITION -'
 580 PREVIOUS CUM LOSS DED
 590 *
 600 CUM LOSS ADDITION = IF PROFIT AFTER TAX.LT.0 THEN -1* PROFIT AFTER TAX'
 610 ELSE 0
 620 *
 630 * NOTE: TAX DEDUCTIONS ARE TAKEN SEQUENTIALLY AND ONLY WHEN NEEDED
 640 *
 650 TAX1 = (PROFIT BEFORE TAX - '
 660 ADDITIONAL TAX CREDITS) *TAX RATE
 670 TAX2 = IF TAX1 .GT.0 THEN TAX1 - CUM LOSS DED ELSE 0
 680 TAX3 = IF TAX2 .GT.0 THEN TAX2 - CCA SHIELD ELSE 0
 690 *
 700 *
 710 * BREAKDOWN OF INITIAL INVESTMENT COST
 720 *
 730 *
 740 LAND = 10000
 750 CAPITAL EQUIPMENT = 10500000,21000000
 760 WORKING CAPITAL = TONNES SOLD PER ANNUM*PRODUCT SELLING PRICE PER T/12
 770 *
 780 INITIAL INVESTMENT = SUM(LAND THRU WORKING CAPITAL)
 790 TAXABLE PROPERTY = 10700000, LAND + CAPITAL EQUIPMENT,PREVIOUS*(1.00+INFLAT
 800 *
 810 * TO GENERATE PROJECTED ECONOMICS SOLVE FOR LINES 850 THRU 1360
 820 *
 830 *
 840 *
 850 * INCOME STATEMENT

860 * _____
 870 * _____
 880 REVENUE = TONNES SOLD PER ANNUM*PRODUCT SELLING PRICE PER T
 890 * _____
 900 * DIRECT MANUFACTURING EXPENSES:
 910 SUBSTRATE COST = TONNES SOLD PER ANNUM/YIELD*SUBSTRATE PRICE PER T
 920 VARIABLE CHEMICALS COST = CHEMICALS COST PER T PROD*'
 930 TONNES SOLD PER ANNUM
 935 DIRECT LABOUR = DIRECT LABOUR COST PER T OF PRODUCT*'
 936 TONNES SOLD PER ANNUM
 940 UTILITIES COST = UTILITIES COST PER T OF PRODUCT*'
 950 TONNES SOLD PER ANNUM
 960 PACKAGING COST = UNIT PACKAGE COST * TONNES SOLD PER ANNUM
 970 * _____
 980 * OTHER EXPENSES:
 990 BLOCK UTILITIES = 0,18000,PREVIOUS*(1.00+INFLATION)
 1000 INDIRECT LABOUR = 0,62985,125970,PREVIOUS*(1.00 + INFLATION)
 1010 TRANSPORTATION = 0,100000,200000,PREVIOUS*(1.00+INFLATION)
 1020 PROPERTY TAX = 0,0,0,0,0,TAXABLE PROPERTY*.025
 1030 MAINTENANCE = 0,CAPITAL EQUIPMENT*.05,PREVIOUS*(1.00 + INFLATION)
 1040 INTEREST PAYMENTS = INTEREST RATE * LOAN BALANCE
 1050 * _____
 1060 TOTAL EXPENSES = SUM(SUBSTRATE COST THRU INTEREST PAYMENTS)
 1070 * _____
 1080 * _____
 1090 PROFIT BEFORE TAX = REVENUE - TOTAL EXPENSES + WASTE DISPOSAL CREDIT
 1100 * _____
 1110 TAX = TAX3
 1120 * _____
 1130 PROFIT AFTER TAX = PROFIT BEFORE TAX - TAX
 1140 * _____
 1150 * _____
 1160 * _____
 1170 * CASH FLOW INFORMATION
 1180 * _____
 1190 * _____
 1200 CAPITAL EXPENDITURES = INITIAL INVESTMENT,INITIAL INVESTMENT -'
 1210 PREVIOUS INITIAL INVESTMENT
 1220 LOAN BALANCE = INITIAL LOAN, INITIAL LOAN,PREVIOUS-PRINCIPAL PAYMENTS
 1230 PRINCIPAL PAYMENTS = 0,0,.05*INITIAL LOAN
 1240 INITIAL LOAN = INITIAL INVESTMENT,INITIAL INVESTMENT,PREVIOUS
 1250 * _____
 1260 * _____
 1270 * _____
 1280 * SUMMARY STATISTICS
 1290 * _____
 1300 * _____
 1310 TOTAL INVESTMENT = TAXABLE PROPERTY + WORKING CAPITAL'
 1320 , TAXABLE PROPERTY + WORKING CAPITAL
 1330 ROI = PROFIT AFTER TAX/TOTAL INVESTMENT
 1340 ATCF = PROFIT AFTER TAX - PRINCIPAL PAYMENTS -'
 1350 CAPITAL EXPENDITURES
 1360 PRESENT VALUE ATCF = NPVC(ATCF, COST OF CAPITAL, 0)
 1370 *****

1380 *****

END OF MODEL

The data contained in the base model is the 'best case' cost data.

5. CONCLUSION AND RECOMMENDATIONS

The objective of this report was to present alternative routes in microbial protein production with the ultimate goal to focus on a most promising approach. Even though there is a great deal of general and laboratory type information available on the potential of the SCP production, exact pilot plant and industrial data were accessible to the authors only on a limited basis. It was therefore necessary to make best estimates of some technological and economic parameters for this production.

Concerning raw materials these are available in Canada on a relatively large scale and represent

- a) materials containing readily fermentable sugars
- b) polysaccharide-containing feedstocks
- c) petrochemically derived feedstocks
- d) various complex industrial and municipal waste materials

Various processes based on yeasts, bacteria, fungi, and algae have been developed on the basis of the above substrates. The most important are presented in this report. One could possibly single out some substrates that would be of particular interest for the Canadian environment. These may be

- i) lignocellulosics, either as forestry crops or as agricultural residues
- ii) industrial 'waste' materials such as cheese whey or waste sulfite liquors
- iii) synthetic petrochemical substrates such as methanol
- iv) municipal wastes such as sludges from waste water treatment processes

Lignocellulosics definitely represent one of the most interesting alternative substrates. The major problem in the utilization of lignocellulosics for the SCP production is that these are not fermentable in their native form. The quantity of this biomass is spectacular on a yearly basis. The preparation step prior to fermentation is generally costly and not yet efficient enough to warrant a production of a relatively inexpensive product such as SCP. Acid hydrolysis processes are not ideal to create a fermentable substrate (by products, corrosion of equipment etc.) and the enzymatic processes are still too slow and inefficient. In this respect new developments by some companies are very exciting. As an example, ICI are actively developing a process, which utilizes inorganic catalysts and converts within 5 - 20 minutes different types of cellulosic materials into fermentable sugars and intact lignin (ICI-personal communication). It is also reported that the process is relatively mild and does not result in formation of such by-products that would inhibit subsequent microbial growth on this hydrolysate. Provided this process gets developed on a large scale with the right economics this development will certainly completely revolutionize the whole approach to lignocellulosics utilization and create an abundance of fermentable cheap sugars for SCP or any other biotechnological production.

Of particular interest is also utilization of industrial 'wastes' as substrates for protein production. In this respect, the economics of such processes is considerably improved as waste water treatment costs are balanced by the value of the final product. This is well demonstrated in our economics analysis. If economics is of major concern, a coupling of the SCP production with a waste stream, that normally has to be treated makes the approach much more attractive. It should, however, be kept in mind that a product derived from such waste materials would generally not be suited to direct human consumption but it would be intended for animal feed.

Methanol is another substrate that could be of interest in Canada which does have an abundance of natural gas. Chemical synthesis of methanol from natural gas is a well developed process and large quantities of methanol can be produced industrially. It is again a question of economics whether SCP production from this substrate would be profitable. The ICI large scale methanol-to-SCP process does not seem to be economically attractive although large installations and production are realized. It is hoped that the firm will recover capital cost losses by selling technology to developing countries. The Hoechst-Uhde methanol process is also at the standstill because of economics.

Another very important factor when industrial production of a product is concerned is that the raw material must be available in sufficient quantity and at acceptable quality on a continuous basis for the plant to operate. Some minor fluctuations are acceptable but if the availability of the raw material cannot be unified, the operation of the industrial plant becomes questionable. This consideration is particularly warranted when industrial waste materials are considered. With these materials one cannot guaranty either a uniform quality or quantity. This is a very serious limitation when industrial wastes are considered as substrates for SCP production. On the other hand, as already mentioned such materials may be of considerable interest due to favourable economics by coupling waste water treatment with SCP production.

In order to obtain as much information on potential alternatives as possible, and due to lack of data from existing industrial plants (very few) an attempt was made to develop and pre-design processes that may be of interest. In this respect, two alternatives are presented

- a) production of algal SCP from poultry waste
- b) production of fungal SCP from agricultural waste

The algae SCP are of interest because of the algae efficiency in protein production per unit area and nutritional quality of the algae biomass. The coupling of this production with a poultry operation makes this approach attractive for a farming community. The process is based on the evidence that poultry manure is a satisfactory substrate for algae culturing while the algae in turn is a suitable feedstock for chickens. A medium sized farm operation of 20,000 laying hens is considered. The algae pond to be utilized has an overall surface area of approx. 0.5 ha with a total productivity of about 165 kg of dry algae per day. According to a preliminary economics analysis, 165 kg of algae solids (with 55% protein) could displace about 227 kg of soy meal which would result in a daily saving of US \$45,6 or about US \$6,650 per year. In addition to this, production of methane gas in an anaerobic digester from the settled manure is considered and represents a further benefit to the farmer. The above approach is attractive and further research in this field on a farmer's scale would be warranted.

The other process, which also was presented and developed in detail is production of fungal SCP from lignocellulosics. An analysis of process parameters, based on data from the Waterloo SCP process (Moo Young, et al.) was performed to establish industrial feasibility of this operation.

The availability and the allowable substrate withdrawal from land for this operation is an important factor to be established to determine the amount of crop and cattle residues that can be removed from the soil without a detrimental effect on future crop productivity. This is a very important parameter for the farmer who should be involved in such operations. The portion of residues which may be removed was calculated in terms of each beneficial effect, i.e. erosion control, nutrient replacement and soil structure stabilization. The calculations and methodology are well presented in the report. On this basis the total amount of crop residue and manure available per year was calculated.

The total cost of this SCP product (fixed and direct operating costs without profit) is \$1.10/kg and on a protein basis (33% protein) the cost is \$3.30/kg protein. Recent data has placed the price of 1 kg protein (from distillers dried grains) at a value of \$0,75 Can. This makes this process, on the protein basis, highly uneconomical. Scaling up of the operation may serve to bring down the cost of the product. The comparison of our analysis of this process to the data presented by Moo Young and associates is further presented in the report.

In order to arrive at a reasonable economic analysis an attempt was made to evaluate a process developed by the Czechoslovak Academy of Science which utilizes waste sulfite liquor as a substrate for a yeast SCP. Data were generously supplied to Prof. Kosaric during his visit to the Czechoslovak Academy of Sciences under the Canadian National Research Council and the C.A.S. exchange scientific program. For this process sufficient data was available to be utilized in a specially developed computer program for economic analysis.

It is interesting to note that with design parameters developed for Czechoslovak industry, an operation of this nature would not be economical under the Canadian environment. Various alternatives were considered and are presented. It was concluded that the above SCP process would not be economical in Canada unless either the cost of the plant can be heavily subsidized by grants or equipment allowances or the plant can be subsidized by a pulp and paper mill operation. Even with this level of subsidy, there is no guaranty of profitability of the plant which will depend on the achieved output level of SCP, the % protein in the product and actual cost realizations.

This analysis, however, is an original and unique approach to evaluate the production of a novel product for the market such as SCP. The developed methodology can thus be applied for any other production where sufficient operating data are available. The authors suggest application of this model on other processes and would be interested to further collaborate in such developments.

One very important aspect which should never be neglected when new bio-products for food or feed are concerned is the nutritional biological and particularly toxicological value of the final product. It must be, without any doubt, proven that the product is not toxic either to animals or to human. Only then can this product be accepted for food or feed.

As it could be seen from this study, there are a number of alternatives for production of the microbial proteins. In the first instance animal feeds should be considered. The technology is generally available, however, actual operations worldwide are limited. The major reason is acceptability of this 'new type of feed', lack of data on biological and toxic effects of all products of interest, and the unfavourable economics. The economics of SCP production is very dependent upon the substrate cost and to make this production a profitable venture, alternative and well accepted feed materials (e.g. soy bean) must be more expensive than the SCP in question. The soy bean and other feed market is subject to a number of variations.

In any case the SCP alternative is available and when this production is coupled to 'waste' treatment processes, by product utilization and possibly production of another valuable fermentation metabolite (e.g. nucleotides, fatty acids, specialty chemicals, etc.) this whole approach is expected to be an attractive alternative for fast production of valuable nutritional proteins.

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