# SINGLE CELL PROTEIN AND OTHER FOOD RECOVERY TECHNOLOGIES FROM WASTES

Municipal Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

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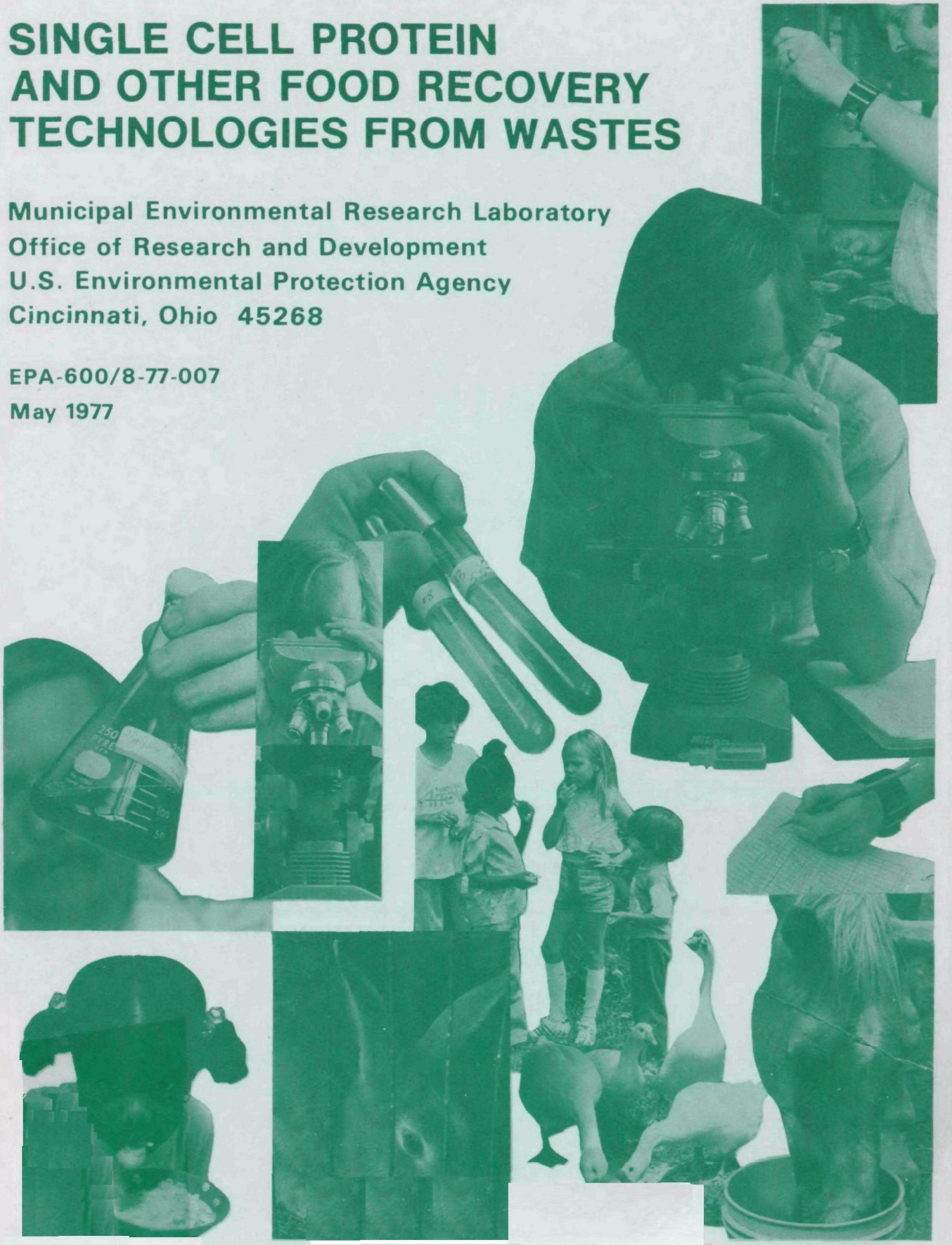

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# SINGLE CELL PROTEIN AND OTHER FOOD RECOVERY TECHNOLOGIES FROM WASTE

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# FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communication link between the researcher and the user community.

This study presents a comprehensive review of the techniques available for the bioconversion of waste materials to single cell protein or other animal feed, feed supplements, and human food.

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Director
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## ABSTRACT

In the field of waste management, much research has focused on the stabilization of wastes with formation of a marketable product to defray the costs of treatment before land disposal. Some wastes are already being commercially exploited for their energy potential. It is also possible to produce a human food or an animal feed through a number of biological waste management technologies.

Very few of the methods of producing proteins or edible carbohydrates from wastes have been explored beyond the pilot plant stage. Much of the research now underway is still at the bench-top scale.

Growth of single cell protein (SCP) on non-wastes is entering commercial exploitation, and companies are now testing and developing markets for microbial products. There are a number of technological refinements necessary to produce SCP acceptable for human consumption. Growth of feed-protein on waste cellulose while technologically feasible, because of incomplete utilization of the waste and poor yields of product, is not now economically competitive with more conventional proteins. Growth of protein on various food-processing wastes appears economically and technically feasible at present. SCP production on any substrate generally consumes more energy than production of vegetable proteins, but less that animal proteins when compared in terms of energy input per gram of protein produced.

Enzyme hydrolysis of cellulose to produce glucose, while an environmentally acceptable method of stabilizing wastes is currently more costly than acid hydrolysis of cellulose to glucose. The process is not energy intensive.

Anaerobic digestion of cellulose produces methane and a sludge with potential as a fertilizer or feed. Extensive nutritional and toxicological testing of the feed-potential of the sludge has yet to take place. If the sludge proves valuable as a feed or feed-supplement, this process is especially valuable, as it also produces energy in the form of methane.

In addition to the technological and economic problems associated with treating and recycling wastes for their nutritional value, there is also the problem of sociological acceptance, especially with human consumption of SCP.



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# LIST OF ABBREVIATIONS AND SYMBOLS

ADF — acid detergent fibre
ATCC — American Type Culture Collection
BOD — biochemical oxygen demand
$BOD_5$ — 5 day biochemical oxygen demand
Btu — British thermal unit
COD — chemical oxygen demand
cm — centimeter
°C — degrees Celsius
°C — degrees Fahrenheit
FPU — filter paper unit
FSL — fermented sludge liquor(brewing)
g — gram
GPL — grain press liquor(brewing)
IVRD — in vitro rumen digestibility
K cal — kilocalorie
kg — kilogram
$K_s$ — saturation constant

BP — British Petroleum
EPA — Environmental Protection Agency
ERCO — Energy Resources Company
ERDA — Energy Research and Development Administration
FAO — Food and Agriculture Organization, United Nations
ICI — Imperial Chemical Industries
IFP — Institut Francais du Petrole

kw — kilowatt
kwhr — kilowatt hour
l — liter
M — molar (solution)
mg — milligram
ml — milliliter
MMBtu — Btu x $10^6$
MSW — municipal solid waste
NDM — nonfat dry milk
NPU — net protein utilization
PER — protein efficiency ratio
ppm — parts per million
$Q_{O_2}$ — specific oxygen demand
scf — standard cubic feet
SCP — single cell protein
TPL — trub press liquor
WPC — whey protein concentrate

MIT — Massachusetts Institute of Technology
NASA — National Aeronautics and Space Administration
PAG — Protein Advisory Group, United Nations
RHM — Ranks Hovis McDougall
SHWRD — Solid and Hazardous Waste Research Division, EPA
SHWRL — Solid and Hazardous Waste Research Laboratory
UN — United Nations

## METRIC EQUIVALENTS OF U.S. CUSTOMARY UNITS

| | | |
|---|---|---|
| 1 short ton | = | 0.907 metric ton |
| 1 long ton | = | 1.016 metric tons |
| 1 short cwt | = | 45.4 kilograms |
| 1 lb. | = | 0.454 kilogram |
| 1 cubic foot | = | 0.028 cubic metre |
| 1 gallon | = | 3.79 litres |
| 1 acre | = | 0.405 hectare |
| 1 bushel | = | 35.24 litres |
| 1 Btu | = | 252 calories |

Note: U.S. customary units are used throughout the text where their use is most familiar. This was done for ease of comprehension of certain data.

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# SECTION 1

# INTRODUCTION

## Purpose

The management of solid wastes in the USA is complicated by increasing quantities of wastes, rising energy costs for disposal and treatment prior to disposal, limited land available for fills and lagoons, and stringent enforcement of environmental legislation. The estimated quantities of wastes from various sources is given in Table 1. Cellulosic residues are the most plentiful and available of all solid wastes. Large amounts of cellulose are found in crop residues, the forest industries, manures, industrial wastes, sewage solids and urban refuse. Hence, much research has focused on stabilizing wastes through utilization of the cellulose content, both to prevent pollution and to offset the costs of disposal through production of marketable goods. These products include methane from anaerobic digestion of organic wastes, ethanol from acid and enzyme hydrolysis of cellulose, various chemicals from corncobs, animal feed pellets from pea vines, etc.

Not only are cellulosic wastes a continuing and increasing disposal problem, but other organic wastes, from the food-processing industries in particular, have an extremely high biochemical oxygen demand (BOD) and therefore place a very heavy burden on the environment if discharged into rivers and streams (see Table 2).

The Solid and Hazardous Waste Research Division (SHWRD), U.S. Environmental Protection Agency, has encouraged and sponsored many research efforts designed to handle all waste materials in a more efficient, economic and responsible fashion. As alternative technologies develop, they are potentially in competition for available wastes. It becomes necessary to establish which processes are the most technologically and economically viable as well as the most environmentally acceptable. It is the purpose of this report to provide the Solid and Hazardous Waste Research Division with an intensive review of the techniques available for the bioconversion of waste materials to single cell protein (SCP) or other animal feed, feed supplements, or human food. Both cellulosic and other organic wastes will be discussed with an emphasis on the former, because in terms of volume, they present the greatest disposal problem, and in terms of resource recovery, they hold the greatest promise.

As cellulosic wastes are already being exploited commercially for their energy potential, it is important to compare and contrast food/feed production from cellulosic wastes with energy production from the same wastes. Although the general public in this country is well aware of the depletion of fossil fuel

TABLE 1

ESTIMATES OF ORGANIC WASTES GENERATED IN THE U.S.
(MILLIONS OF DRY TONS ‡PER YEAR) (1)

| Source | 1971 | 1980 |
|---|---|---|
| Manure | 200 | 266 |
| Urban refuse | 129 | 222 |
| Uncovered logging and wood manufacturing residues | 55 | 59 |
| Agricultural crops and food wastes* | 390 | 390 |
| Industrial wastes † | 44 | 50 |
| Municipal sewage solids | 12 | 14 |
| Miscellaneous organic wastes | 50 | 60 |

* Assuming 70% dry organic solids in major agricultural crop waste solids.

† Based on 110 million tons of industrial wastes per year in 1971, of which 40% were organics.

‡ Short ton. U.S. weights and measures are used throughout this report where still customary. Metric equivalents may be found listed on page x.

TABLE 2

CHARACTERISTICS OF SELECTED FOOD INDUSTRY WASTES (2)

| Type of Waste | 5 day BOD (ppm) | Suspended solids (ppm) | Reference |
|---|---|---|---|
| Packing house and stockyards | 595 | 606 | Hurwitz & Jonas |
| Meat products | 1,141 | 820 | ——— |
| Rendering | 1,180 | 634 | ——— |
| Dairies and milk products | 674 | 387 | ——— |
| Soft drinks | 430 | 220 | ——— |
| Cannery | | | Sanborn |
| Asparagus | 100 | 30 | ——— |
| Beans, green or wax | 160 to 600 | 60 to 85 | ——— |
| Beets | 1,580 to 5,480 | 740 to 2,188 | ——— |
| Corn, whole kernel | 1,123 to 6,025 | 300 to 4,000 | ——— |
| Corn, cream style | 623 | 302 | ——— |
| Cherries, sour | 700 to 2,100 | 20 to 605 | ——— |
| Grapefruit | 310 to 2,000 | 170 to 287 | ——— |
| Peaches | 1,350 | 600 | ——— |
| Pears | 2,250 to 4,700 | 1,200 to 6,700 | ——— |
| Peas | 380 to 4,700 | 272 to 400 | ——— |
| Potatoes, sweet | 295 | 610 | ——— |
| Potatoes, white | 200 to 2,900 | 990 to 1,180 | ——— |
| Pumpkin | 2,850 to 6,875 | 785 to 3,500 | ——— |
| Tomatoes, whole | 570 to 4,000 | 190 to 2,000 | ——— |
| Tomatoes, juice or products | 178 to 3,800 | 170 to 1,168 | ——— |

resources and perceives the energy crisis as more imminent, the 'impending' global protein shortage is already a reality in many Third World countries (see Figure 1). Ultimately, it is possible that a choice will have to be made between energy and food production, based on survival priorities rather than technological and economic data. Meanwhile, it is important for officials in decision making positions and the general public to appreciate the many factors that will hasten or impede the utilization of organic wastes for their potential nutritive value.

## Need

Protein-calorie malnutrition is the most serious and the most common cause of infant mortality and morbidity in the emergent nations, as well as among lower socio-economic groups in the industrialized countries. Kwashiorkor and marasmus may directly cause the death of an infant, or indirectly result in fatality upon infection, when the body's meager protein reserves are depleted further. Long term effects of protein-calorie deficiency in utero or in early infancy may include retarded physical and mental development.

Malnutrition and starvation are facts of life to countless millions in the Third World. Even in parts of the United States, a recent nutritional survey has found evidence of inadequate nutrition. Despite development of new genetic strains of wheat and rice with double or triple yields of grain and improved amino acid profiles, the protein gap between the developed nations and the Third World remains as wide as ever. This is partly a result of the continuing geometric increase in population. Also a factor is the increased demand for more and better quality protein as income rises in the industrialized nations. In the United States alone, per capita beef consumption has more than doubled since 1940 (4).

World supplies of high quality protein are not meeting the rising demand. Fishing was once considered an ever plentiful source of protein. Due to overfishing and coastal pollution, world fish catches are well down. The failure of the anchoveta catch off Peru in 1972, not only caused a rapid escalation of fish meal prices, but also drove up prices for all alternative feeds (see Figure 2).

A decrease in world production of legumes in favor of high yield cereals has also contributed to the world-wide need for more protein. However, there is a limit to the amount of land suitable for soy bean production in the United States. A shortage of protein for feeding to domestic animals causes slow growth and low productivity of farm animals and thus contributes to the poor diet of the human populace.

In the United States, as can be seen in Figure 1, the majority of essential protein consumed comes from animal sources. The feeding of grains to livestock to produce animal protein is not an efficient way to produce high quality protein. In terms of efficiency of protein production, Thaysen (6) demonstrated that a 1,000 lbs. bullock can synthesize 0.9 lb. of protein every 24 hours, whereas 1,000 lbs. of soybeans synthesize 82 lbs. of protein

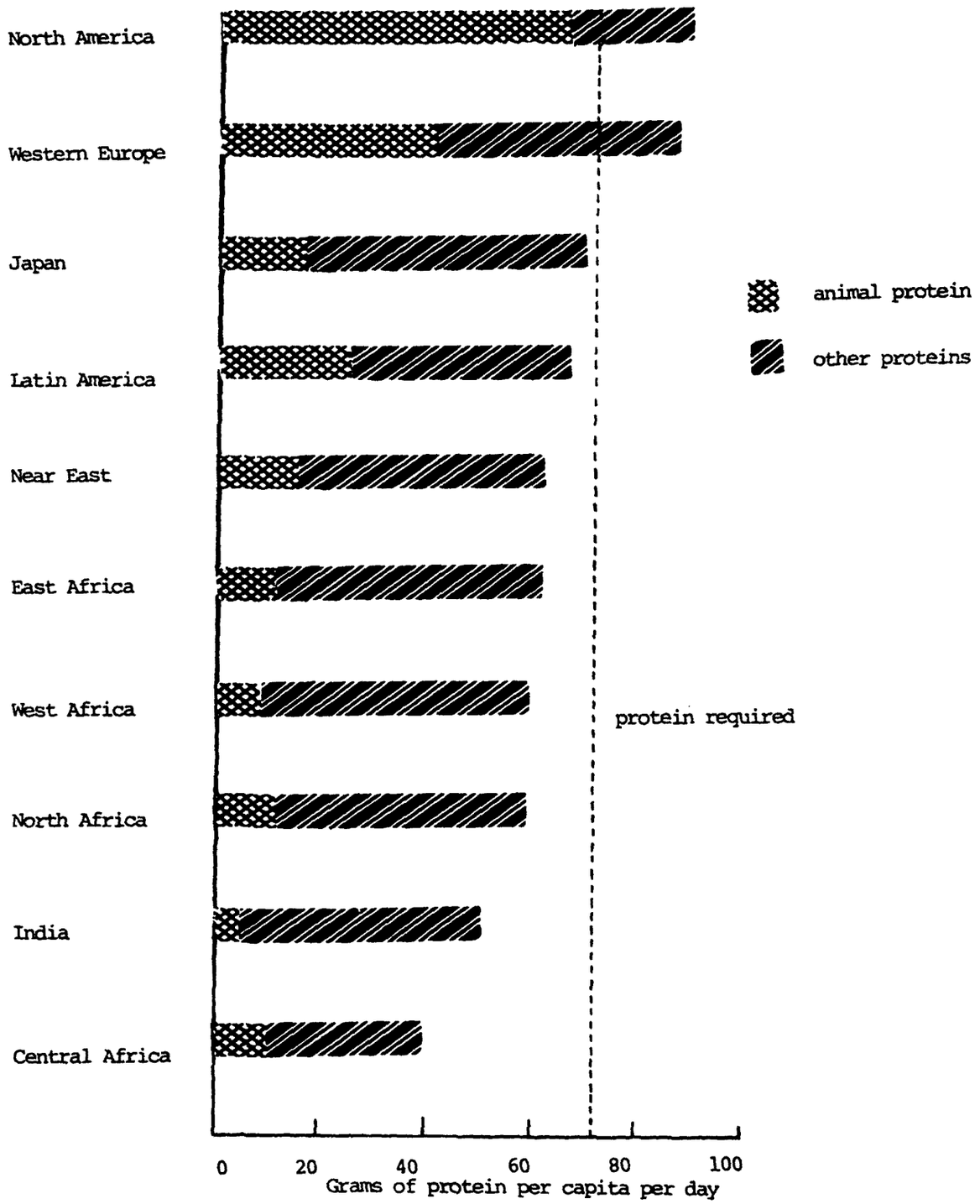


Figure 1. Protein Intake, 1970 (3)

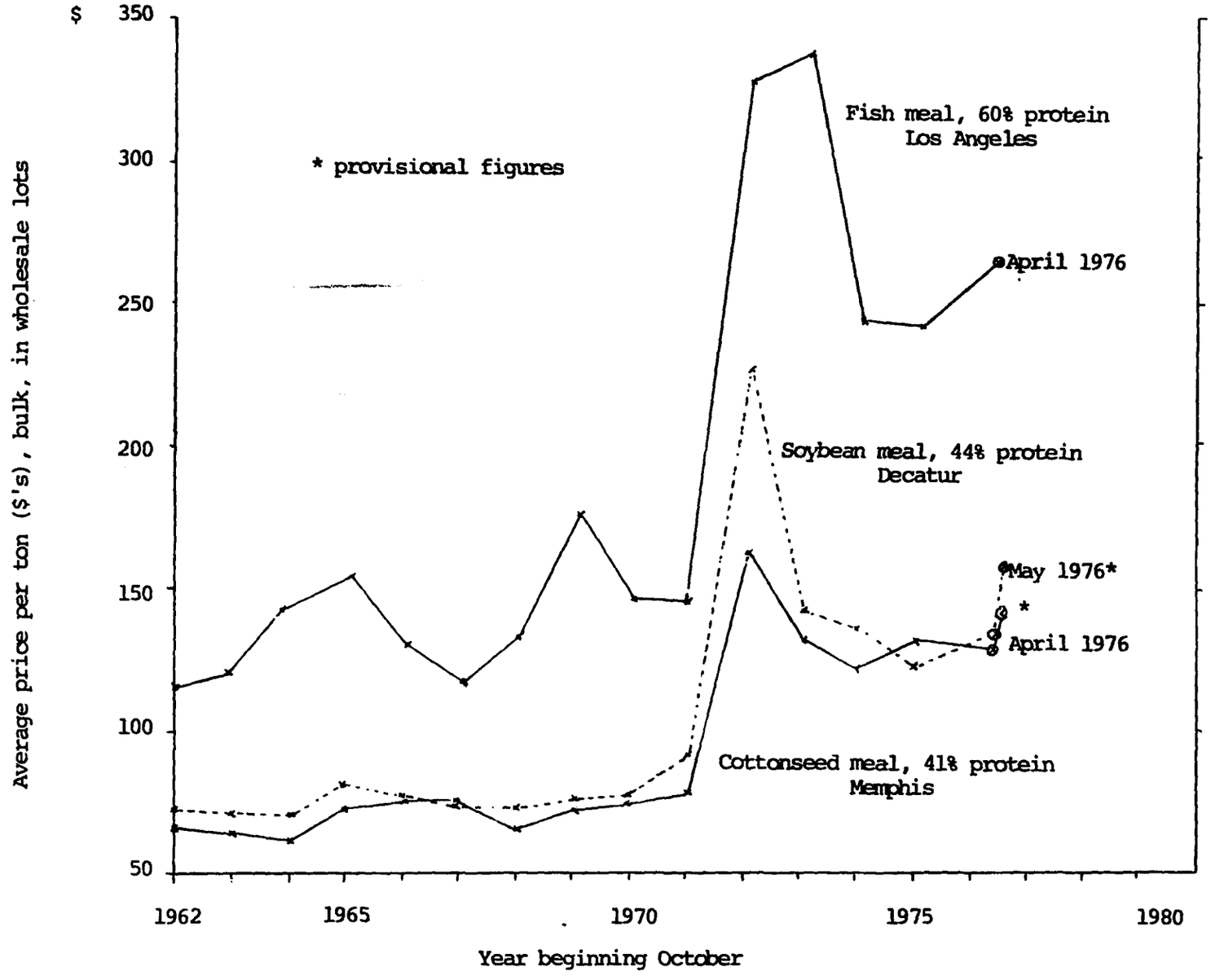


Figure 2. Cost of Selected Feedstuffs in the U.S., 1962-1975 (5)

in the same time, and in theory, 1,000 lb. of yeast could produce more than 50 tons of protein in a day. In terms of quantity, the amount of feed concentrates fed per animal unit in the United States has risen from a value of 1.98 tons in 1959 to 2.41 tons in 1973 (5). The quantity of feed purchased represented 17.5% of total farm production expenses in this country in 1959 and slightly over 20% of expenses in 1973 (5). It is obvious that not only is the feeding of high quality grains to farm animals an inefficient method of producing protein, but it is also costly in both human and economic terms.

The primary resource ultimately limiting expansion of agriculture is availability of suitable land. In terms of land utilization, Gray (7) calculated that an acre of corn could produce 261 lbs. of corn gluten protein, 280 lbs. of fungal protein and only 72 lbs. of beef (see Figure 3). Meadows and Meadows *et al* (8) estimated that even if all possible land available is utilized for farming, there will be a desparate land shortage before the turn of the century, if per capita land requirements and population growth rates remain as they are today.

Modern agriculture is an extremely energy intensive industry (see Comparative Energy Usage section of this report). Not only are our available energy resources rapidly dwindling, but the cost of unregulated fuels has skyrocketed since the advent of the political energy crisis of 1973. A recent report prepared for the National Science Foundation illustrates the impact of energy costs on the total variable costs of crop production in this country. The report stated that for every crop (14 field crops throughout the continental United States) the energy component of production costs has risen between 50 and 75% from 1970 to 1974 (9).

The need and concern for more efficient and economic means of producing high quality protein has stimulated research into unconventional sources of protein (including microbial protein) in the past twenty years. New protein sources will not replace the more traditional methods of protein production, but will be compared to modern farming practices and prices. In the case of protein production from waste materials, the technologies available must prove more viable than alternative waste management systems.

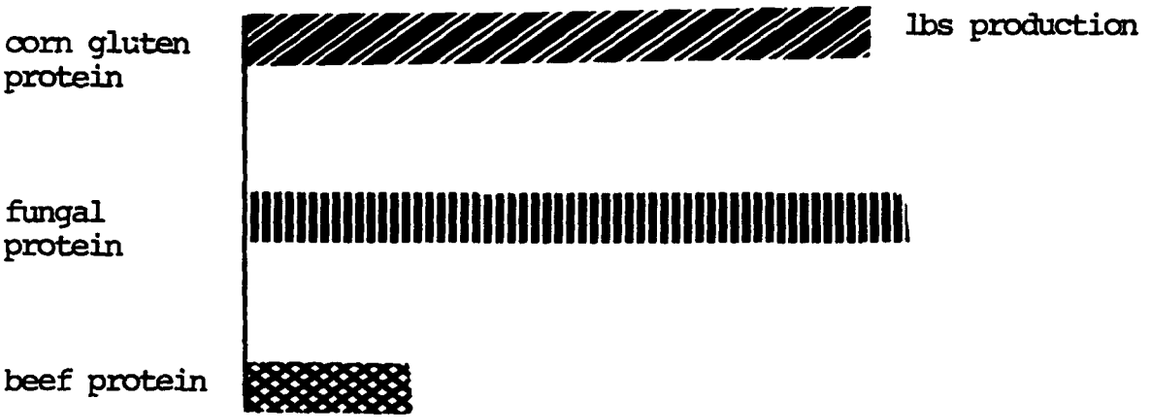


Figure 3. Land Use Efficiency of Several Protein Sources, Quantities for One Acre (7)

TABLE 3

EFFICIENCY OF PROTEIN PRODUCTION OF SEVERAL PROTEIN SOURCES IN 24 HOURS (5)

| Organism (1,000 lbs) | Amount of Protein |
|---|---|
| Bullock | 0.9 lb |
| Soybeans | 82.0 lbs |
| Yeast | 50 tons |

## SECTION 2

## CONCLUSIONS

Much of the research into food/feed production from solid wastes is still at the bench-top scale. A few pilot plant projects have confirmed the need for more research and development. Therefore, it is not possible to fully evaluate these technologies as methods for solid waste management at present.

Recycling waste cellulose for its nutritive potential is an environmentally acceptable method of stabilizing wastes. Effluents generated by any of the processes discussed are easier to treat than large volumes of solid waste.

Food/feed production from waste cellulose does not appear to be economically competitive with fuel production from the same wastes, at least in the near future. In the long run, the situation could well be reversed. Processes for producing energy from wastes are now entering commercial exploitation, and are now economically and technically practicable.

Pilot plant studies with food industry wastes have indicated the technical and economic feasibility of SCP production on wastes such as whey, potatoes, citrus and other fruit wastes, corn and pea-canning residues, etc.

Anaerobic digestion of agricultural wastes, especially manures, is a very promising waste management technique. Methane is generated and the sludge has potential value as either a fertilizer or a feed. However, there is a need to determine the safety, digestibility and palatability of the sludge to livestock. Several ongoing studies should clarify all possible uses of this sludge.

Not only will protein products derived from wastes have to compete with conventional food sources for potential markets, but with other "unconventional" protein sources now under development. A comparison of the status of research into all protein resources will prove useful.

Finally, while reclamation of the nutritive potential of wastes is not an immediate possibility, it does appear that such a reclamation will take place, perhaps within 10 years. Funding of research in this area should be considered an investment in the future. While the *Green Revolution* continues, the *Garbage Revolution* has just begun.

## SECTION 3

## RECOMMENDATIONS

There are a number of research areas requiring attention before full-scale exploitation of the nutritive potential of solid wastes is possible:

(1) Pilot plant studies are needed, especially for SCP processes and enzyme hydrolysis. Only larger scale projects will provide the raw data necessary to solve technological problems and to clarify the economic future for these processes.

(2) Computer simulations are needed to identify areas of economic sensitivity. Economic data must include variables associated with collection and transportation of wastes and the seasonality of some crop residues.

(3) An in-depth energy analysis should be performed based on at least pilot plant experience.

(4) The sludge of anaerobic digestion of manures has potential as a feed or fertilizer. There is a need to determine the safety, acceptability and digestibility of this sludge to livestock.

(5) Research into pre-treatment of wastes to improve their digestibility should continue and expand. Genetic engineering approaches to improve yields and composition of protein products should receive attention.

(6) All food or feed products derived from wastes (or any other unconventional food source) must undergo stringent and exhaustive safety evaluations before reaching the market place.

(7) Methods for improving the digestibility and functionality of single cell protein for human consumption need more investigation.

Though much research is still to be accomplished, and the economic future of some of the processes described is unclear, it does appear that commercial exploitation of the nutritive potential of wastes will become a reality.

*Financial support for such an undertaking, however, must come from sources possessing vision in the future, patience in the present, and understanding of the experimental efforts of the past.*

Nyiri and Tannen, 1975.

## SECTION 4

## SINGLE CELL PROTEIN — OVERVIEW

### Introduction

Single cell protein is the name given to the non-viable dried cells of micro-organisms such as yeasts, mycelial fungi bacteria and microalgae grown on various carbon sources. The name was coined by Professor Caroll Wilson of Massachusetts Institute of Technology in May 1966, to present a neutral image of the product to a general public which might react unfavorably to the idea of a "microbial" or "bacterial" protein source.

In addition to a source of carbon, the substrate, microorganisms require added nutrients, principally nitrogen, phosphorus, calcium, magnesium and iron in order to maintain growth. Algae with few exceptions require light as a nutrient.

While the composition of different single cell proteins varies depending on the choice of organism, substrate selected and conditions of growth, single cell proteins generally contain more than 50% high quality protein, together with smaller amounts of nucleic acids, carbohydrates, fats, ash, calcium, phosphorus and water.

Various companies, institutions and universities throughout the world have widely researched most aspects of SCP production, particularly in the last twenty years. However, man has a familiarity with the food and feed usage of microorganisms stretching back for centuries. Yeasts have been utilized in the brewing and baking industries since antiquity. Bacteria are eaten in products as diverse as sauerkraut, sausages and yoghurt. Haylage and silage are prepared through the activities of anaerobic bacteria and have proved a very satisfactory energy source for ruminants. A blue-green algae, *Spirulina maxima*, has been eaten by nationals of the Republic of Chad for centuries, and various species of marine algae have been used to prepare soups and to make glacé cherries. Higher fungi have been widely used for food purposes, as well as finding a place in religious ritual. According to Robert Graves, the mythological "ambrosia of the gods" was probably a species of mushroom.

The first modern effort to grow microorganisms for food/feed purposes was in Germany during the First World War. Over 15,000 tons of Torula yeast was added to human foods per year up to the mid thirties. The yeast was grown on beet molasses, wood sugar hydrolyzates, and later, following development of the process by Heijkenskjold in Scandinavia in 1925-1928, on spent sulfite liquor (10).

TABLE 4

FEED GRADE SCP PLANTS (12)

| Type of plant | Country | Substrate | Organism | Tons/year |
|---|---|---|---|---|
| Demonstration | | | | |
| BP | U.K. | n-Paraffin | Yeast | 4,000 |
| Chinese Petroleum | Taiwan | n-Paraffin | Yeast | 1,000 |
| Dianippon | Japan | n-Paraffin | Yeast | |
| ICI | U.K. | Methanol | Bacteria | 1,000 |
| Kanegafuchi | Japan | n-Paraffin | Yeast | 5,000 |
| Kohjin | Japan | | Yeast | 2,400 |
| Kyowa Hakko | Japan | n-Paraffin | Yeast | 1,500 |
| Milbrew | U.S. | Whey | Yeast | 5,000 |
| Shell | Holland | Methane | Bacteria | 1,000 |
| Svenka-Socker | Sweden | Potato Starch | Yeast | 2,000 |
| Semi-Commercial | | | | |
| BP | France | Gas Oil | Yeast | 20,000 |
| United Paper Mills | Finland | Sulfite Waste | Yeast | 10,000 |
| USSR | USSR | n-Paraffin | Yeast | 20,000 |
| Commercial | | | | |
| BP | Italy | n-Paraffin | Yeast | 100,000 |
| Liquichimica | Italy | n-Paraffin | Yeast | 100,000 |

TABLE 5

FOOD GRADE SCP PLANTS (12)

| Plant | Country | Substrate | Organism | Tons/year |
|---|---|---|---|---|
| Amoco Foods | U.S. | Ethanol | Yeast | 5,000 |
| Boise Cascade | U.S. | Sulfite Waste | Yeast | 6,000 |
| St. Regis Paper | U.S. | Sulfite Waste | Yeast | 5,000 |
| Slovnaft-Kojetin | Czech. | Ethanol | Yeast | 1,000 |

TABLE 6

OTHER SCP SYSTEMS UNDER CONSIDERATION (12)

| Substrate | Organism | Corporation |
|---|---|---|
| Animal Feed | | |
| Cellulose Waste | Bacteria | LSU-Bechtel |
| Citric Acid Waste | Fungi | Tate & Lyle |
| Coffee Waste | Fungi | ICAITI (Guatamala) |
| $CO_2$-Sunlight | Algae | IFP |
| Feed Lot Waste | Actinomycete | General Electric |
| Methanol | Yeast | Mitsubishi |
| Paper Pulp Waste | Fungi | Finnish Pulp & Paper |
| Human Food | | |
| Ethanol | Bacteria | Exxon-Nestle |
| Molasses | Yeast | Dianippon |
| Starch & Carbohydrates | Fungi | RHM - Dupont |
| Whey | Yeast | Kraftco |

In 1920, Pringsheim and Lichtenstein (11) reported feeding animals with the fungal mycelium, *Aspergillus fumigatus*, grown on straw with added inorganic nitrogen as a nutrient. After World War II, growth of fungi in submerged cultures for production of antibiotics, led to an investigation of the potential of microfungi as flavor additives to replace mushrooms.

In the fifties, the petroleum industry began experiments on the biological removal of wax and sulfur containing fractions from crude oil. The microorganisms used were found to contain over 50% protein, and became of primary interest themselves as either an animal feed or a human food.

Today, many demonstration and semi-commercial plants for SCP production are found throughout the world, as well as several full-scale commercial plants (see Tables 4, 5 and 6). As can be seen from these tables, the majority of producers are growing yeasts on a variety of petro-chemical based substrates.

An understanding of the status of SCP growth on any substrate is a necessary background to understanding the additional problems associated with use of a cellulose substrate.

Further details of the market status of food and feed grade SCP appear towards the end of this section.

## General Overview of the Process

With the exception of algal protein, SCP is grown in a fermenter which contains the culture, the carbon substrate, air to supply oxygen, water, added nutrients and ammonia to supply nitrogen and to adjust the pH. All inputs to the fermenter must be sterilized to prevent contamination of the culture, which is a particular problem with the slower growing fungi (13).

The fermentation is continuous, that is, a volume of broth and suspended cells is continually removed from the fermenter as fresh medium is added to keep the volume constant. A steady state growth rate of the microorganisms can be made to approach maximum values by adjusting the limiting nutrient input (flow rate). The productivity of a continuous culture expressed in grams of cells/unit volume per unit time, is higher than in a similar batch culture. Continuous cultivation of microorganisms has been limited on a large scale because of problems of maintaining sterility, changes due to harmful mutation and a lack of knowledge of microbial behavior (14). Knowledge advances in both biochemistry and engineering have made the design of large scale continuous plants feasible. Computer-coupled systems provide an immediate ongoing analysis of environmental conditions within the fermenter.

The limiting step in the growth of the organism is the rate of oxygen uptake from the airstream. Aerobic organisms require oxygen for respiration and growth up to a certain critical concentration of oxygen, which ranges from 0.1 to 1.0 ppm. for homogeneous cultures grown at 20-50° C (14). Oxygen utilization is related to growth and/or cell concentration. At a steady state, the oxygen transfer rate is equal to the oxygen uptake rate, which

varies depending on the organism and substrate. For example, bacteria, because of their lower lipid and higher nitrogen content require less oxygen than yeasts. The cost of oxygen enrichment can add significantly to the cost of the process.

The design of the fermenter has a considerable effect on the rate of oxygen transfer (15). Different types of fermenter include conventional stirred, draft-tube and air lift. An important new concept in fermenter design has come from Imperial Chemical Industries (ICI) in England. Their pressure cycle fermenter has operated on a continuous basis accepting runs of 2,000 tons of broth per hour, for periods up to eight weeks. The design of the fermenter optimizes the rate of oxygen transfer by forcing air bubbles through the vessel from the base without the problem of foaming. It is also designed to minimize heat shock of the organisms as they pass through the heat exchangers (16).

As previously implied, the reaction is exothermic and therefore heat exchangers are required to remove excess heat. Heat removal is especially costly for mesophilic organisms grown in hot climates where ambient water temperatures are high and refrigeration is required. Hence in warmer climates, microbes with temperature optima greater than or equal to 40°C would prove economically useful. Optimal temperatures for operation of fermenters will depend on whether the specific organisms involved are thermophilic or mesophilic in nature (*vide infra*). Departures from the optimal temperature for a particular organism will result in reduced rates of growth according to the Arrhenius equation (17):

$$k = A \exp(\mu/RT)$$

where
- k is the rate of reaction
- R is the caloric Gas Constant
- T is the absolute temperature
- $\mu$ is the temperature characteristic of the organism
- A is a constant

The pH of the fermenting broth is also an important factor in the yield and crude protein content of the harvested cells. The product is removed from the broth by centrifuging or filtering, perhaps after an initial dewater or with addition of a flocculent. The supernatant may be recycled to the fermenter to reduce sterilization costs, to utilize substrate and nutrients more efficiently, and to lower the biochemical oxygen demand of the effluent (12).

Harvesting can be a problem depending on the size of the cells and their concentration in the broth. Microalgae, for example, are so small and present in such dilute suspensions that they cannot be removed by sedimentation or flotation (18). Exceptions include some blue green algae which float to the top of the fermenter and may be removed by skimming. If the biomass is to be used as an animal feed, it is washed and roller or spray dried. If the protein is to be incorporated into a human food, then it is likely that further

treatment is required to remove nucleic acids which could cause kidney damage. Also, it may be necessary to treat the SCP to rupture the cell wall to improve digestibility and to texturize the protein. After treatment, the product is washed, centrifuged and spray dried (12).

The recovery of cells from n-alkane substrates presents a particular problem in that residual hydrocarbons adhere to the cells. The techniques for harvesting from n-paraffins are therefore modified to remove contaminants from the SCP.

## Choice of a Substrate

As can be seen in Table 7, there are many factors to be considered when choosing a substrate for SCP growth. Historically, as previously mentioned, the major oil companies began their research as part of a program to dewax fractions of crude oil. Hence in Europe and Japan, where oil companies were in the vanguard of research into single cell proteins and are now marketing feed grade SCP, gas-oil and n-alkanes are favored substrates. The cost of these substrates has escalated considerably since 1973, so that the advantage of high yields, which reduce production costs, has been offset by the cost of the substrate. This cost has delayed the further expansion plans of British Petroleum and Liquichimica (19).

There are a number of technological disadvantages to the use of a paraffin substrate. The oxygen requirements of the system are greater than for a partially oxidized substrate, the heat generated is greater and hence heat transfer more costly (12). The possibility of absorption of the substrate into the cell wall caused a furor in Japan where organized consumer groups forced the postponement of plans for production of feed-grade SCP (20). The fear was that carcinogenic polycyclic aromatic compounds, present in apparently harmless concentrations in Japanese "petroprotein" samples, might accumulate in livestock tissue.

The lower alcohols have several advantages as substrates. Apart from their free availability in a very pure form, their partially oxidized state means that less oxygen is required for growth of the microorganisms and less heat is generated, thus lowering heat transfer requirements and hence cost (21). Methanol and methane are currently considered the most economically attractive of the petro-chemical substrates (22) (see Comparative Economics section of this report). ICI has chosen methanol as a substrate partly for the reasons listed in Table 7 but also because the Corporation is a big producer of methanol from methane. Similarly, the Mitsubishi Gas Chemical Company which has also chosen methanol as a substrate, is a major producer of methanol for world markets. Amoco Foods which is currently selling food grade yeast to about 50 food companies in the United States (23) chose methanol partly because of its availability in a pure form, but also because of its psychological acceptability to the general public.

In Scandinavia, where forest industries are of major importance, sulfite waste liquors of the paper and pulping industry have been well utilized.

TABLE 7

FACTORS INFLUENCING THE CHOICE OF A SUBSTRATE

| Item | Methanol | Ethanol | Methane | Higher paraffins | Cellulosic wastes |
|---|---|---|---|---|---|
| Availability | widely available from many hydrocarbon sources | available from petrochemicals or sugar fermentation | well-head gas now flared could be used to grow SCP at low cost | freely available in the petroleum producing countries | plentiful and renewable resource |
| | available at high purity | available at high purity | could be easily manufactured should natural resources run short | requires purification before use | some wastes are easily collectable |
| | | | | | some wastes only available seasonally |
| Cost | lowest cost petrochemical available | relatively high cost | costs low but likely to rise, shortage of natural gas | cost of substrate rising, only economic for oil producing nations | low or negative cost of wastes |
| Versatility | restricted use by microorganisms, hence low possibility of contamination | versatile substrate, can be used by many organisms, contamination problems | no known yeast will grow on methane, thus eliminating one source of contamination | versatile substrate, thus contamination problems | fungi and bacteria |
| Properties | completely miscible w. water | completely miscible w. water | gas, insoluble in water | insoluble in fermentation media | fermentation slow as lignin shields the cellulose, problems w. solubilizing substrate |
| | explosion hazard less than methane | explosion hazard low | danger of explosion w. concentrations of $O_2$ | possible explosion problems | |
| | easy to store & handle | easy to store & handle | as above | as above | storage of some wastes not feasible |

TABLE 7 (continued)

| Item | Methanol | Ethanol | Methane | Higher paraffins | Cellulosic wastes |
|---|---|---|---|---|---|
| Properties | partially oxidized, therefore reduced oxygen requirement | partially oxidized, therefore reduced oxygen requirement | contains no oxygen, therefore added oxygen requirement | contains no oxygen, therefore added oxygen requirement | contains oxygen, reduced $O_2$ requirement |
| | | | | greater heat of fermentation, needs more cooling | conversion to SCP reduces BOD of waste |
| Toxicity | more toxic than ethanol | less toxic than methanol, most psychologically acceptable because of long use as a beverage | as a gas unlikely to contaminate substrate | need to purify substrate & product-fear of carcinogenic cmpds. | in themselves never toxic, but many contain pesticides, heavy metals other materials harmful to microorganisms |
| Status | semi-commercial plant | commercial plant | demonstration plant | commercial, historical development on paraffins | some pilot plant scale, most bench scale, some R & D still to complete |
| Yields | acceptable yields | acceptable yields | acceptable yields | especially high yields | low yields |

In the U.S., Boise Cascade and the St. Regis Paper Company are each producing 5,000 to 6,000 tons of food grade yeast per year, and are likely to expand their facilities.

In countries with a strong agricultural base such as the United States, all forms of agricultural waste are being examined as substrates for SCP growth. The Louisiana State University pilot plant used bagasse as a substrate for bacterial SCP growth. The process is applicable to most cellulosic wastes and Bechtel has shown interest in promotion of the process. General Electric has investigated growth of thermophilic actinomycetes on feed lot wastes in a demonstration plant at Casa Grande in Arizona. For a variety of technical reasons including heavy metal contamination of the substrate, animal feeding trials were stopped, and the plant ceased operation. It has not reopened for economic reasons (24).

In terms of large scale development, growth of SCP on cellulosic and non-cellulosic carbohydrate wastes is several years behind petrochemical substrates. The renewable nature of these wastes, together with escalating prices for non-renewable petroleum derived substrates, makes the future appear promising for SCP growth on wastes, though a number of technical difficulties must be overcome (*vide infra*).

While the choice of a carbon source depends very largely on availability and cost of substrate, other factors, including psychological acceptance of the product at the market place, are also important.

## Choice of an Organism

The choice of an organism is based on several selection criteria. The most important factor is the safety of the organism. It must be non-pathogenic and must not produce toxins. If the product is to be incorporated into human food, it must be acceptable to the palate, both in taste, texture and form, and it must not alter the functional properties of the food to which it is added.

The growth rate and the maintenance requirements should permit maximum cell yield, lower operating costs and reduced contamination potential. As most continuous fermentations are limited by the oxygen transfer rate, maximum yields can be achieved by either high cell density at low growth rate or a low cell density at a high growth rate (25).

Another factor of importance when choosing an organism is the efficiency with which it converts the raw materials into protein, and the stability of the culture as it grows.

The ease with which the protein can be processed is also an important criterion in choice of an organism. Table 8 summarizes the advantages and disadvantages of the four classes of organism commonly used. Table 9 compares the mass doubling times of different organisms, illustrating one of the important advantages of SCP production over other protein sources.

TABLE 8

SUMMARY OF DESIRABLE CHARACTERISTICS AND PROBLEM AREAS
ASSOCIATED WITH SINGLE CELL PROTEINS (27)

| Desirable characteristics | Problem areas | Comments |
|---|---|---|
| | ALGAE | |
| - growth in outdoor ponds<br>- simple nutrient requirements | illumination | Artificial illumination is too costly. Outdoor cultivation is practical only below 35° latitude. |
| - possibility of utilizing sewage or industrial wastes | $CO_2$ limitation | $CO_2$ must be supplied. Sometimes simple aeration will supply an adequate quantity of $CO_2$. |
| - | contamination | Pathogenic bacteria and viruses may contaminate algae growth in sewage oxidation ponds. |
| - | harvesting | Cells settle at slow rate, centrifugation too costly, flocculants contaminate product. |
| - | nutritional value | Poor digestibility in man and non-ruminants. |
| - | product quality | Without pre-treatment cells tend to be deficient in methionine: algae toxins might be present. |
| - | sensory quality | Bitter flavor is not acceptable to man or some animals: may require special treatment. |
| - | costs | Estimated production cost of $0.24 per kilo make algal protein non competitive. |

TABLE 8 (continued)

| | | |
|---|---|---|
| | YEASTS | |
| - long history of human and animal use | substrate concentration | Low concentration of carbohydrates must be used to avoid carbon losses. |
| - possibility of utilizing low cost hydrocarbons or waste carbohydrates as substrates | oxygen requirements | Large amounts of oxygen are required especially with hydrocarbons. |
| | contaminants | Aseptic conditions may be required especially in tropical regions. |
| - favorable protein and amino acid contents | cooling | Large amounts of heat released during growth, especially with hydrocarbons: refrigeration required. |
| - good nutritional value | harvesting | Separation of trace residues of hydrocarbons from cells may be troublesome. |
| - | waste | Spent growth medium may have high BOD which must be treated. |
| - | nutritional value and product quality | Hydrocarbons may depress growth, methionine limiting; only limited amounts can be added to human diets without imparting adverse flavor. |
| - | cost | Production costs using hydrocarbon substrates would make product uncompetitive in animal feed markets in U.S.A. |

TABLE 8 (continued)

| | | |
|---|---|---|
| | FUNGI | |
| - Mushrooms have long history of human consumption | growth rate | Growth rates are lower than bacteria or yeast; poorer productivity. |
| - Possibility of utilizing waste carbohydrates as substrates | contaminants | Contaminants may have higher growth rates and take over. Aseptic conditions are required. |
| - Mycelium may be harvested by filtration | cooling | Fungi, of interest as protein sources, do not grow well above 35°C, refrigeration may be required. However, many potentially useful fungi which have not been examined closely will grow optimally at $\pm$ 35°C. |
| - | wastes | Spent growth medium may have high BOD which must be treated. |
| - | nutritional value and product quality | Toxins may be produced by some fungi. Mycelium tends to be deficient in methionine. Nutritional value for mycelia for domestic animals and man have not been established. |
| - | cost | Slow growth rates and requirements for aseptic conditions and cooling make fungal protein non-competitive in U.S. for use in animal feed. May be useful as flavoring additive. |

TABLE 8 (continued)

| BACTERIA | | |
|---|---|---|
| - rapid growth rates | oxygen requirements | Large quantities of oxygen are required especially with hydrocarbons. Sterility must be maintained since pathogenic bacteria may grow under same conditions as desired. |
| - ability to utilize low cost substrates such as methane, paraffins, methanol, cellulose | genetic stability | May vary widely among different species. |
| | cooling | Large amounts of heat released during growth. Refrigeration may be required. |
| - generally good yields, protein contents (70-80%) and amino acid profile | harvesting | Small size of bacterial cells makes centrifugation too costly. |
| | nutritional value | Endotoxins may be present in cells, acceptability to humans and animals yet to be established. |
| - | cost | Achieving production costs of less than $0.20 per kilo will require improved oxygen transfer system and product separation techniques. |

TABLE 9

MASS DOUBLING TIMES (19)

| Organism | Time for one mass doubling |
|---|---|
| Bacteria and yeast | 10-120 min. |
| Mold and algae | 2-6 hours |
| Grass and some plants | 1-2 wks. |
| Chickens | 2-4 wks. |
| Pigs | 4-6 wks. |
| Cattle | 1-2 mo. |
| People | 0.2-0.5 yrs. |

Other advantages of SCP production include:

- Genetic experimentation to improve protein quality is possible.
- Production of SCP is not limited by land surface or sunlight except for algae.
- Microorganisms not dependent upon agriculture or climatic conditions (except algal growth in ponds) (14).

The microorganisms available for growth to single cell protein are conveniently classified according to the temperature range for optimum growth. Mesophilic organisms grow well between 20-45° C and thermophilic organisms grow best between 45-60° C. They are all aerobic microorganisms, i.e., they require oxygen for growth. Temperature control of the fermenter using ground water at ambient temperatures is more easily accomplished with thermophiles (26).

When utilizing cellulosic wastes as a substrate, there are additional advantages in using thermophilic organisms (26):

(a) Thermophiles have a higher rate of cellulose and lignin digestion than mesophiles;

(b) Thermophiles grow at pasteurization temperatures, thus eliminating pathogens present in the wastes.

Further Treatment for Human Consumption

As previously mentioned, single cell proteins contain percentages of nucleic acids ranging from 6 to 25% depending on conditions of growth and species of microorganism (28). Ruminants possess enzymes which metabolize nucleic acids to the soluble excretable allantoin. Man lacks these enzymes and metabolizes nucleic acids to the sparingly soluble uric acid, which increases in concentration in the urine and plasma (28). Increased plasma levels of uric acid result in deposition of uriate crystals in the joints, as in gout. The Protein Advisory Group of the United States (PAG) has established an upper limit of 2 grams of nucleic acid per day for the healthy adult (29). This figure is based on research by Waslein *et al* at Berkeley (30) and Edozien *et al* at the Massachusetts Institute of Technology (31). Individuals on a low purine diet cannot tolerate this high a level of nucleic acids.

Therefore, if SCP is to be marketed as a human food, it must be processed to remove the nucleic acids, or at least to reduce their percentage to an acceptable level. Several methods are under investigation for modifying the nucleic acid content of single cell proteins. They include(28):

(a) Control of growth rate - the RNA content of cells is dependent on growth rate;

(b) Base-catalysed hydrolysis - treatment with sodium hydroxide or potassium hydroxide has been proposed for microalgae;

(c) Chemical extraction - hot sodium chloride reportedly removes nucleic acids from yeast;

(d) Cell disruption - physical, chemical and biological (enzymatic) treatments have been described as methods for cell disruption when the protein isolate is desired. High pressure homogenization has been proposed by Sucher *et al* (32) as a means of producing protein, a glycan and a yeast extract from bakers' yeast. Ultrasonic techniques have successfully solubilized the protein from heat-treated soybean products (33), and cottonseed products (34) and could be applicable to release of protein from SCP cells.

(e) Treatment with exogenous or endogenous enzymes - Castro *et al* (35) used exogenous pancreatic RNase to reduce the nucleic acid content of yeasts, and found that the efficacy of the process was independent of either the

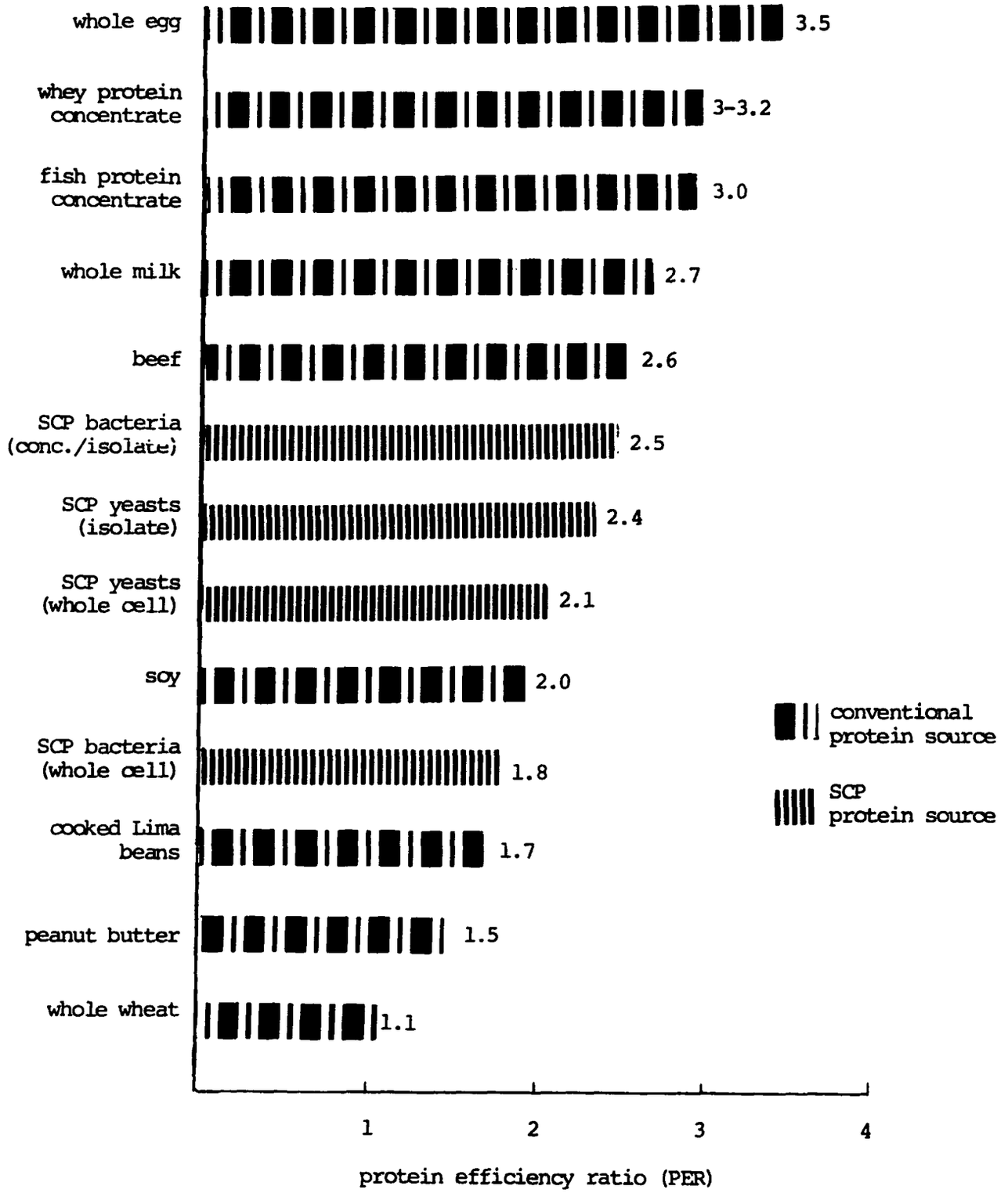


Figure 4. Protein Efficiency Ratios of a Number of Protein Sources (40), (12)

age or species of yeast.

Following either heat or chemical shock, activated endogenous RNase will reduce the RNA content of *Candida utilis* from 7 to 8% to 1 to 2%.

The effectiveness of these treatments in removing nucleic acids must be balanced against the cost of each treatment and its effect on the functional properties of the SCP. Much research and development work is still underway both to reduce the nucleic acid content and/or to release the protein from the cells. Sinskey and Tannenbaum (28) are looking into the possibilities of genetic engineering to produce temperature sensitive mutants which stop nucleic acid synthesis above a certain temperature.

In man and mono-gastric mammals, the digestibility of single cell protein is thought to be hindered by the cell wall (36). The production of protein concentrates from whole cells is known to improve the biological availability of the protein (37).

The manner in which the SCP is treated and dried also alters its functional properties, such as gel formation, whipping and foaming abilities and water and fat absorption characteristics (38). More research is required into the functional properties of variously processed single cell proteins. This requirement applies to SCP grown on any substrate including solid wastes.

Protein Quality and Amino Acid Composition

The protein content of a product as measured from a nitrogen assay (Kjeldahl nitrogen X 6.25) is inaccurate because of the non-protein nitrogen in the cell. Amino acid analysis provides a more accurate indication of the nutritive value of the SCP. If the protein has an amino acid composition corresponding to the ideal pattern required by the body, it may be well utilized. The Food and Agriculture Organization (FAO) has established a reference protein with a particular percentage of essential amino acids, to which other proteins are compared. The value of a particular protein source is limited by the amino acid present in the lowest proportion. For example, in the case of wheat gluten, lysine is the limiting amino acid, reducing the utilization of wheat protein to 33% that of an ideal protein. The amino acid distribution in single cell proteins varies, depending on the microorganism and the substrate, as well as the environmental factors of production. Some single cell proteins have been found deficient in the sulfur-containing amino acids, methionine and cystine.

Empirically, the quality of a given protein is established through nutritional trials to establish how much weight gain results per gram of protein consumed. This figure may be expressed as protein efficiency ratio (PER); as a biological value (BV) or as net protein utilization (NPU). Figure 4 compares the protein efficiency ratio of yeast and bacterial SCP, with the PER values for more conventional protein sources.

Certain amino acids which cannot be synthesized by the human body are considered essential in the diet. They are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (39). Histidine is essential for growth in infants.

## Nutritional Studies

However favorably the amino acid analysis compares with the FAO reference protein, the true digestibility or acceptability of the SCP can only be established by short and long-term nutritional trials of laboratory animals. Every single cell protein must undergo stringent toxicological and nutritional trials before marketing can be considered. In 1972, PAG reissued updated guidelines in a series relating to the clinical testing of all novel proteins as potential animal feeds or as human food (29), (41). These guidelines are extremely comprehensive and are adhered to in principle in laboratories throughout the world.

## SCP for Human Consumption

Various researchers have reported adverse gastrointestinal effects on human consumption of even small quantities of yeast, though at least a dozen reports have indicated a human tolerance of up to 100 grams/day (g/day) (4). Similarly, according to some reports, algae have been tolerated up to 100 g/day without gastrointestinal disturbance, while other nutritional trials have demonstrated a poor tolerance of microalgae even in small amounts (4).

Calloway *et al* at Berkeley, found that small quantities of several bacterial proteins produced stomach cramps, nausea and vomiting in human volunteers, when larger doses have indicated no adverse effects in a variety of experimental animals. Researchers at MIT have similarly noted gastrointestinal problems and the development of sensitivity among two groups of fifty human volunteers ingesting 12 g of a bacterial SCP or 20 g of a yeast. Both organisms were grown on food-grade ethanol. A MIT study feeding fungal protein to human volunteers at the rate of 40 g/day for one week also reported abdominal discomfort (4).

Though published results of nutritional trials with human subjects have been limited, the mixed results so far reported are discouraging. The reasons for the poor human tolerance of single cell proteins are not clearly understood, though a number of possibilities exist. It is clear that there is a need for more basic research into the acceptability of single cell products as human food. Because a product has successfully completed a series of animal feeding trials, no assumptions can be made with regard to human acceptability.

In spite of these general difficulties, Amoco Foods is now marketing a food-grade yeast, "Torutein". "Torutein" is Torula yeast grown on ethanol and spray dried. This yeast has long been established and accepted as a food ingredient and Amoco Foods has already obtained government clearances to market "Torutein" in Canada and Sweden as well as in the United States (23).

The yeast is about 52% protein and due to its relatively low methionine level has a PER value of about 1.7. The yeast has a high lysine content and as little as 9% added to wheat will improve the PER value of wheat from 1.1 to 2.0. "Torutein" is being marketed as a flavor enhancer of high nutritional value, with very acceptable functional properties. Three grades of "Torutein" are available:

(a) "Torutein" - especially well suited for addition to meat products;

(b) "Torutein-LF" - low flavor "Torutein" especially acceptable where a low yeast flavor is desirable, and valuable as a replacement for nonfat dry milk;

(c) "Torutein-94" - which can replace 20 to 100% of whole egg or egg yolks in salad dressings, bakery goods, etc. (23).

Other major firms interested in manufacturing food-grade SCP include Exxon/Nestle and Ranks Hovis McDougall/Du Pont. Exxon Enterprises has looked into growth of both bacteria and yeasts on food-grade ethanol. They are investigating strains of the bacterium, *Acinetobacter calcoacetius*, in a pilot plant in Switzerland. Exxon/Nestle protein has undergone extensive acute and chronic toxicological testing with a variety of experimental animals (species not indicated). The material was well tolerated when fed to 25 adult males at a level of 10 g/day for two months (43). Their most recently improved product, treated to reduce nucleic acid content to 3%, contains 80% crude protein and has a PER value close to casein (43).

RHM/Du Pont have chosen to grow microfungi on carbohydrates such as molasses, beans or cassava. Their pilot plant has a capacity of 150 tons/year but the process will not be commercial in the near future (44).

## SCP for Animal Consumption

While the nutritional trials of SCP for human consumption have so far been limited, a great deal more is known and understood concerning acceptance of SCP by higher animals. Firms with an interest in SCP production since the late fifties have completed extensive feeding trials with pigs, poultry, calves and other young mammals. There seems little doubt that once the non-toxic nature of the product has been established, if the nutritional characteristics are proved adequate, that a variety of SCP proteins are acceptable as replacements of at least a part of the soy or fish meal component of high protein feeds (45).

Both "Toprina", the yeast grown on gas-oil by British Petroleum (BP), and "Pruteen", the bacterial SCP grown on methanol by Imperial Chemical Industries (ICI), have undergone short and long-term feeding trials. "Toprina" is marketed as a replacement for fish meal in high protein feeds, and as a replacement for skimmed milk powder in milk replacers. Therefore, there

has been a special emphasis on nutritional trials with pigs, poultry and calves though other animals (fish, quails, etc.) have also taken part in the testing program (46). The BP Cap Lavera plant is on-line at 20,000 tons/year. A 100,000 tons/year plant is under construction in Sardinia.

"Pruteen", the ICI feed-grade protein, analyses as 72% crude protein at normal moisture levels. It will be marketed as a source of energy, vitamins and minerals as well as a highly balanced protein source. The methionine and lysine content of "Pruteen" (1.8 and 4.9% respectively) compare very favorably with white fish meal. Extensive feeding trials have indicated that "Pruteen" is better than fish meal in many diets. A commercial plant of capacity 100,000 tons/year is in the final stages of design (47).

The construction of a 100,000 tons/year plant by Liquichimica Company of Milan in Calabria, Italy, was delayed by financial problems. A special feature of their process is the sea-water cooling system using DeLaval titanium plate heat exchangers of special surface design (48).

In the next ten years, it is anticipated that there will be many feed-grade SCP plants of at least 100,000 tons/year capacity in Czechoslovakia, France Great Britain, Japan, Italy and the USSR.

Future Prospects.

There is a difference of opinion as to future directions for single cell protein manufacture. While the technology for production of food-grade SCP is not as advanced as that for feed-grade protein, some experts believe that the potential of SCP as a food is greater than its potential as a feed for economic reasons (12). The marketing of "Torutein" by Amoco as a food additive is seen as an attempt to test and develop markets for food-grade SCP. Many companies have expressed an interest in "Torutein", and Amoco is "encouraged" bu the response of its marketing efforts (23). Marketing a new product in well-established markets is the preceding stage to development of new markets and uses for the product.

The acceptability of a bacterial, fungal or algal protein for human consumption probably suffers more psychological constraints than does consumption of yeasts. Certain agricultural wastes are also likely to be "unacceptable" substrates for growth of food-grade SCP, e.g., manures. A number of cultural, religious and sociological taboos limit the consumption of a new food even though it has an acceptable taste and "mouth-feel".

Some experts believe that consumer resistance to products considered "artificial or imitation" food will delay acceptance of SCP products in sophisticated markets, at least until vegetable-based proteins are more widely accepted by the general public (49).

European markets are currently more promising outlets for feed-grade SCP than the U.S., as Europe has to import the bulk of its oil-seeds while the United States is the world's leading exporter of soy products. In this country, long term prospects for marketing of feed-grade SCP from low cost cellulosic

wastes are very attractive. Table 10 projects the U.S. demand for high protein feed-grains to 1990 (50).

Government regulations relating to usage of SCP in animal feed or as a human food vary from country to country. As previously mentioned, most companies are adhering in principle to the PAG guidelines for clinical testing of novel proteins. In Europe, the European Association of Single Cell Protein Producers (UNICELPE) was inaugurated on November 27, 1974. One of the functions of UNICELPE is to develop a common policy toward testing and regulation of single cell proteins in Common Market countries.

The future for SCP in sophisticated markets looks promising both as a food or feed. Whether food-grade SCP will solve problems of the hungry in Third World Countries is another question. All processes so far mentioned are high technology, high investment projects. Tate and Lyle believe that a low cost, "village-level" technology is needed for the emergent nations. They are building a pilot-plant in Belize, Central America, to examine the feasibility of utilizing citrus industry wastes to produce fungal protein for feeding to pigs and poultry. They have investigated growth of *Aspergillus niger* (M1) and a *Fusarium sp.* on carbohydrates (especially carob pods and spoiled papaya) in the laboratory for some years (51). Their calculations indicate that a low technology plant in a country with cheap labor could be economic on the scale of 100 tons of SCP product/year. The citrus industry in Belize produces about 2300 tons/annum of a high BOD waste, most of which is dumped (52).

Whether a "village-level" technology, manufacturing a relatively expensive feed-grade SCP to support an expanding livestock industry, will in fact solve the food problems of the very poor, is also questioned by many.

It is clear that though many problems of technology, economics and acceptability are not solved, that a new industry has been born which will continue to work towards production of high quality, inexpensive protein for human and animal consumption.

TABLE 10

FORECAST OF ANIMAL FEED CONSUMPTION
IN THE UNITED STATES, 1960-1990
(Millions of Tons) (50) *

| Item | 1960 | 1970 | 1980 | 1990 |
|---|---|---|---|---|
| Feed Grains | 121.80 | 144.90 | 176.00 | 200.00 |
| High-Protein Supplements: | | | | |
| Soybean Meal | 8.84 | 13.46 | 17.80 | 22.85 |
| Other Oilseed, Animal and Grain | 7.95 | 7.81 | 8.95 | 8.55 |
| Total High Protein | 16.79 | 21.27 | 26.75 | 31.40 |
| Other Processed Feeds | 11.24 | 13.31 | 15.85 | 17.80 |
| Total Feed | 149.83 | 179.48 | 218.60 | 249.20 |

* 1980 and 1990 estimated. 1960 and 1970 derived from USDA Economic Research Bulletin No. 410, Feed Statistics through 1966, as supplemented through 1969, Feed Situation, November 1971 and certain unpublished data.

## SECTION 5

## THE NUTRITIVE POTENTIAL OF WASTES

### CELLULOSE

Cellulose is a major component of the municipal, industrial and agricultural waste streams both in terms of volume and weight. From 1.4 to 2.0 billion tons of cellulose are discarded each year (53). Traditional methods of disposal of solid wastes, principally landfill and incineration, are not strictly controlled by environmental legislation, and are becoming increasingly expensive. In order to defray cost of treatment and disposal of cellulosic wastes, attention has focused on the reuse of these materials. Products of commercial value which could be obtained from cellulosic wastes include various chemicals, energy, animal feed or human food (see Figure 5).

However the waste is to be commercially exploited, there are problems connected with the collection and seasonality of certain wastes, notably the agricultural field residues which are generated in the largest quantities. Certain agricultural wastes are found in large enough volumes in specific locations to make collection a simple and relatively economic proposition. These wastes include sugar cane bagasse, corn cobs and stalks, milling wastes from wheat, rice and other grains, and prunings from orchards and vineyards. Large volumes of manures concentrated at feedlots are a pollution problem, and because of market limitations on the use of manure as a fertilizer, are negative cost wastes for resource recovery systems. Logging slash and the wastes of the pulping industries are also readily available at certain locations.

An extensive state-by-state inventory of all agricultural wastes was undertaken by Stanford Research Institute. Their findings are the most complete and accurate record of the nature, magnitude, distribution and availability of agricultural wastes across the United States (115).

Municipal solid waste (MSW) is of course generated daily and is collected regularly for disposal. A number of cities are operating or planning energy recovery systems using MSW as a fuel source, either through co-firing, water-wall incineration or pyrolysis (St. Louis, Missouri; Ames, Iowa; Wilmington, Delaware; Seattle, Washington, etc.)

Since cellulose-containing materials are naturally biodegradable by a variety of microorganisms, much of current research into stabilization of organic wastes has focused on biological methods for decomposing cellulose to useful products. Biological attack of cellulose is impeded by impurities in the

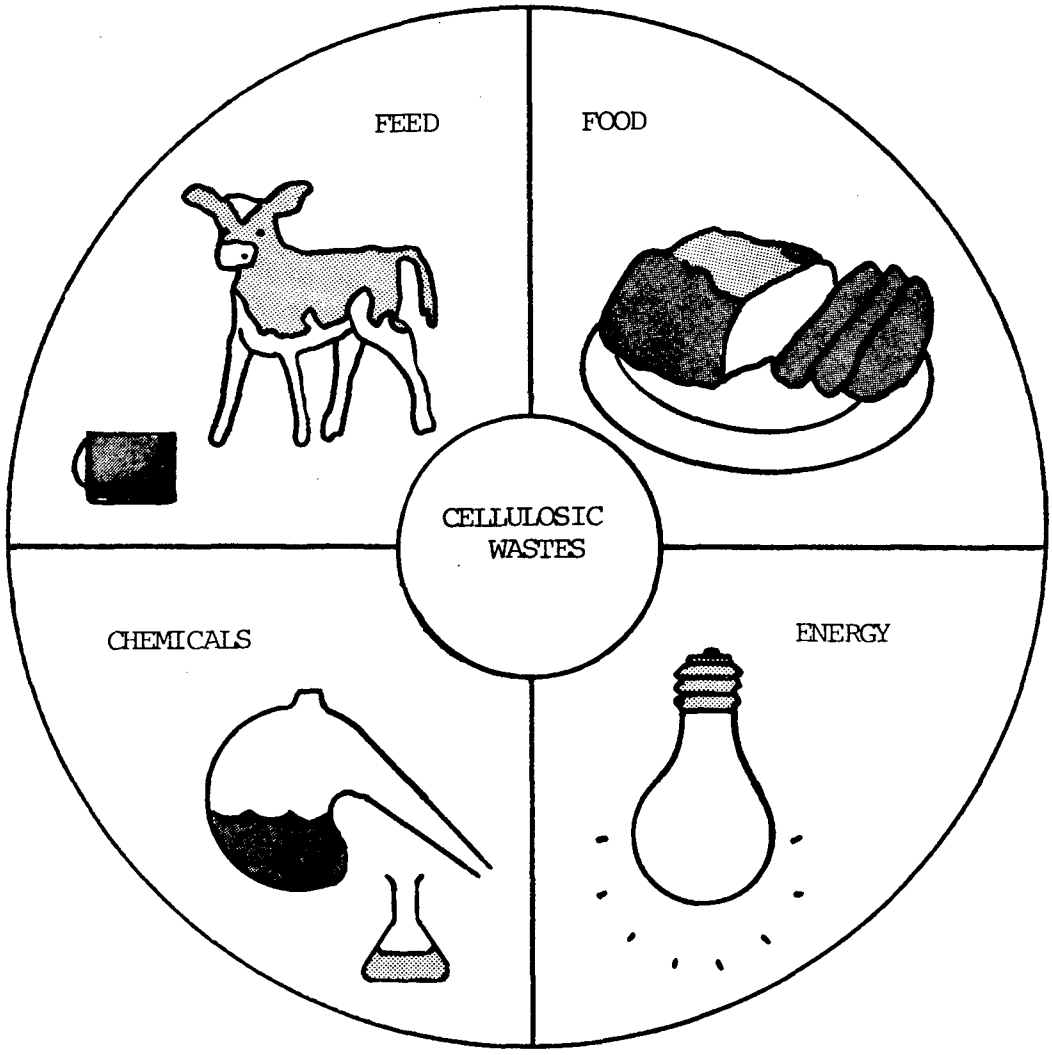


Figure 5. Alternative Products from Salvage of Cellulosic Wastes

wastes, heavy metal contamination, high salt content, herbicides and pesticides (54). Variable composition wastes are also a problem when establishing optimal reaction conditions and concentrations for a commercial process. Table 11 gives the cellulose content of various wastes (55).

## What is Cellulose?

Cellulose is an insoluble linear polymer of anhydroglucose which exists as both an amorphous and a crystalline solid. The composition of cellulose is illustrated in Figure 6. In nature, cellulose is associated with hemicelluloses and lignin. The ease with which the cellulose is attacked by microorganisms is limited by the percentage of lignin in the sample (56).

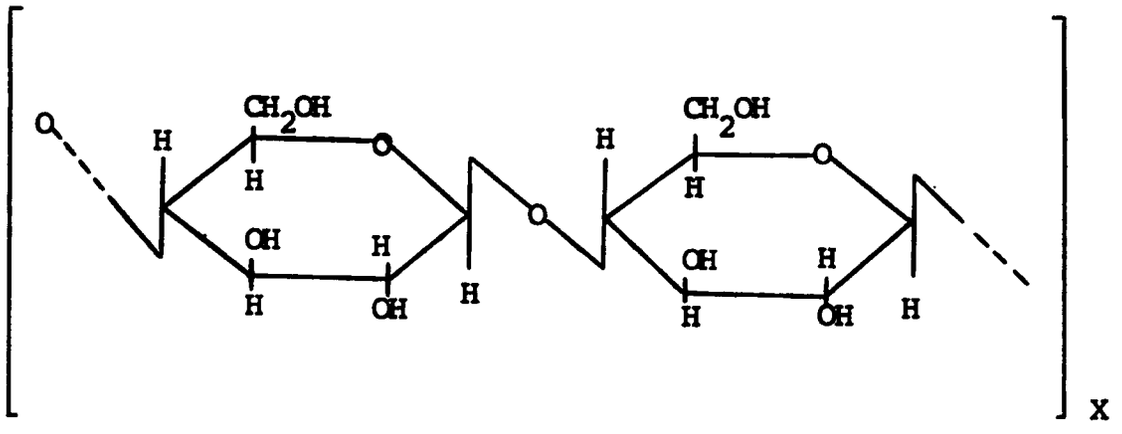


Figure 6. Structural Formula of Cellulose

TABLE 11

CELLULOSE CONTENT OF VARIOUS MATERIALS (55)

| Material | Cellulose, % dry weight |
|---|---|
| Filter paper | 98+ |
| Newsprint | 85 |
| Sugar cane bagasse | 51 |
| Rice straw | 34 |
| Corn cobs | 37 |
| Prairie grass | 37 |
| Cotton linters | 90 |
| Cottonseed hulls | 50 |
| Paper-pulp bagasse | 96+ |

Cellulolytic organisms manufacture enzymes called cellulases which attack either or both cellulose and hemicellulose to form mono- or di- saccharides which are then used as food by the microorganisms (50). The rate of reaction of the cellulases depends on the surface available for attack. A decrease in crystallinity of the cellulose, which increases its surface area, will increase the rate of reaction (56). Lignin is a three dimensional polymer formed by condensation of radicals of cinnamyl alcohol. Various forms of lignin are recognized and while the nature of the lignin-cellulose complex is not fully explained, the lignin appears to form a "cage-like" structure around the cellulose which reduces its availability to cellulase attack (26).

Whether the cellulosic waste is attacked by anaerobic organisms to produce methane and carbon dioxide, or by various aerobic organisms to grow SCP, rates and yields of reaction are limited by the crystallinity of the cellulose, but more especially by the lignin "net". Also, the lignin content of several field residues makes for poor digestibility by ruminants and hence limits the possibilities of directly refeeding certain straws and grasses to cattle (57).

Various chemical and physical methods to strip the lignin or in some way modify the structure of the lignocellulose add significantly to the cost of all biological resource recovery systems from cellulosic wastes.

## Pre-treatment of the Wastes

Table 12 summarizes some of the pre-treatments tested and found acceptable by investigators currently active in nutrient recovery from wastes. Chemical and physical methods have received more attention, but biological methods for pre-treatment of cellulosic wastes to improve biodegradability are also possible. As research is ongoing into various forms of pre-treatment, newer methods may prove more technologically and economically feasible than the methods presented in Table 12. It should also be noted that there will probably never be just one most effective method, but that the most feasible treatment will continue to vary depending on the nature of the wastes.

Another approach to improving the utilization of the cellulose by the microorganisms is to select an organism better adapted for growth on lignocellulose. The cellulolytic thermophilic filamentous actinomycete, *Thermomonospora fusca* has been found capable of significantly degrading substrates with up to 18% lignin (58). An additional possibility is to find an organism which will attack the lignin prior to attack of cellulose by cellulolytic organisms. Crawford and co-workers at the University of Idaho are beginning systematically to screen various white-rot fungi and bacteria (expecially the actinomycetes) for their ability to attack lignin (59). Preliminary to this screening, it was necessary to develop a new and more accurate procedure for determining the degree of lignin biodegradation than the commonly used Klason procedure.

An organism known to be a lignin degrader (*Polyporus versicolor*) grew on $^{14}C$-lignin labeled lignocellulose and $^{14}CO_2$ was evolved as a result of growth. A cellulolytic organism with no lignolytic activity (*Thermomonospora fusca*) grew off the lignocellulose without evolving $^{14}CO_2$. Natural lignocelluloses (twigs) fed $^{14}C$-tagged phenylalanine incorporated the $^{14}C$ only into their lignin components. The percentage of radioactive carbon dioxide evolved was taken as a measure of the lignin-degrading activity of the organism. A minimum value of approximately 2% conversion to $^{14}CO_2$ is considered acceptable as identifying lignolytic activity (63).

There are a number of other problems connected with commercial exploitation of bioconversion processes to stabilize wastes and exploit their nutrient

TABLE 12

VARIOUS METHODS OF PRETREATING CELLULOSIC WASTES SO AS TO IMPROVE BIODEGRADABILITY

| Substrate | Organism | Treatment | Results | Reference |
|---|---|---|---|---|
| Sugar cane bagasse | *Cellulomonas sp.* | alkaline swelling at moderate saturated stream pressure, uv irradiation, severe milling, partial acid hydrolysis | alkaline swelling at moderate saturated steam pressure most effective at lowest cost | Dunlap (54) |
| Feed lot wastes | *Thermoactinomyces sp.* | acid treatment, alkali treatment, oxidation with $HNO_3$m | NaOH, 0.05M @ 23°C for 4 hrs. 74-81% degradation | Bellamy (60) |
| | | enzymes for 15 hrs. @ 80°C | NaOH, 0.05M @ 23°C for 20 hrs. 76-80% | |
| | | | anhy. $NH_3$ @ 10 ats & 23°C 72-80% | |
| | | | enzymes, acid & oxidations relatively ineffective | |
| Newspaper (Boston Globe) | *Trichoderma viride* | ball milling, pot milling, cuprammonium, alkali, viscose, soak in water, boil in water, shredding, hydropulping | ball milling very effective, pot milling acceptable too, preliminary hydropulping would cut costs | Spano (61) |

TABLE 12 (continued)

| Substrate | Organism | Treatment | Results | Reference |
|---|---|---|---|---|
| Various pure cellulose substrates | *Trichoderma viride* | pot and ball milling | ball milling effective in improving % of saccharification | Spano (61) |
| Agricultural wastes:<br>Rice hulls<br>Bagasse<br>Manures, etc. | ---- | ---- | ---- | ---- |
| Paper:<br>Computer printouts<br>Milk cartons<br>Corrugated fibreboard<br>etc. | ---- | ---- | ---- | ---- |
| MSW:<br>Black Clawson<br>Bureau of Mines | ---- | ---- | ---- | ---- |
| Ground refuse, wood pulp, water-pulped refuse | various mycelial fungi | high temperature hydrolysis, electron irradiation, nitrate photochemical treatment, alkali oxidation | nitrite photochemical treatment superior to others tested<br><br>alkali oxidation also improved rate of degradation<br><br>others not effective | Rogers (53) |

TABLE 12 (continued)

| Substrate | Organism | Treatment | Results | Reference |
|---|---|---|---|---|
| Waste paper (Newsprint without ink, newspaper) | *Myrothecium verrucaria* | ball milling | % cellulose utilized varied from 34% to 81% depending on paper, reaction conditions | Updegraff (62) |
| Pulped wood (3, 8 & 18% lignin) | *Thermomonospora fusca* | dried and ground (40 mesh) | total cellulose loss after 5 days: 3% lignin - 92% 8% lignin - 74% 18% lignin - 44% | Crawford (58) |

value. The handling of an insoluble solid substrate presents engineering difficulties, but the technology exists to overcome these problems. However, more experience of large scale fermentation is required.

## Alternative Food/Feed Production Systems

As indicated in Figure 7, there are a number of alternative processes for food/feed production from cellulosic wastes. The aerobic fermentation of wastes to produce SCP is but one possibility. Another feasible system is anaerobic fermentation of the wastes to produce methane and a sludge with potential value as an animal feed. Certain agricultural wastes could be fed to ruminants without further treatment (manures) or with treatment to improve digestibility (grasses, straws, and manures). A brief overview of each possibility follows.

## Aerobic Fermentation of Wastes to SCP

Although the commercial growth of SCP on cellulosic wastes is not as advanced as SCP growth on other substrates, partly for historical reasons, and partly because of the special limitations mentioned above, there is much active research ongoing in the field.

The growth of a symbiotic culture of the mesophilic bacteria, *Cellulomonas sp.* (ATCC #21399) and *Alcaligenes faecalis* (ATCC #21400), on sugar cane bagasses has been studied at Louisiana State University. The process is applicable to a variety of wastes.

The bagasse was treated with alkali at moderate saturated steam pressure to increase digestibility. The solids which survived pre-treatment were washed to remove excess alkali and the lignin and hemi-celluloses which had been solubilized. The washing improved color and texture of the final product as well as improving reaction kinetics.

The fermentation took place under aseptic conditions at 34° at a pH controlled by addition of either anhydrous ammonia or caustic soda to 6.6 - 6.8. Yeast extract or lysed yeast cells were added with an inorganic salts solution to provide nutrients. Cell yields were in the range of 44% to 50%. Fermenters of 7.14 and 50 litres were operated on a batch on continuous basis. Different sterilization techniques were employed depending on the scale and mode of fermentation.

For a ruminant animal feed, a polyelectrolytic flocculent was added to the culture media which was then either centrifuged or filtered. For a human food, the broth was screen-filtered and centrifugally desludged to produce a clear cell liquor. After addition of a polyelectrolytic flocculent and centrifuging of the floc, the cells were drum or spray dried to a cream to yellow colored product (54), (55).

Growth of *Cellulomonas sp.* and *Alcaligenes faecalis* on rice straw is under

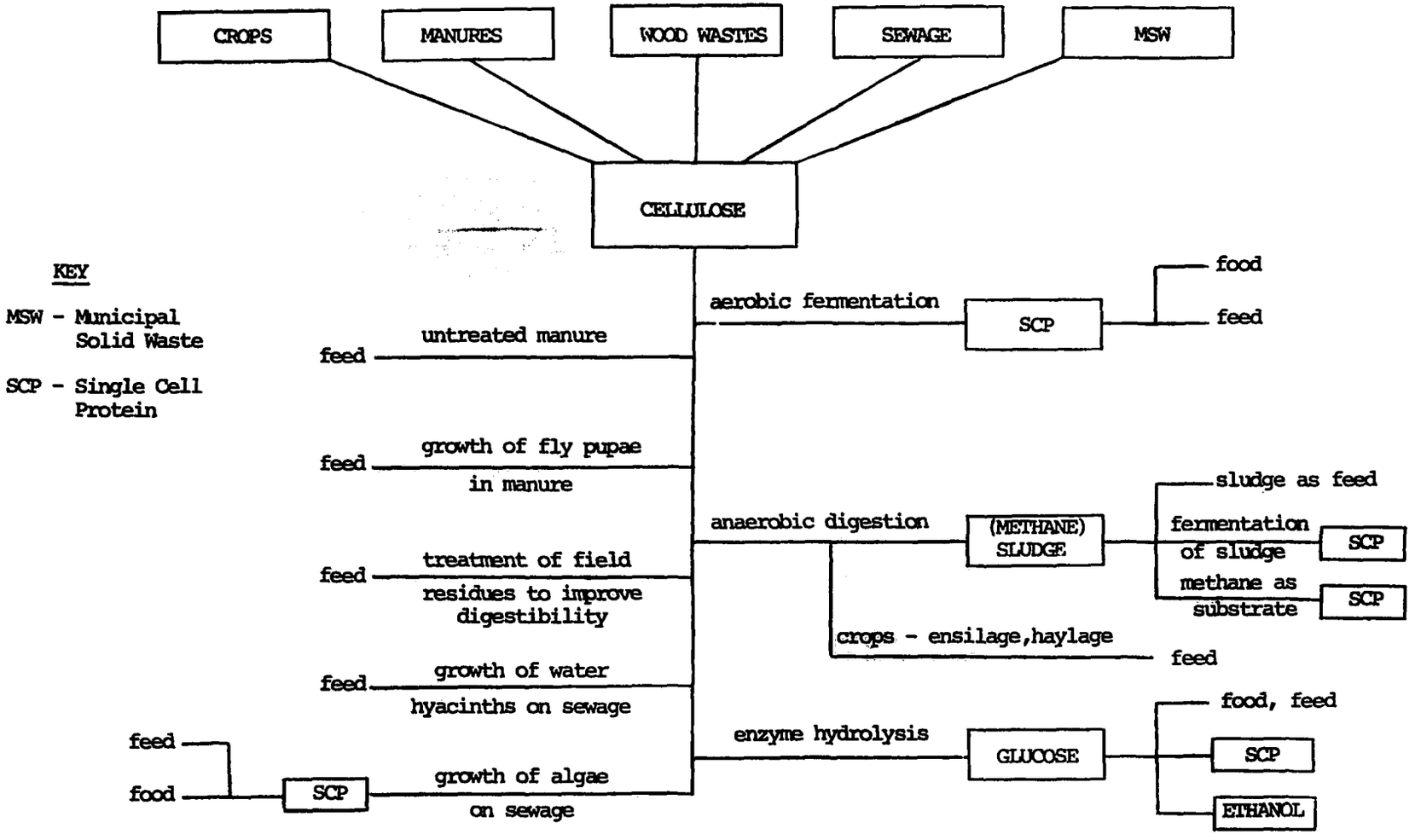


Figure 7. Possible Food/Feed Recovery Systems from Cellulosic Wastes

study by the Western Regional Research Laboratory, U.S. Department of Agriculture. As the burning of rice straw is now limited by pollution legislation, efforts are being made to find an economic use for the millions of tons of rice straw generated each year (an estimated 7.6 million metric tons in 1970 in the United States (57)).

As generated, rice straw is a low-quality feed, poorly digested by ruminants with only 4.5% crude protein (57). Both the lignin and the high silica content of rice straw (up to 15% dry weight) limit the digestibility of rice straw (30% digestibility, c.f. alfalfa 50%).

At the Western Regional Research Laboratory, after pre-treatment with either soda or aqueous ammonia, the rice straw was fermented with both mixed and individual cultures of *Cellulomonas sp.* and *Alcaligenes faecalis*. The mixed cultures utilized 75% of the substrate and produced 18.6% of total substrate weight as microbial protein. The cells were 38% crude protein and the residue contained 12% protein (64). In vitro rumen digestibility (IVRD) of the cellulose residue increased to 52%, while the digestibility of the cell was 41.2% to 55%. Both the cells and the residue had a high ash content, 25% and 21% respectively. Data indicated that the residues could be used as a high protein feed for ruminants, and the microbial cell fraction could be fed to nonruminants.

The Western Regional Research Laboratory has also investigated the semisolid fermentation of *Candida utilis*, *Aureobasidium pullulans* and *Trichoderma viride* on ryegrass straw (65). The straw was pre-treated with 0.5N sulphuric acid at various temperatures to hydrolyze the cellulose and hemi-cellulose to sugars. The acid hydrolyzates were neutralized with ammonia prior to fermentation. The greatest increase in crude protein content was obtained from growth of *A.pullulans* preheated at 121° C (from 3.1% protein untreated to 13.9%). The greatest increase in digestibility was demonstrated by *C. utilis* preheated at 121° C (46.7% ± 4.7, c.f. untreated unfermented ryegrass straw at 32.7% ± 5.1).

A number of advantages of semi-solid fermentation are cited by Han and Anderson (65):

(a) Unlike submerged culture, there is no need to harvest the cells. The entire residue can be fed to ruminants.

(b) No heating or cooling of the revolving fermenter is needed.

(c) It is not necessary to maintain strictly sterile conditions.

(d) Complicated controls and equipment are not required.

(e) As the entire residue is fed, the process does not generate quantities of polluting effluents.

The General Electric Company has studied growth of a series of thermophilic actinomycetes on feedlot wastes. Several hundred species of microorganism collected from a number of sources were screened for growth on cellulose and lignocellulose. The organisms chosen grew optimally at 55° C and pH 7.5 - 7.8. The Casa Grande demonstration plant was built recognizing the need to investigate high solids fermentation on a larger scale than the bench-top fermenter. Feeding trials were stopped because of problems including culture stability and variability. Experience with large-scale high solids or "solid-state" fermentation is still very limited.

At the University of Pennsylvania, Humphrey and co-workers have investigated growth of a *Thermoactinomyces sp.* obtained from General Electric. The initial substrates chosen were feedlot wastes, cryogenically ground Oregon grass, seed hay and bagasse. The high solids concentration of the wastes caused problems with mixing, sampling and line plugging.

Because of problems in obtaining uniform feedlot samples for laboratory study and because of the variability of data obtained from the wastes, it was decided to concentrate on growth of *Thermoactinomyces* on pure cellulose. Though the substrate under study (AVICEL/FMC PH102) is highly crystalline, rates of degradation as high as 5-10 g/litre-hour were recorded (66). Both batch and continuous runs were studied. Few of the runs produced total cell yields higher than 25% with complete cellulose digestion. Economic analysis indicated 35% yields as the break even point.

Both oxygen transfer and glucose concentration limited growth of the cells. Preliminary results have indicated that yields of 0.45 g. cell/g. cellulose utilized and cell growth rates of 0.45 hr-1 are possible. The key problem was identified as optimizing cell productivity and yield as well as achieving high cellulose digestion.

The Northern Regional Research Laboratory, U.S. Department of Agriculture, has investigated growth of *Trichoderma viride* on feedlot wastes. They found that not only did the fungus produce high yields of cellulases but that after rapid growth of the fungus for four days, the unpleasant odor of the wastes was replaced by an earthy odor (67). After seven days, the cellulase activity of the fungus approached its highest value and the wastes were stabilized. It was found that 66% of the carbohydrates were utilized.

The fermented waste retained its nitrogen and improved its amino acid profile, increasing the percentage of lysine and retaining the sulfur-containing amino acids. Crude protein content of the fermented solids increased from 18.8% in the undigested manure to 22.6% (67). The highest cellulase activity coincided with a final pH of 5 and an initial substrate concentration of 2.5%. Tween 80 (polyoxyethylene sorbitan monooleate) and oleic acid (0.1%) were added to stimulate cellulase production. The stabilized enriched wastes are being considered for refeeding as a protein supplement.

The Solid and Hazardous Waste Research Laboratory (SHWRL), Environmental Protection Agency, has studied growth of fungi on waste cellulosics, including ground or water pulped municipal refuse. A summary of pre-treatments investigated by SHWRL is found in Table 12.

The Laboratory has also looked at ways to improve the quantity of protein synthesized by the fungus, by adding various agents to the cultures which might stimulate the metabolism of nitrogenous compounds. A number of auxins and herbicides were added to fungal cultures, individually or with zinc sulfate. The effect of zinc sulfate alone was also evaluated. After three days incubation, each flask was analyzed (Kjeldahl nitrogen) for increased protein synthesis. The results were statistically compared using Dunnet's t-statistic test (68).

The results indicated that for the fungi evaluated (*Trichoderma viride, Aspergillus fumigatus, Aspergillus awamori*), grown on glucose or whey as a substrate, zinc sulfate in combination with growth regulators increased both mycelial mass and fungal protein content over both the control and growth stimulators alone. The improvement was more noticeable for fungi grown on the glucose than on the whey (68).

As the economics for production of SCP from cellulosic wastes are marginal, any improvement in protein quality and quantity would increase the economic feasibility of the process.

Updegraff at the Denver Research Institute has studied growth of *Myrothecium verrucaria* on ball-milled newspaper. The maximum rate of cell growth recorded was 0.3 grams/litre/day (g/l/day) and the maximum protein yield was 1.42 g/l (62). Urea (0.3 g/l) and yeast autolysate (1.0 g/l) stimulated growth rate and protein production. It does not appear that this process is economically feasible because of the low yields and growth rates.

Pulp and paper industry sludge presents a definite disposal problem. The sludge is difficult to dewater as the pulp fibers retain water to form a gel-like structure. Incineration of the sludge cake and landfill are both increasingly expensive and are associated with various pollution problems. Growth of *Thermomonospora fusca* on pulp and paper mill sludge could be a process which would not only stabilize the wastes, but also defray the costs of disposal by production of a marketable product.

(For an approximate protein content of selected organisms grown on waste materials, see Table 13).

The University of Wisconsin has investigated growth of *T. fusca* on a number of sludges. Growth of the bacteria on aspen sulfite fines for six days gave a 30 - 40% yield of a product containing 30 - 35% protein. The amino acid profile is good (see Table 14), and preliminary feeding trials with test and control groups of chicks indicated that replacement of 25% of their feed with *T. fusca* was well accepted. In three weeks, the average weight gain for the test group was 123g and for the control, 125g (70). Growth of bacteria reduced the $BOD_4$ by 90%. Effluents from the process would require some treatment prior to disposal, perhaps in aereated lagoons (59).

The aim of the Swedish Forest Products Research Laboratory is to develop a process based on solid lignocellulose waste in which hydrolysis and protein production are carried out simultaneously. They are investigating conver-

TABLE 13

APPROXIMATE PROTEIN CONTENT OF SELECTED ORGANISMS GROWN ON WASTE MATERIALS

| Organism | Substrate | % Protein | Source |
|---|---|---|---|
| Fungi | | | |
| *Myrothecium verrucaria* | newsprint | 30 | Updegraff (62) |
| *Aspergillus fumigatus* | glucose | 31 | Rogers *et al* (53) |
| *Aspergillus niger* | ag. carbohydrates | 25-35 | Imrie (51) |
| *Fusarium sp.* | ag. carbohydrates | 41-51 | " |
| *Trichoderma viride* | glucose | 34 | Rogers (53) |
| | rye-grass straw | 10.9 | Han (65) |
| | glucose | - | Mandels (56) |
| *Paecilomyces varioti* | sulfite waste liquor | 55-60 | Forss (69) |
| Bacteria | | | |
| *Cellulomonas sp.* | bagasse | 50-55 | Dunlap/Callihan (55) |
| | rice straw | 38 | Han (64) |
| *Actinomyces sp.* | feedlot waste | 50 | Bellamy (60) |
| *Thermomonospora fusca* | woodpulp fines | 30-35 | Harkin *et al* (70) |
| | (carboxymethyl-cellulose) | 54-59 | " |
| | (cellobiose) | 59 | " |
| Yeast | | | |
| *Candida utilis* | sulfite waste liquor | 50 | Wiley (10) |
| | rye grass straw | 12.4 | Han (64) |
| | acid food wastes | 45-46 | Hang (71) |
| *Candida steatolytica* | brewery wastes | 46 | |
| *Geotrichum candidum* | acid food wastes | 39.3 | Hang (72) |
| *Saccharomyces fragilis* | cheese whey | 54-56 | Knudsen Wheast |
| Algae | | | |
| *Spirulina maxima* | secondary waste water | 50 | |
| *Euglena gracilis* | sewage or swine manure digest | 60-70 | Waygood (73) |

TABLE 14

AMINO ACID PROFILE OF SELECTED ORGANISMS GROWN ON CELLULOSIC WASTES

| | Organisms | | | | | | |
|---|---|---|---|---|---|---|---|
| Amino acid | 1 | 2 | 3 | 4 | 5 | 6 | FAO reference |
| isoleucine | 4.74 | 7.76 | 4.2 | 3.20 | 7.30 | 4.00 | 4.2 |
| leucine | 11.20 | 9.15 | 7.3 | 6.10 | 8.80 | 5.10 | 4.8 |
| lysine | 6.84 | 3.29 | 12.3 | 3.63 | 4.40 | 4.40 | 4.2 |
| methionine | 1.86 | 0.62 | 1.5 | 2.06 | 7.30 | 2.00 | 2.2 |
| phenylalanine | 4.36 | 5.45 | 5.15 | 2.64 | 6.10 | 5.50 | 2.8 |
| threonine | 5.37 | 4.88 | 2.1 | 4.00 | 7.00 | 4.40 | 2.8 |
| tryptophan | | 6.38 | 16.2 | | | | |
| valine | 10.71 | 4.16 | 3.4 | 12.97 | 2.60 | 4.80 | 4.2 |
| histidine | 2.30 | 1.13 | 4.3 | 1.96 | 2.20 | 1.60 | |
| tyrosine | 2.67 | 3.55 | 4.1 | 1.87 | 3.90 | 3.70 | 2.8 |
| alanine | | 7.71 | 3.6 | 13.92 | 5.90 | 5.10 | |
| arginine | 9.21 | 3.19 | 3.0 | 5.62 | 3.70 | 4.00 | |
| aspartic acid | | 9.51 | 4.9 | 6.74 | 8.80 | 7.70 | |
| cystine (1/2) | | 2.06 | | 0.41 | | | |
| glutamic acid | | 15.89 | 6.8 | 18.03 | 11.00 | 9.90 | |
| glycine | | 4.47 | 2.4 | 4.42 | 5.30 | 5.90 | |
| proline | | 1.68 | 2.2 | 6.10 | 2.90 | 3.70 | |
| serine | | 4.27 | 1.8 | 2.57 | 5.50 | 2.60 | |

Key:

| organism | substrate | reference |
|---|---|---|
| 1 - *Cellulomonas sp.* (ATCC #21399) | - bagasse | - (74) |
| 2 - *Thermoactinomyces* # 26 | - feedlot waste | - (26) |
| 3 - *Thermoactinomyces* # 24 | - feedlot waste | - (26) |
| 4 - *Thermomonospora fusca* | - fines | - (70) |
| 5 - *Aspergillus fumigatus* # 3 | - waste paper | - (53) |
| 6 - *Trichoderma viride* # 9 | - waste paper | - (53) |

sion of waste fibers from a sulfite mill to protein using the white rot fungi, *Sporotrichum pulverulentum*. The cultivation starts on glucose and continues on cellulose. Unlike Wilke *et al* in California (q.v.), the Swedish group found that the change in substrate did not result in a long induction period (1.5 hours) (75).

The mycelium contains only 25 - 30% protein when grown batch-wise on waste fibers. It grows in pellets (0.2-0.4mm.) which are easy to filter. In order to improve the protein content, the Laboratory has investigated a symbiotic process with C. *utilis*. It is felt that symbiotic cultures on water-soluble oligomeric materials are more feasible while utilizing immobilized enzymes.

Erikson and co-workers have also mutated white rot fungi into cellulase-free mutants. These fungi produce enzymes which attack lignin in wood chips to permit defibration of the wood with less energy expenditure (76).

## Enzymatic Hydrolysis of Cellulosic Wastes

Researchers at Natick have developed a mutant strain of the fungus, *Trichoderma viride*, which can manufacture enzymes with both $C_1$ and $C_x$ activity, i.e., the enzymes manufactured by the fungus will attack both the crystalline ($C_1$) and the amorphous ($C_x$) structure of cellulose. The cellulases (enzymes) produced by the fungus convert the cellulose to the water-soluble disaccharide, cellobiose, which is then converted to glucose. If the organisms are left in the broth, then they would consume the sugar. To produce glucose, the following procedures are employed (61):

(a) The fungus is grown on a starving diet of cellulosic wastes (0.75%) at 25 - 28° C and a pH carefully controlled to prevent hyperacidity;

(b) When the cellulases are in solution, the broth is filtered and the filtrate enters the fermenter;

(c) At 50° C and atmospheric pressure, the enzymes break down a cellulose rich slurry into sugar at pH 4.8;

(d) After 24 hours, the syrup is harvested and some of the slurry is recycled.

The glucose solution obtained could either be used for a human food or an animal feed, or alternatively as a substrate for yeast growth to produce ethanol or SCP.

At Natick, they have grown *T. viride* on pure cellulose substrates, agricultural wastes (rice hulls, bagasse, manure), various paper wastes (including newspapers, computer printouts, milk cartons, etc.) and municipal trash fractions (Black Clawson and Bureau of Mines). The rate and extent of the hydrolysis are dependent on the nature and pre-treatment of the substrate

as well as the quality of the enzyme. Milled cotton was 6% hydrolyzed compared to over 90% saccharification for milled pulp, SWECO 270 (61).

The pre-pilot plant operating at Natick is interfaced with a computer for rapid data analysis and process estimation. Capacity will increase steadily from 1,000 lbs/month to 4,000 lbs/month.

Continuous cellulase production is under study at the University of California at Berkeley. In the first stage of a multi-stage operation, *Trichoderma viride* (QM9414) utilized glucose for growth and in the second stage grew on pure spruce wood cellulose (77). Determinations were made of the specific oxygen demand ($QO_2$) of exponentially grown fungus; the maximum specific growth rate ($\mu$ max) and the saturation constant ($k_s$). The maximum specific growth rate = 0.294/hr. and the saturation constant = 0.083 mg/ml. There was a substantial time lag (approximately 30 hours) during which the fungus adapted from growth on glucose to cellulose. Average batch productivity assuming 48 hours down time was 13.80 X $10^{-3}$ filter paper units (FPU/ml. X hr, (c.f. calculated from Mandels and Weber, 5.39 X $10^{-3}$ FPU/ml. X hr.).

Wilke has produced a complete economic analysis of the capital and operating costs of a commercial process to hydrolyze domestic wastes to glucose with *Trichoderma viride* (78).

## Acid Hydrolysis

The bench scale production of sugar by acid hydrolysis of MSW was investigated by the Thayer School of Engineering. The process was carried out continuously to produce about 52% - 54% yield of glucose. The reaction took place isothermally at 230° C using 1% weight of sulfuric acid and with a reaction time of 20 seconds. The yield was found to be a function of time of reaction, temperature, concentration of the acid and solid-to-liquid ratio in the slurry. As the hydrolysis conditions also favor the decomposition of glucose, higher yields cannot be expected (79).

## Anaerobic Processes

Digestion of cellulosic wastes in the absence of air produces the gases methane and carbon dioxide, and leaves a biodegraded sludge. The emphasis of research and development activities relating to anaerobic digestion of manures has been on maximization of methane production. However, many investigators have mentioned the possible utilization of the sludge as either a fertilizer or a feed. The sludge retains its nitrogen and is superior to undigested manures in that it is odorless and biologically stable. Also, the sludge is probably pathogen free (80).

The marketing of the sludge as a feed (or fertilizer) would significantly improve the economics of the process. Jewell *et al* at Cornell University have determined the number of animals required to make methane generation from cow manure economically feasible for a New York dairy farm (80). They found that if the sludge could be commercially exploited for its nitrogen

content, that the number of animals required to support methane generation was surprisingly small (see Table 15). Their estimates were based on selling the sludge as a fertilizer, but presumably utilization of the sludge as a feed would show similar results.

Hamilton Standard Division, United Aircraft, for the Northern Regional Research Center, USDA, has completed the first phase of a study of anaerobic digestion of cattle wastes. This study included both economic feasibility and preliminary trials of the feed quality of the sludge (81). The 20 litre fermenters were maintained in continuous stable operation at thermophilic temperatures, with loading rates as high as 16.0 g volatile solids/litre/day.

Analysis of the effluents indicated that the crude protein content had risen to 25% or double the protein of the fed material. At the same time, there was a mass reduction of 50%. A four-fold increase in the amino acid concentration (to 20%) indicated that non-protein nitrogen was converted to protein. The effluents were non-toxic to baby chicks. An inhibition in feed efficiency was attributed to high levels of ammonia in the sludge. As ruminants can assimilate large amounts of ammonia, this inhibition is not expected to occur when feeding to cattle.

TABLE 15

NUMBER OF ANIMALS REQUIRED TO ECONOMICALLY SUPPORT METHANE GENERATION TREATMENT OF ANIMAL WASTES (80)

| Animal | Number of Animals | |
|---|---|---|
| | Energy Only | Energy & Nitrogen Value |
| Beef | 570 | 155 |
| Dairy | 380 | 80 |
| Poultry | 57,000 | 5,200 |
| Swine | 2,800 | 585 |

The second phase of the project involves the building of a more sophisticated digester in Nebraska. Extensive feeding trials with cattle hopefully will clarify the feasibility of utilizing the sludge of digested manures as a ruminant feed. The new digesters will be batch fed; one tenth of the volume of the fermenter will be withdrawn each day after the reaction has reached the steady state (82). The solid/liquid residue will be centrifuged and dried. In extensive feeding trials, the feed-cake will replace all but the corn and wheat portion of the animals' present feed (83).

SHWRD is funding a study at the University of Wisconsin which will assess anaerobic digestion and other bioconversion processes applicable on the farm in terms of technical and economic feasibility as well as energy consumption (84). The study will examine possible uses of the effluent of digestion:

(a) as a refeed;

(b) as a substrate for algal growth;

(c) in aquaculture;

(d) as a fertilizer.

Methodical systems analysis applied to the bioconversion processes will do much to clarify the options available for waste utilization on the farm.

Commercial methane production from Montford Feedlot waste is slated to be in the range of 120 million cubic feet of gas/month. Seventy million cubic feet of gas will be sold to Colorado Interstate Gas and fed directly into interstate pipelines (85). The sludge will be evaluated as a feed and a fertilizer.

Because anaerobic digestion of manures produces both energy (as methane) and a potential feed (the sludge), it looks potentially a very attractive waste management technique. Ongoing research into the nutritive potential of the effluents should give a clearer indication of the feasibility of this process.

Anaerobic fermentation of the soluble portion of manures (as opposed to the insoluble cellulose) is also under study. A number of companies are investigating fermentation of manures in a silo or covered ditch. Digestion of the solubles by streptococci and lactobacilli leaves the fibers untouched so that the wastes require further treatment to degrade the cellulose. The Ceres Ecology Corporation of Chino, California, will ferment the manure from over 100,000 dairy cattle to produce a refeed, and to minimize salt pollution of ground water. It is reported that a 10 - 20% savings in feed costs can be attained by replacing 8 - 10% of the protein in the regular feed with the ensiled manure (24).

## Direct Feeding of Wastes and Related Methods to Improve the Digestibility of Cellulosic Wastes

### Manures

A measure of the crude protein potential of animal wastes can be found in Table 16 which gives estimates of the amount of nitrogen in livestock excreta. This nitrogen comes from undigested feed, by-products of digestion, microbial cells and sloughed tissue (86).

The simplest way to recover the nitrogen is to refeed excreta to either the same or a different species. Scavenging of manures occurs in nature, and cattle are known to lick their own excreta with no apparent ill-effects. On the farm, there are a number of problems associated with refeeding manures, especially the possibilities of accumulation of feed additives, pesticides, toxins and/or pathogens in animal tissues.

Fontanet *et al* (87) reported a copper toxicity in ewes fed poultry litter and Griel *et al* (88) reported a high rate of abortion in heifers fed poultry litter. Nevertheless, poultry litter has been successfully fed to gestating/lactating ewes, fattening steers, beef heifers and calves, and dairy cows (89) as well as laying hens and broilers (50). Feeding trials with ruminants indicate that the litter is slightly less digestible than soybean meal (50).

Prior to feeding, the wastes must be dewatered (thermal or air-drying) and sterilized. Heat treatment at 150° C for three hours effectively destroys pathogens in poultry litter (89). Though heat processing reduces crude protein content of the litter, acidification with sulfuric acid to pH 6 lowers nitrogen loss.

Refeeding dehydrated poultry manure to laying hens is an incomplete waste management system. The hens can tolerate up to 25% of manure in their diets before egg production is affected. Therefore, about 75% of the manure must still be disposed of (90).

In the past, the U.S. Department of Agriculture has discouraged feeding of poultry litter to other species of livestock for health reasons. More recently, the Department has encouraged and sponsored research into refeeding of all types of manures.

Cattle manure can be dehydrated and fed to ruminants (86). Alternatively, the solid and liquid components of cattle wastes can be physically separated and the washed fiber fed to cattle (91). A series of feeding trials has indicated that the fibrous component of manure could successfully replace a portion of the basal diet with no ill-effects. No advantage was demonstrated by heat treating and washing the manure prior to feeding. As washing resulted in production of polluting waste-water, this treatment was kept to a minimum (91).

Both poultry and cattle excreta may be ensiled to produce acceptable feeds.

TABLE 16

ESTIMATED EXCRETA N FROM U.S. LIVESTOCK IN 1972 --
BASED UPON LIVESTOCK PRODUCTS MARKETED,
THEIR PROTEIN CONTENT, AND FEED-TO-PRODUCT
PROTEIN CONVERSION EFFICIENCY (86)

| Product | Amount * | Product Protein | Conversion efficiency † | Excreta protein factor ‡ | Excreta N § |
|---|---|---|---|---|---|
| | lbs x $10^3$ | % | % | kg x $10^3$ | |
| Milk | 120,278,000 | 3.5 | 30 | 2.3 | 704,173 |
| Chicken | 1,118,723 | 21 | 25 | 3 | 51,259 |
| Broiler | 11,477,931 | 21 | 25 | 3 | 525,898 |
| Turkey | 2,424,145 | 21 | 20 | 4 | 148,093 |
| Eggs # | 8,725,500 | 10 | 20 | 4 | 253,833 |
| Sheep and lamb | 1,081,254 | 16 | 10 | 9 | 113,237 |
| Hogs | 20,258,557 | 15 | 20 | 4 | 884,010 |
| Cattle | 37,185,295 | 15 | 15 | 5.6 | 2,271,683 |
| Calves | 767,496 | 15 | 15 | 5.6 | 46,887 |
| Total | | | | | 4,999,073 |

* Production and slaughter estimates from U.S. Department of Agriculture, Agricultural Statistics, 1973, Tables 536, 582, 595, 602, 485, 467, and 453.

† Source: Philips, R.W., 1967

‡ Excreta protein factor = $\frac{\text{kg of excreta protein}}{\text{kg of product protein}} = \left(\frac{1}{\text{conversion efficiency}} - 1\right)$

§ Excreta (kg) = lb of product x % product protein x excreta protein factor x 2.2 kg/lb. x 0.16 kg N/kg protein

\# Eight eggs assumed to weigh 1 lb.

The University of Auburn has investigated a number of ensiled mixtures containing cattle manure and hay and/or corn. An ensiled mixture of cattle manure (57%) and ground hay (43%) was fed to breeding ewes and beef-breeding cattle. During the former study, lasting 389 days, "wastelage" fed ewes were noticeably more alert than the control fed hay. The breeding cows reproduced and lactated similarly on either a "wastelage" or corn silage diet. Animals on corn silage gained more weight than the test group. No harmful effects were noted in either feeding trial. Chemical studies have indicated parasitic nematodes were destroyed in the ensiling process.

An ensiled mixture of corn (48%), bermuda grass hay (12%) and manure (40%) was fed to yearling cattle for 152 days prior to slaughter. The cattle were of the same live weight as a control group fed a conventional feed. Feed costs for the test were 8% less than the control. The meat was of the same high quality and reportedly tasted delicious. Anthony of Auburn University stresses that the manure was collected from "healthy cattle fed approved feed mixtures" (91).

Composting cattle manures to feed to ruminants has received little attention to date. Experience with refeeding swine manures to swine is also limited, though nutrient recovery from swine oxidation ditches has been investigated (92).

## Crop Wastes

As previously mentioned, rice straw is a poor quality feed for ruminants because of its low digestibility and protein content, poor palatability and bulk. Both the lignin and silica content of the straw limit its acceptability.

One method of improving the digestibility of rice straw is to heat the straw with an alkali. Mild sodium hydroxide treatment has proved most effective. Holding rice straw at 100° C for 15 minutes increased digestibility from 30% to 73% (57). It is also possible to acid or enzyme hydrolyse the straw to sugars which are then used as a substrate for yeast growth. Ensilage of rice straw with various additives to supply nitrogen also improves the quality of the feed.

Caustic soda treatment improves the in vitro rumen digestibility of ryegrass straw. Han, Lee and Anderson have demonstrated that if the hemi-cellulose is first removed from the straw that the caustic soda treatment is ineffective (93). Dioxane treatment was found to improve the microbial digestibility of the hemi-cellulose free fibers (acid detergent fibers).

## Wood and Wood Based Residues

Wood and wood based residues have been evaluated as a source of energy for livestock and as a roughage extender for ruminants. Because of the low digestibility of woods by ruminants (see Table 17) untreated wood wastes are

TABLE 17

*IN VITRO* DRY-MATTER DIGESTIBILITY
OF VARIOUS WOODS AND THEIR BARKS (94) †

| Substrate | Digestibility* Wood | Bark | Substrate | Digestibility* Wood | Bark |
|---|---|---|---|---|---|
| | % | % | | % | % |
| Hardwoods | | | Hardwoods | | |
| Red alder | 2 | - | Soft maple small | | |
| Trembling aspen | 33 | 50 | Twigs | 37 | - |
| Trembling aspen | | | Sugar maple | 7 | 14 |
| (groundwood fiber) | 37 | - | Red oak | 3 | - |
| Bigtooth aspen | 31 | - | White oak | 4 | - |
| Black ash | 17 | 45 | | | |
| American basswood | 5 | 25 | Softwoods | | |
| Yellow birch | 6 | 16 | Douglas-fir | 5 | - |
| White birch | 8 | - | Western hemlock | 0 | - |
| Eastern cottonwood | 4 | - | Western larch | 3 | 7 |
| American elm | 8 | 27 | Lodgepole pine | 0 | - |
| Sweetgum | 2 | - | Ponderosa pine | 4 | - |
| Shagbark hickory | 5 | - | Slash pine | 0 | - |
| Soft maple | 20 | - | Redwood | 3 | - |
| Soft maple buds | 36 | - | Sitka spruce | 1 | - |
| | | | White spruce | 0 | - |

* For comparison: Digestibility of cotton linters 90%; of alfalfa, 61%.

† Reprinted from Baker, A.J. et al. In: Cellulose Technology Research, ACS Symposium Series, Vol. 10, p.78, 1975.

not useful as an energy source. Wood and paper mill sludges could be used as a roughage extender, as a partial replacement for hay in the ruminant diet (94).

Many methods of improving the digestibility of both woods and pulp and paper mill sludges have been investigated. These methods include:

electron irradiation;
vibratory ball-milling;
gaseous and liquid ammonia;
gaseous sulfur dioxide;
dilute sodium hydroxide;
white rot fungi.

Baker *et al* (94) have measured the effects of the above pre-treatments on a number of woods and wood based wastes. In vitro rumen and cellulase digestion assays were followed by digestibility and palatability studies on goats.

Sulfur dioxide treatment improved digestibility without removing the lignin. Hardwood sawdust was maintained for two hours in an atmosphere of sulfur dioxide at 3 lb/sq. ins. and a temperature of 120° C. Softwood sawdust reacted for three hours. After removal of the gas, the woods were neutralized with caustic soda and then air-dried. The results of the treatment are summarized in Table 18. The treated woods were well accepted by ruminants in extensive feeding trials.

Delignification of pulp and papermill residues and wood pulp improved their in vitro digestibility. The degree of improvement depended on the extent of removal of the lignin. IVRD of the residues was from 45 - 60% In vivo digestibilities were of the same order of magnitude. Hardwoods showed a greater improvement in digestibility on removal of lignin than did the coniferous species. Some residues were unacceptable as a feed because of a high ash content and heavy metal contamination, but as indicated below, the parenchyma cell fines were well accepted by the test animals.

Up to 50 - 75% of parenchyma cell fines were successfully included in the feed of ewes for one year. During gestation and lactation, additional grain was fed to the pregnant animals. Ewes fed a diet containing aspen bark also maintained satisfactory growth. Beef cows fed a ration of hay, parenchyma fines, grain and mineral supplement for seven months maintained growth. Both ewes and cows in the test group consumed more rations than the controls fed hay.

## Municipal Solid Waste

Van Soest and Mertens (95) have investigated the composition and digestibility of low quality cellulosic wastes including MSW and various types of paper. They point out that as lignin remains unattacked by anaerobic organisms, that it will accumulate in anaerobic systems and ultimately have to be removed. Hence, anaerobic fermentation of municipal solid waste, while

TABLE 18

COMPOSITION AND CELLULASE DIGESTION
OF VARIOUS WOODS BEFORE AND AFTER $SO_2$ TREATMENT (94) *

| Species | Lignin | | Carbohydrate | | Digestibility | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After |
| | % | | | | | |
| Quaking Aspen | 20 | 7 | 70 | 71 | 9 | 63 |
| Yellow birch | 23 | 9 | 66 | 67 | 4 | 65 |
| Sweetgum | 20 | 5 | 66 | 64 | 2 | 67 |
| Red oak | 26 | 8 | 62 | 60 | 1 | 60 |
| Douglas-fir | 30 | 24 | 65 | 63 | 0 | 46 |
| Ponderosa pine | 31 | 19 | 59 | 58 | 0 | 50 |
| Alfalfa | 17 | - | 51 | - | 25 | - |

*

Reprinted from Baker, Andrew J. et al. Wood and Wood-based Residues in Animal Feeds. In: Cellulose Technology Research, ACS Symposium Series, Vol. 10, p.90, 1975.

increasing the protein content of the wastes (see Table 19) also slightly increases the lignin concentration, and so reduces digestibility.

Feeding edible garbage to swine has been tried, but cannot be considered a waste management technique with any future potential. Residential wastes contain inedible and harmful materials, and are not a favored hog feed (50).

The digestibilities of paper products are usually acceptable, as they have already been treated and at least partially delignified (see Table 20). Van Soest and Mertens point out that as the composition of papers from different sources varies widely, so does their digestibility. If papers are fed to livestock, various additives including heavy metals, chlorinated organics, inks, clays, plastics, etc. may accumulate in animal tissues and eventually enter the human system. It is therefore suggested that future research should concentrate on the development of non-toxic easily removable additives.

Other Methods of Converting Animal Wastes to Nutrients

Colorado State University has investigated the stabilization of poultry wastes by the larvae of the common house fly, *Musca domestica* (96). Tubs of poultry manure were inoculated with fly eggs at rates of 2 to 5 g eggs/4000 g of manure. The tubs were subjected to different temperatures and humidities to determine the best conditions for hatchability of the eggs and stabilization of the wastes. Pupae were harvested from the manure by flotation, and then force air dried at 65°C overnight. At 27°C, it was found that the optimum yield of dry pupae was obtained with an inoculum of 3 g fly eggs/4000 g of fresh poultry excreta at a humidity of 41%. Too high a concentration of eggs resulted in lower yields of pupae.

Within a week, the moisture content of the feces dropped from 78.5 to 55%. The manure was stabilized with a slight ammonia odor still remaining. The treated manure had a granular consistency and was much easier to dry than the untreated excreta.

The protein quality of the fly pupae was similar to meat and bone or fish meal, and superior to soybean meal. In a series of feeding trials, hens and broilers were fed rations containing up to 30% of either the pupae, the stabilized manure or a mixture of both pupae and manure. Chicks fed pupae as all or part of their protein ration showed no statistically significant difference in weight from controls fed soybean meal. New Hampshire and Indian River chicks fed both pupae and stabilized manure at levels of 5 to 10% also maintained their weight and feed conversion comparative to the control.

The study concluded that fly pupae have potential as a protein supplement for chick starter and broiler rations.

As a waste management technique, growth of larvae under caged layers reduces

TABLE 19

EFFECT OF FERMENTATION ON COMPOSITION AND *IN VITRO* DIGESTIBILITY OF MUNICIPAL WASTES (95) *

| Description | Composition % protein | cellulose | hemicell | lignin | Digestibility % dry matter | cell wall |
|---|---|---|---|---|---|---|
| Raw waste | 9 | 38 | 16 | 5.0 | 73 | 64 |
| Treated waste | 26 | 43 | 10 | 6.3 | 65 | 44 |

* Unpublished data of Robertson and Van Soest.

TABLE 20

COMPOSITION AND DIGESTIBILITY OF PAPERS (95) *

| Paper type | Ash % | Organic matter true dig. % | Hemi-Cellulose % | Lignin % | Cellulose % | Lignin-to-cellulose ratio |
|---|---|---|---|---|---|---|
| Office bond | 6 | 96 | 10 | 4 | 77 | 0.05 |
| Brown paper | 1 | 91 | 9 | 7 | 81 | 0.10 |
| Cardboard | 4 | 72 | 10 | 12 | 70 | 0.17 |
| Glossy magazines | 23 | 62 | 6 | 11 | 50 | 0.23 |
| Newsprints | 0.5 | 33 | 14 | 24 | 56 | 0.42 |
| Coarse magazines | 7 | 33 | 14 | 21 | 53 | 0.41 |

* Unpublished data of Robertson and Van Soest.

the weight by 50% as well as stabilizing the wastes and reducing odors. Before land disposal, the compost-like wastes would have to be heat-treated to kill any fly larvae present (50). If the manure is to be used as a specialty soil conditioner or a feed, it would have to be dewatered to 10% moisture. The costs of treatment, labor and land-disposal are not attractive (50).

The USDA at Beltsville, Maryland, has considered growth of fly larvae on manure held in a rotating drum, as well as other automated techniques to reduce labor costs. Clear-cut cost estimates for such a process are not yet available. It is felt, however, that the complexity of the system would eliminate its use by the small farmer (50).

## Algal Systems and Aquaculture

### Growth of Algae

Controlled growth of algae in oxidation ponds developed as a waste management technique to provide oxygen to stabilize organic wastes. The value of the algae as a protein supplement was recognized, though not at first exploited. Improvement in growth, harvesting and processing techniques made it appear that large scale production of algae could provide a low cost protein of food or feed quality.

In 1969, Oswald and Golueke (18) prophesized that if world research into controlled algal cultures continued to progress at past rates of logarithmic growth, that by the mid 1980's, it would be possible to satisfy the entire protein requirements of the United States.

Today, it appears that the costs of production of algae and the land requirements for growth, together with other factors summarized in Table 8, make algal growth for protein much less promising, at least in the immediate future. The maintenance of a specific algal culture in an outdoor pond has proved difficult. Growth of algae is limited by available sunlight to a geographic region between latitudes 35° N. and S.

While a number of companies are actively funding research and development projects into bacterial, fungal and yeast production, only the Institut Francais du Petrole (IFP) with Sosa Texcoco, S.A. are investigating algal cultivation. *Spirulina* algae have been grown in culture basins in Southern France, in Algeria and near Mexico City (97). The surface area of the basins was from 5 to 700 sq. metres. The culture medium contained mineral salts as nutrients and was maintained at pH values from 8.5 - 11, with addition of sodium carbonate and bicarbonate. Continuous circulation of the culture was produced by alternately injecting combustion gas into one of two connected compartments in the basin.

Semi-natural basins were constructed in Lake Texcoco to exploit the algae which normally grow in the Lake. On harvesting, it was necessary to preconcentrate the *Spirulina* by filtering over an inclined plane prior to

horizontal strip filtering and drying, either on heated rollers or by spray-drying. In 1972, a semi-industrial plant began operation with a capacity of 1 ton of dry algae/day.

Long range comprehensive feeding trials and toxicity assays with rats, chickens and pigs, have so far been very encouraging. In Mexico, the algae have been successfully incorporated into beverages, cereals, soups and other human foods. Though clinical tests of *Spirulina* as a human food are not complete, the Mexican government has cleared the algae for human consumption (97).

All varieties of waste can be stabilized by algal growth in the presence of bacteria. The bacteria oxidize the carbon wastes to nutrients which are assimilated by the algae in photosynthesis. Oxygen produced by the algae is used by the bacteria to oxidize more wastes (18).

In the United States, Oswald and Golueke in California have investigated growth of unicellular chlorophytes (*Chlorella* and *Scenedesmus*) on sewage, animal wastes and MSW. The California group is interested in combined energy/food production systems. They have operated a pilot plant to digest manure from a poultry house, in order to stabilize the solids and produce methane. The supernatant liquid was used as a nutrient, the clear liquid was recycled to the poultry house as wash water. The methane generated could be used to dry the algae. Gas production from the 400 gallon digester averaged 12 cu. ft. of gas per lb. of volatile solids added (98).

The California group has also studied the possibilities of algal growth on wastes for digestion to methane. Again the supernatant could be recycled as a nutrient for further algal growth, and the methane could be used to dry harvested algae (98), (99).

The projected cost of algae produced in such an integrated energy/food system would depend on the method of harvesting. As of 1973, the cost of harvesting by centrifugation followed by spray drying was estimated at 6 cents per pound (98). Alum flocculation, centrifugation to dewater and heat drying would produce a slightly cheaper product. The most economical method of harvesting would be alum precipitation followed by drying on a sand bed and recovery on a shaker screen, a process which would tend to denature the protein.

Dr. Waygood of the University of Manitoba has investigated the mass culture of *Euglena gracilis*. *E. gracilis* was chosen for a number of reasons including:

- It contains no cellulose cell wall and should therefore be readily digestible;
- It is motile, eliminating the need for expensive stirring;
- It can utilize the organic wastes and/or carbon dioxide from the air during photosynthesis, i.e., it is both photomixotrophic and heterotrophic (73).

The algae was grown on sewage, secondary effluents, abattoir waste and various livestock manures. The manures were diluted depending on the source, while the other wastes were used as generated.

The cultures were grown in trays (8 litres, 7.5 cm. depth) or ponds (275 to 550 l., 15 to 30 cm. deep) with no oxygen or carbon dioxide enrichment. At a pH of 4.0 bacterial and protozoal contamination was restricted though fungal growth was enhanced. As the fungi were metabolized by E. *gracilis*, this was not a problem.

After four to five days, growth of algae ceased, even though nutrients remained in the culture. Addition of whey to the media improved growth of the algae and utilization of nitrogen and phosphorus nutrients.

For sewage or swine manure, optimal growth of E. *gracilis* gave a dry product containing 60 to 70% protein, 15 to 20% fat, and 15 to 20% carbohydrates. The yield of algae was between 10 to 40 g dry wt./sq. metre/day. In Manitoba, this corresponds to a productivity of 5 to 20 tons/acre per 100 frost free growing season.

Aquaculture

Mass production of marine algae in outdoor cultures has been studied at Woods Hole, Massachusetts, and Fort Pierce, Florida. Duplicate pools of 2,000 litre capacity were used to grow diatoms on a substrate of sea water (50%) and secondary waste effluent (50%). The water was continuously pumped through the constantly agitated culture, and approximately steady state conditions were established for each flow rate (100). At Woods Hole, the phytoplankton dominant in the culture depended on the season; *Phaeodactylum tricornutum* predominated in the late spring, with other successions of diatoms predominating the rest of the year. In Florida, *Nitzchia closterium* was the prevailing species.

Table 21 shows the effect of dilution rate on the mean algal yield in the two locations. Note that the yields clearly show the effect of latitude on algal growth.

Inorganic nitrogen and phosphorus were added as nutrients. The nitrogen uptake for all species in the culture was up to 99.9% (101).

The phytoplankton were fed to oysters and clams in raceways maintained at 15° to 20° C. It was estimated that 92 tons of oyster meat/acre could be produced during the summer months at Woods Hole (101). Separate studies with seaweeds have shown that though the seaweeds grow faster than the phytoplankton, they are less efficient at nitrogen removal (90%).

There is a pilot estuarine aquaculture system at The Tallmans Island Pollution Control Plant in Queens, New York. The study is sponsored by the

TABLE 21

EFFECTS OF DILUTION RATE ON MEAN ALGAL YIELD IN TWO LOCATIONS (100)

| Dilution rate | Mean algal yield (g dry weight $m^{-2}$ $d^{-1}$) Woods Hole (lat. 41° 52'N) | Ft. Pierce (lat. 27° 28'N) |
|---|---|---|
| 0.25 | 7.6 (2)* | |
| 0.50 | 12.4 (8) | 18.0 (6) |
| 0.75 | 13.3 (2) | 15.3 (1) |
| 1.00 | 12.4 (1) | 23.6 (1) |
| 1.50 | | 0 † (1) |

* Values in parentheses represent number of replicate experiments. Each experiment consisted of determining the steady-state algal yield at the stated dilution rate. The mean algal yields where applicable were determined by averaging the data from the replicates. Variations from the means were less than ± 20%.

† Steady-state algal growth could not be maintained and the culture washed out.

City University of New York and the New York City Department of Water Resources. The project is similar to the Woods Hole study but greater concentrations of secondary sewage effluent are being used in order to reduce space. Fresh water phytoplankton are also under study (101).

For a 50:50 ratio of secondary sewage effluent to harbor water, phosphorus removal was about 90%. Nitrogen removal was also regularly more than 90% before phosphate detergent use was restricted in the area. The nitrogen: phosphorus ratio should be between 10:1 and 15:1 for most effective uptake of nutrients. The phytoplankton culture is diluted with harbor water before removal by filter-feeding mussels and clams.

In feeding trials with chicks, the shellfish proved nutritionally superior to commercial feed (101). Toxicology studies are underway to determine the pesticide and heavy metal concentrates in both the shellfish and the chickens.

A number of marine and fresh water phytoplankton have been screened for their ability to utilize the nitrogen and phosphorus in the wastewater. In addition, a number of shellfish species including: *Mytilis edulis, Modiolus demissus, Mya arenaria, Lampsilis sp.* and *Corbicula manilensis,* have been screened for their phytoplankton-stripping and protein-producing abilities.

Various aquatic weeds have been investigated for their ability to clean up waste water, and for possible use as a high protein animal feed. The most well-known species is probably the water hyacinth, previously regarded as an aquatic nuisance. At Bay St. Louis, Mississippi, National Aeronautics and Space Administration (NASA) scientists have conducted research into controlled growth of water hyacinths. The vascular plant has the ability to absorb and concentrate organic and heavy metal pollutants found in sewage. The plant grows extremely rapidly; it is estimated that a one acre sewage lagoon could produce 8 to 16 tons of plants per day (102). A three acre lagoon growing water hyacinths on raw sewage will be expanded to twelve acres this summer.

Dried, ground plants have been included in a corn silage ration for cattle. Feeding trials with water hyacinths at the University of Florida showed that cattle would voluntarily consume the plant at a low level (1 to 1.5% body weight). The ration was more acceptable when the plants were hammer-milled and blended with sugar cane molasses. The protein and nutrients in the plant were poorly utilized by the animals (103).

It is also possible to digest the plants to methane. About one lb. of dry plants yields six cu. ft. of methane. The sludge from digestion could be used as a fertilizer or soil conditioner (102).

## The Economics of Food/Fuel Recovery from Wastes

Since the use of waste products for food or fuel depends on cost and market

considerations, the economic dimensions of SCP production will be compared to waste conversion into glucose, into a variety of fuels, and to direct fuel use of waste (co-firing with coal or oil).

In the following discussion of waste conversion costs, it should be remembered that, for the most part, cost estimates used are derived from laboratory studies or computer simulations on technologies that are, at best, in a formative stage. Some authors claim to compute costs for "optimum" plant size, or to present some estimate of a "minimum cost". It is better, in the absence of greater commercial experience with waste conversion, to look on these cost estimates as reasonable first approximations, base line figures to be used for rough ordering among the various techniques, (for further comments on making and using cost estimates, see Appendix 2).

To measure the cost of using waste in different ways, it is necessary to remember the distinction between private and social costs and returns. This distinction is important in judging profitability of a private venture that buys waste from a public disposal authority. For example, if the waste disposal authority sells waste to a private user at $1.00/ton, and the private user replaces another fuel (at $2.00/ton) with waste, the fuel input component of his cost will be $1.00. This is wholly private cost, and his additional private return will depend on conditions in the market for his product (in the short run, he should enjoy a profit thanks to cheaper fuel). At the same time, if it costs the disposal authority $5.00/ton to discard the waste, social costs of disposal have been reduced by a subsidy of $1.00/ton, for every ton sold to the private user. The social returns of reduced disposal costs may accrue directly to the taxpayer, or indirectly in reallocation of resources from waste disposal to private production with the possibility of greater total product and/or lower product prices. If, of course, waste disposal and waste conversion are both public activities, social costs are "internalized" and "credits" in the form of reduced costs of waste disposal and gains from final products sale (resource recovery) should be weighed by the public authority in decisions on a choice of waste recycling technology.

It is important at this point to clarify a misapprehension. Many environmentalists feel that the stress placed upon the private profit motive in resource recovery from waste materials is improper. This is basically because they believe that such an emphasis encourages the generation of more wastes (where wastes = money) instead of resource conservation at source. The Environmental Protection Agency agrees with the principles of conservation and is of course mandated by law to uphold these principles. At the same time, the Agency recognizes that polluting wastes are generated which must be treated at a central disposal facility. Being realistic, no local authority is going to opt for a resource recovery scheme, however environmentally sound, if the economic costs of the technology are prohibitive.

Process Cost Comparisons - Protein Production

Waste materials may substitute for other sources of food including:

- conventional animal and vegetable food sources;
- substrates that might be used to produce food, such as hydrocarbons and alcohol;
- oilseed proteins
- other unconventional sources such as leaf proteins.

When discussing the choice of a substrate for SCP production, cellulose is often described as a low or negative cost material. As is made clear in Table 22, many forms of cellulosic waste have a value. The cost of cellulose depends on its quality, note the comparatively high value of paper pulp. Dunlap predicts that as the resource recovery industry develops and expands, no waste cellulose will have a zero value (104). He expects that the higher cost cellulosic "wastes" will be used for the higher cost products. Table 23 gives the value of products produced from cellulose, updated by a 10% inflation factor.

For the private producer, it is critical to compare costs of food stuffs produced from wastes with the costs of competing sources of protein and carbohydrates. Costs for production of feed grade SCP vary by substrate as shown in Table 24. If these figures are reasonable, then feed grade SCP grown on any substrate including cellulose, is not competitive with soybean meal, fish meal or any of the other high-protein feeds listed at approximate U.S. market prices. Dunlap has calculated that feed-grade SCP must sell at about $220/ton (1975 figure updated 10% for inflation), while Humphrey has used the value of $275/ton (1976 updated figure) (105).

However, yeast grown on hydrocarbons sells on European markets for two to three times this value (106). In a nutritional evaluation of "Pruteen", ICI used the figure £ 245/tonne for British markets, i.e. approximately $400/ton at current rates of exchange. In the same evaluation, it was concluded that even with soybean meal at £ 100/tonne, a 50/50 "Pruteen"/soy mixture was more economic than the 100% soy control, because of increased egg production in test hens (47).

The ICI study makes the valid point that not all proteins are created equal! Table 24 makes no distinction between the quality of the proteins listed. Peanut meal may cost the least per pound of crude protein, but the PER value is low compared to processed SCP products, see Figure 4. As far back as 1971, Callihan and Dunlap stated that it would be most desirable to compare the cost of protein sources in terms of their nutritional quality. The need for such a study is even more obvious today. As Table 25 indicates, as "Torutein" is upgraded in protein quality, its cost rises from 42¢/lb. to

TABLE 22

COST OF CELLULOSE WASTES*

| Source | Value $/ton | Cellulose Content | $/ton Cellulose |
|---|---|---|---|
| Hay | | | |
| alfalfa | 52-78 | 13-34 | 154-604 |
| grass | 26-52 | 20-35 | 74-261 |
| clover grass | 39-65 | 11-27 | 145-594 |
| Sugar cane bagasse | 11-14 | 50-55 | 20-38 |
| Waste paper | | | |
| News | 10-30 | 75-80 | 12-41 |
| Mixed | 5-18 | 70-85 | 6-26 |
| Paper pulp | | | |
| bleached sulfite | 315-363 | 95-98<br>95-98 | 322-382 |
| unbleached sulfite | 266-306 | 95-98 | 271-322 |
| Ground wood | 240 | 80-85 | 285-302 |
| Wheat straw | - | 45-50 | - |
| Rice straw | - | 45-50 | - |
| Corn stalks & cobs | - | 35-50 | - |
| Rice hulls | - | 35-50 | - |
| Municipal refuse | - | 50-55 | - |

* Figures adapted from Dunlap (104) and Dunlap (106) by updating to 1976 $'s to allow for inflation.

TABLE 23

VALUE OF PRODUCTS PRODUCED FROM CELLULOSE (104)*

| Product or Use | Units | Value ($/unit) | Units produced from 1.0 lb dry cellulose | Value of product from 1.0 ob dry cellulose |
|---|---|---|---|---|
| Direct burning as fuel | $10^6$Btu | 0.33-0.88 | 0.010 | 0.33-0.88 |
| Methane | $10^6$Btu | 0.88-2.2 | 0.004 | 0.33-0.88 |
| Ethanol | lb | 10 | 0.56 | 5.6 |
| Single cell protein | lb | 16.5-27.5 | 0.45 | 7.5 -12.3 |
| Glucose | lb | 10.8-28.6 | 0.80 | 8.6 -23 |

* Note: These figures are not related to cost of production. They are derived from current market prices multiplied by an efficiency factor for each process. The figures have been adjusted from the original source to reflect an inflation factor of 10% per annum.

TABLE 24

COSTS OF NUTRITIONAL PROTEIN

| Source | Cost ¢/lb | Crude Protein % | Cost of Crude Protein ¢/lb |
|---|---|---|---|
| Gelatin | 165-275 | 100 | 165-275 |
| Casein | 77-110 | 100 | 77-110 |
| Flaxseed | 16.7 | 24 | 69.6 |
| Hominy feed | 4.1 | 10 | 41.0 |
| Bran, wheat | 4.2 | 17 | 24.7 |
| Fishmeal | 12.9 | 60 | 21.5 |
| Alfalfa pellets | 3.9 | 18 | 21.7 |
| Linseed meal | 7.4 | 35 | 21.2 |
| Feather meal | 9.9 | 55 | 18.0 |
| Meat-bonemeal | 8.7 | 50 | 17.4 |
| Corn gluten feed | 4.2 | 25 | 16.8 |
| Brewers grains | 3.9 | 24 | 16.2 |
| Cottonseed meal | 6.3 | 42 | 15.0 |
| Soybean meal | 6.7 | 46 | 14.6 |
| Peanut meal | 5.6 | 46 | 12.2 |

Note that all of the above figures were derived from Dunlap (106) They have been updated 10% for inflation, and do not represent current market values of the products which fluctuate a great deal.

SCP from:

| | | | |
|---|---|---|---|
| n-paraffins * | 28.1 (37.5) † | 61 | 14.1 (61.5) † |
| gas-oil * | 25.2 (36.3) † | 68 | 37.1 (53.4) † |
| methanol * | 27.5 (34.5) † | 83 | 33.1 (41.6) † |
| natural gas * | 21.2 (29.2) † | 75 | 28.3 (38.9) † |
| cellulose ‡ | 16.5-27.5 | 50 | 33.0-55.0 |
| bagasse § | 17.3 | 50 | 34.6 |
| MSW (yeast via # acid hydrolysis) | 22.8-40.2 | 45 | 50.6-89.3 |

* Adapted from Brownstein & Constantinides (22) - inflation factor 0.19.
† 10% return after 50% taxes added by Maclaren (12)
‡ Adapted from Dunlap (104) - based on value of product x efficiency of process, does not indicate production costs.
§ Adapted from Callihan & Dunlap (55), Bechtel figure said to be lower than this.
\# Adapted from IRTC (50).

67¢/lb. As a high grade food protein with unique functional properties, this cost competes very favorable with casein and the low quality protein, gelatin. The value of any product is the highest price the market can command. Therefore, the extent of acceptance of "Torutein" and similar products derived from petrochemicals must be some indicator of future prospects for high grade functional proteins grown on waste cellulose.

If SCP grown on cellulose is to compete with conventional feeds, then there must be an improvement in utilization of the cellulose and increased yields of products with a higher protein content. Note that while Table 24 uses a figure of 50% crude protein for SCP grown on cellulose, as is made clear in Table 13, the protein content is often much less than this.

TABLE 25

CURRENT TRUCKLOAD PRICES F.O.B. FOR "TORUTEIN"
HUTCHINSON PLANT (1976) (23)

| Product | $/cwt Prices |
|---|---|
| "Torutein" | 42.00 |
| "Torutein-LF" | 50.00 |
| "Torutein-94" | 67.00 |

* Note: Torutein is food-grade yeast grown on ethanol. The prices reflect the additional processing required to upgrade the yeast to a replacement for non fat dry milk (Torutein-LF) or further treatment to Torutein-94, a replacement for eggs.

Mention has been made of the potential value of refeeding the sludge of anaerobic digestion, especially to ruminants. Many scientists feel that exploitation of the nutritive value of the sludge will be more profitable than selling the methane (80), (81), (107). Weisberg and Krishnan (107) have stated that for a 200 ton/day plant processing manures of 50,000 head of feedlot cattle, the price of high quality, high pressure methane can drop from $2.87 per 1,000 cubic feet (mcf) of gas to $2.43/mcf. This figure would still realize a 10% after-tax return on investment. Weisberg and Krishnan reported a 13% protein content of sludge, while other scientists have indicated a 25% protein content. It would be premature to make any predictions of cost for a product still undergoing evaluation.

As research into anaerobic digestion has concentrated on the optimization of gas production, little attempt has been made to evaluate the protein content or quality of the sludge. Certainly the possibility of manipulating reaction conditions to optimize protein production has been neglected. Ongoing studies of the digestibility and acceptability of the sludge to livestock have been mentioned previously. The results and recommendations of these investigations are awaited with interest.

Process Cost Comparisons - Edible Carbohydrates

The food value of organic wastes is not confined to protein production. Cellulose may be enzymatically or acid hydrolyzed to glucose. The glucose syrup may be crystallized and sold as an edible carbohydrate, fermented to ethanol, or used as a substrate for microbial growth. A maximum production cost of $50 to $60/ton glucose is considered essential in order to produce either ethanol competitive with the ethylene process, or SCP competitive with soybean meal (108), (109).

As is clear from Table 26, the cost estimates for acid hydrolysis of cellulose are much lower than the current estimates for enzyme hydrolysis. Fermentation of glucose from acid hydrolysis of MSW is probably competitive at 9.2 - 16.3¢/lb ethanol (figures updated from 1971) depending on the size of the plant.

The enzyme hydrolysis to glucose would be more cost effective with enzyme recovery and improved enzyme efficiency (110). It is evident that there is a need to examine the process at the pilot plant level. Engineering studies and computer simulations of sensitive areas of operation would permit an optimization of the reaction, a more valid economic analysis and an improvement in the economic feasibility of the reaction.

In order to improve the economic picture for enzyme hydrolysis, it has been suggested that the process be examined as a source of other marketable products. Humphrey suggests that the intermediate water-soluble di-saccharide, cellobiose, could be produced by genetic manipulation of the fungus producing the enzymes (109). Cellobiose reportedly has 75% of the sweetness of glucose, and may pass undigested through the human system. Currently, the market price of cellobiose is over $100/lb (109). An expansion of markets for cellobiose as a dietetic sugar might be worth investigating.

TABLE 26

COSTS OF NUTRITIONAL CARBOHYDRATES*

| Source | Cost ¢/lb (approx.) | Digestible Carbohydrates % | Cost of Digest. Carbohydrates |
|---|---|---|---|
| Refined sugar † | 36.6 | 100 | $ 36.6 |
| Dextrose † anhydrous | 32.2 | 100 | 32.2 |
| Dextrose † hydrated | 24.0 | 91 | 26.4 |
| Corn syrup † | 16.0 | 81 | 19.8 |
| Rice † | 11.3 | 74 | 15.3 |
| Barley † | 10.0 | 77 | 12.9 |
| Oats † | 6.7 | 57 | 11.8 |
| Wheat † | 6.2 | 67 | 9.2 |
| Corn † | 5.7 | 68 | 8.4 |
| Rye † | 5.5 | 70 | 7.9 |
| Molasses † | 3.0 | 55 | 5.4 |
| Masonex † | 2.1 | 17-22 | 9.5-12.3 |
| Ethanol (from † ethylene) | 17.7 | 95 | 18.7 |
| Ethanol (from acid ‡ hydrolysis MSW/ferment.) | 9.2-16.3 | 95 | 9.7-17.2 |
| Glucose (from cellulose, MSW, acid hydrol) | 8.9 ‡<br>2.7-3.9 § | | |
| Glucose (from MSW, enzyme hydrolysis | 9.4 #<br>12.5 ¶<br>22.4 ¶ | | |

* Updated for inflation to 1976 dollars.

† Source: Dunlap (106)
‡ Source: IR&T (50)
§ Source: Converse *et al*(79)
# Source: Rosenbluth & Wilke, 5% syrup, updated for inflation (78)
¶ Source: Humphrey - 12.5¢ updated figure, syrup. 22.4¢ updated figure, Xalline (109)

## Summary - Food/Feed Production from Cellulosic Wastes

A comprehensive, up-to-date economic analysis of SCP production from cellulosic wastes does not exist in the public sector. Data quoted are derived from either estimations based on small scale studies or are extrapolated from limited pilot plant experience. Similarly, even recent analyses of the economic feasibility of enzyme hydrolysis are based upon data from 1970 (Rosenbluth and Wilke) (78). The economic potential of refeeding the sludge from anaerobic digestion is unclear though promising.

Based on the limited data available, it does not appear that feed-grade SCP produced from waste cellulose is competitive with conventional high-protein feeds at present or for the near future. That being said, it must be remembered that the cost/lb of protein must always be qualified with reference to the protein quality.

The near future prospects for food-grade SCP are much more promising. An additional factor to remember when discussing marketing SCP as a human food must be the psychological acceptability of the substrate to the general public. Crop residues are probably more acceptable than animal excreta as substrates.

Acid hydrolysis to glucose followed by yeast fermentation to ethanol is probably economically feasible at present.

One factor not previously identified as "economically sensitive" is the cost of transporting wastes to a centralized processing plant. Nyiri and Tannen have shown by a computer simulation that transportation costs are unacceptably high beyond a 10 mile radius. This constraint must apply to a greater or lesser extent to all processing utilizing wastes, whether for food or energy production.

While the economic picture is not clear, it must be considered hopeful at least for the long range exploitation of cellulosic wastes as sources of protein or edible carbohydrates.

## Process Cost Comparisons - Energy Resource Recovery

It is not possible to compare costs/unit output between food and fuel recovery from wastes, since measures of output are not comparable. Rather than rank methods by cost per unit output, one can compare costs of processing a ton of waste; in effect the "value added" to waste in obtaining a marketable product. Processing costs vary widely as can be seen in Table 27. Unfortunately, the figures given are for varying sizes of plant, so direct comparisons are not valid.

From these figures it would appear that energy recovery from manures tends to be more expensive than from MSW, in spite of the fact that the MSW costs include an elaborate front end system to reclaim inorganic materials. Credits for the nitrogen value of the sludge from manure digestion will make

TABLE 27

COST OF VARIOUS WASTE PROCESSING TECHNOLOGIES
($/ton dry waste input)

| Process | Scale | $/ton input |
|---|---|---|
| Anaerobic Digestion | | |
| MSW to methane * | 1000 ton/day (TPD) | 12.7(a)<br>15.1(b) |
| MSW to methane † | 1000 | 18.7<br>(8.1) |
| Feedlot waste to methane ‡ | 200 | 12.4(a)<br>25.0(b)<br>28.3(c) |
| Feedlot waste to methane § | 9120 | 22.3 |
| Pyrolysis | | |
| MSW (oil and char) # | 1000 | 8.7 |
| MSW (no. 6 oil) # | 1358 | 7.0 |
| Feedlot waste (29% MC) ¶ | 161.6-1616 | 39.8-18.9 |
| Feedlot waste (50% MC) ¶ | 115 -2960 | 44.9-14.0 |
| Combustion | | |
| Cofiring (coal+MSW) # | 980 | 8.1 |
| MSW to steam # | 1600 | 11.4 |
| SCP Production | | |
| MSW # | 400 | 47.4 |
| Manure # | 400 | 13.2 |
| Carob * | 1763 tons/yr | 42.5 (a)<br>52.7 (b) |
| Acid Hydrolysis | | |
| MSW to glucose # | 190 | 11.2-22.3 |
| MSW to ethanol # | 190 | 15.2-26.8 |
| Enzyme Hydrolysis | | |
| MSW to glucose † | 20 | 223 |

A brief explanation of this table appears on the following page. Except where indicated this cost/ton does not include credits for products formed or reclaimed nor penalties for sludges requiring additional treatment and/or disposal. All values given here have been updated for inflation to 1976 $'s.

Key to Table 27

It is recognized that because of differences in scale of production and because of different methods of estimating capital and manufacturing costs that these figures are NOT directly comparable. They are quoted because these were the figures most readily available, or in some cases the only figures available.

* Pfeffer (111)
(a) Figure with incineration of organic residues
(b) No incineration
Neither figure contains credits for inorganic recovery or sale of methane.
Ash and inorganic disposal are included in the costs.

† Kispert *et al* (112)
First figure does not include any penalties or credits for inorganic recovery.
Figure in brackets includes penalties and credits (except sale of gas).

‡ Weisberg & Krishnan (107)
(a) low purity, low pressure methane
(b) high purity, low pressure methane
(c) high purity, high pressure methane
All cattle manure.

§ Hamilton Standard (81)
Cattle manure

\# International Research and Technology (50)
MSW (oil and char) figure updated from calculations in Ware (113).
Cofiring - includes credit for replacement of coal (1/4 of charge).
SCP - does not include marketing and distribution, nor credit for the product.
MSW to glucose based on Meller(114) and Converse (79).
MSW to ethanol based on Meller (114) and Converse (79).
190? The report does not make clear the scale of these two reactions.

¶ Inman (115)

* Tate and Lyle (51)
The first figure is for labor at $1000/man year (developing country).
The second figure is for labor at $10,000/man year.

† Rosenbluth and Wilke (78)

(Further details relating to these estimates can be found in Appendix III.)

this process more attractive. Pyrolysis of manures is relatively expensive because of the high moisture content (MC) of the excreta. Pyrolysis and co-firing of MSW are in the same range. Pyrolysis may be favored because the fuel produced is transportable.

SCP production from MSW is nearly four times as expensive in terms of cost/ton input as SCP production from manures. Municipal solid waste requires more initial preparation than manures. If the manure is processed close to a feedlot and the SCP is returned for fodder, then SCP production from manures would not incur heavy transportation costs. Both these figures for SCP production are derived from an initial analysis prepared by Meller for the Environmental Protection Agency (114). The initial analysis referred to yeast production. It has been pointed out that bacterial production from MSW will cost slightly more per ton, because of higher yields of protein (50). It should not be assumed that these figures are close to the present economic reality.

The figure for SCP production from carob beans relates to a "village-technology" process. The capacity of the plant is very low and of course the cost/ton would drop significantly for a larger scale operation. It must be remembered, however, that the figures for production in the United States would be much higher due to higher wage scales.

The cost of acid hydrolysis to produce either glucose or ethanol compares favorably with the fuel production technologies. Note that the cost of ethanol production from MSW is probably now competitive with the ethylene process. It has been suggested that ethanol could be used as a fuel extender or even partially replace many petrochemicals as a source of antibiotics, vitamins and other important chemicals. In this event, acid hydrolysis of MSW looks very promising economically.

Although the food production processes cost more per ton input than do the fuel recovery technologies, it must be remembered that both SCP and glucose are potentially more valuable products than fuels derived from wastes (see Table 23).

## Summary

For the immediate future at least in the United States, it is unlikely that SCP production from cellulosic wastes could be considered a commercially viable process. Similarly, it appears that enzyme hydrolysis cannot compete economically with acid hydrolysis/fermentation to alcohol, which may now be commercially feasible.

However, several facts must be emphasized:

(a) Data are limited, out-of-date and incomplete relative to the economics of the above processes. There is a definite need for pilot scale projects to gather fresh data for computer simulations, so to identify the areas

of economic sensitivity for both SCP production and enzyme hydrolysis.

(b) Any economic evaluation of SCP from wastes must recognize the importance of considering the nutritive value of the protein produced before condemning the process as uneconomic for a particular cellulose source.

(c) It is possible to predict that for certain wastes, either anaerobic digestion for methane and refeed, or SCP production to produce a soybean replacement would be favored processes. This is especially true for feedlot wastes, which could be processed in situ to eliminate transportation costs and defray feedlot expenses (for energy and/or food).

Energy Comparisons Between Alternative Food Production Systems

U.S. farming is heavily dependent upon usage of fossil fuels to achieve high productivity. From 1950 - 1970 the general consumption of energy in this country doubled. During the same period, certain sectors of the agricultural industry experienced a threefold increase in demand (116). The reliance of the farming industry upon fossil fuels is illustrated by the following figures: while agriculture consumes about 2.5% of annual production of electricity, it uses about 10% of petroleum products (117). Table 28 gives some indication of the disappearance of energy on the farm and in the food processing industries. Note that after consumption of fuel to manufacture and operate farm machinery, that the energy required to produce fertilizers is the third largest component of on farm energy usage. Transportation and energy consumption within the food processing industry accounted for 1.54% and 1.9% respectively of total U.S. energy expenditure in 1970.

The increased consumption of energy on the farm has been accompanied by a decrease in labor. In 1970, about 2.9 million kilocalories of fuel energy were used to raise one acre of corn (116). In 1945, 23 hours of labor per crop acre were required for corn production. By 1970, this figure had dropped to 9 hours per crop acre, a decrease of 60% (116). During the same period, the number of tractors increased from 2.4 million to nearly 4.5 million, with an accompanying increase in horsepower. For the entire U.S. corn production, there was an increase in energy expenditure for machinery from 15 gallons/acre in 1945 to 22/gallons/acre in 1970 (116).

The use of fertilizers for corn production has also exploded since 1945. Nitrogen use alone increased by a factor of 16, phosphorus usage by a factor of 5, and potassium quantities applied per acre increased 14 fold (116). According to Pimental *et al*, corn production accounted for 17% of all insecticides manufactured for agricultural usage (116).

TABLE 28

ON FARM AND FOOD PROCESSING INDUSTRY ENERGY USAGE
IN THE UNITED STATES FOOD SYSTEM, 1970 (118)

| Item | | Approximate Energy Usage as % of Total U.S. Energy Consumption, 1970* |
|---|---|---|
| On Farm | | |
| Fuel (direct use) | | 1.45 |
| Electricity | | 0.4 |
| Fertilizer | | 0.6 |
| Agricultural steel | | 0.013 |
| Farm machinery | | 0.6 |
| Irrigation | | 0.22 |
| | Sub Total | 3.28% |
| Processing Industry | | |
| Food processing industry | | 1.9 |
| Food processing machinery | | 0.04 |
| Paper packaging | | 0.24 |
| Glass containers | | 0.30 |
| Steel cans and aluminum | | 0.76 |
| Transport (fuel) | | 1.54 |
| Trucks and trailers (manufacture) | | 0.47 |
| | Sub Total | 5.25% |

*Note: Total U.S. energy consumption for 1970 taken as 1.6 x 1016 kilocalories (114).

Transportation and electrical requirements increased 3.5 times, irrigation and the resultant water costs rose 1.8 times and the energy for seeds for planting nearly doubled.

The introduction of strains of hybrid corn after the Second World War resulted in an increase in yield of corn per acre from 34 bushels in 1945 to 81 bushels in 1970. In 1972, the corn yield per acre rose to an all-time high of 97.1 bushels (5). If this increase in productivity is examined in relation to increase in energy consumption, there has been a decrease in the ratio of energy production of the crop per energy expenditure from 1950 to 1970. This decrease in efficiency is around 11% if the input of solar energy is included and an incredible 24% if only the fossil fuel usage is considered. It should be noted that Pimental *et al* chose corn production to make this analysis because not only was more data available for corn than any other crop, but corn production is an "average" crop in terms of energy consumption.

## The Energy Analysis

In this analysis of various methods of protein production, the system was defined as the process itself and did not include marketing of products. The comparative unit employed was the term British thermal units per gram of protein (Btu/g protein). Protein was defined as the crude protein content (Kjeldahl nitrogen X 6.5) of the product.

In most cases the analysis was based on incomplete information with data extrapolated from bench-scale operations. The ultimate scale of fermentation is industrial. While complete energy analyses for commercial single cell protein production undoubtedly exist, the information is proprietary to the individual companies and was not made available for this report.

For single cell protein production, it must be remembered that it is possible to use different cellulosic substrates of varying composition. Also the end product concentration of SCP was not always clearly indicated in the reports studied.

Protein yields depend on mass transfer, oxygen transport to and across the cell surface, environmental conditions within the reactor and the genetic makeup of the growing microorganisms. Protein yields were usually estimated for SCP analyses.

Most of the conventional farming figures presented were extrapolated and indexed for 1976 figures.

Due to the varying units used in the different energy analyses, all of the comparisons were made on the Btu/g protein basis. This permits a ready conversion to fuel consumption if needed. The actual energy cost to process water, electrical power, steam and gas as well as the raw materials input for ammonia, phosphoric acid etc. was included for each of the processes in the energy inventory.

It was assumed that certain economies of scale would hold from one process to another if certain factors were eliminated. For example, for production of more than 500 tons SCP/year, refrigeration is required to remove heat of fermentation. This factor is not included in the analysis.

If specific data were not reported, as a rough approximation, the raw materials energy input, packaging and general overheads were taken as about equal to the electrical energy input. The estimates are rough, but as dimensional figures would impart a variation of no more than ± 15%.

Processes which are extremely energy dependent per gram of protein show up as a larger Btu/g protein figure. This is especially obvious for deep-sea fishing where the fleets sail longer distances in order to obtain their catch compared to coastal fishing.

It should be noted that these figures are dependent upon first principle derivations, data supplied through references, and the best possible extrapolations from these. The reported percentage protein content of each of the listed crops was used in the analysis, as well as the conventional figures for fuel input and fertilizer production and other inputs to the system.

It should be noted that solar energy was not considered a fuel for this analysis. If solar energy becomes a technically useful source of fuel on a large scale, this may involve use of land. If the land could be used for food production then the trade-off between solar fuel, power output and food production must be studied. If the solar device does not take up useful land (e.g. rooftops, etc.) then it competes with nothing and has no meaning in terms of energy costs. Furthermore, including solar energy with fuels and human and animal working energy inputs to food production would overshadow these inputs dimensionally. The energy analysis would be nothing more than a study of photosynthetic conversion from solar energy to food energy and would not illustrate the energy intensiveness of the alternative methods of producing food. For this analysis, the primary energy resources were the fossil fuels; nuclear fuels were not included.

The comparative energy usage table includes corn production, fishing, cattle raising and manufacture of SCP on different substrates. The substrates included are methanol, carob pods, petroleum stock, whey and cellulose. An in-depth analysis of two of these processes (the Wilke system of enzymatic hydrolysis and the Abcor system for SCP production from whey), is included in Appendix IV.

The energy recovered as SCP averages 5500 kilo calories per kilogram (119). This is the energy of the protein which will be utilized by man or animal and is not included in the protein equation of the energy analysis. In addition, the plant energy and the energy used by manual labor is not included as energy input.

The removal of large amounts of heat during metabolism in the microbial processes is assumed to be accomplished by the process water and the heat exchangers. Occasionally, data not available for calculation were derived on the basis of the process water alone.

It is recognized that microbial fermentation is an exothermic process. None of the systems examined appeared to include a heat recycle though energy conservation might usefully be applied to the SCP processes discussed.

## Discussion

It cannot be over-emphasized that the energy analysis presented in this report is a rough approximation. Bench scale operations have not yet reached the stage of a complete energy analysis and much of the industrial scale data is confidential information. It is recommended that a complete energy analysis of fermentation of cellulose to single cell protein be considered a priority item for research and development funds. The energy analysis of bioconversion schemes now underway at the University of Wisconsin should do much to clarify the energy intensiveness of a number of processes applicable on the farm.

That being said, it is interesting to see that the processes designated "Single Cell Protein" fall within a range. The low energy figure for whey as a substrate reflects the fact that the whey is a source of whey protein concentrate through ultrafiltration as well as yeast single cell protein grown on the lactose permeate. The energy figure for SCP production on carob beans is less than the value for growth of petro-proteins, and for production of glucose from cellulosic wastes. This is expected as the Tate and Lyle process is being developed as a "village-level" or "intermediate" technology, less energy intensive and more reliant on labor.

The figure for enzymatic hydrolysis is artificial, note the unit - Btu input/g potential protein. The main product of enzyme hydrolysis is of course glucose with only small amounts of fungal protein recovered. The glucose amount has been converted to the quantity of protein potentially available using glucose as the growth medium. At the same time, the energy required to make this conversion is not included in the analysis. Hence, the figure given is unreal but is included for comparative purposes only.

Amoco Foods now marketing the food grade yeast "Torutein", has indicated that the energy consumption of their process is about one-half the energy required to produce an equivalent weight of beef protein processed to the wholesale level of marketing. They did not indicate whether the beef was range-fed, grass-fed or raised on a feedlot. If it is assumed that the comparison was made considering the energy input/protein production for grass-fed beef, then the figures presented in Table 29 look to be in the correct range -- note the value for the petroleum based process.

Appendix IV contains an approximate analysis of the anaerobic digestion process. As digestion of agricultural residues produces both a fuel and

TABLE 29

A ROUGH APPROXIMATION OF ENERGY CONSUMPTION
FOR A NUMBER OF FOOD PRODUCTION SYSTEMS
INCLUDING MICROBIAL PROTEIN

| Item | Energy input per protein produced (Btu in/g protein) | | Reference |
|---|---|---|---|
| Commercial Fishing | | | |
| Coastal Fishing | 124 | | Steinhart & Steinhart (118) |
| Distant Fishing | 1240-1500 | | + Leach (120) |
| Prawn Fishing | 2500-5000 | | " |
| Fish Protein Concentrate | 900-1000 | | " |
| Commercial Beef | | | |
| Range Fed Beef | 60 | | " |
| Grass Fed Beef | 360 | | " |
| Feed Lot Beef | 1500-2000 | | " |
| Single Cell Protein | | | |
| SCP from Methanol (ICI)* | 161 | | Leach (120) |
| SCP from Petroleum (Italy)* | 185 | | " |
| SCP from Whey | 48 | | Pace & Goldstein (121) |
| SCP from Carob Pods | 87 | | Tate & Lyle (51) |
| SCP from Cellulose | 98 | Btu/ potential protein | Rosenbluth & Wilke (78) |
| SCP from Bagasse † | | | |
| Corn Production (all U.S.) | | | |
| Corn (1970) | 59 | | Leach (120) |
| Corn (1945) | 75 | | " |
| Corn (1945) | 47 | | Pimental (116) |
| Corn (1950) | 55 | | " |
| Corn (1954) | 65 | | " |
| Corn (1959) | 61 | | " |
| Corn (1964) | 57 | | " |
| Corn (1970) | 62 | | " |
| Corn (1970) | 44 | | Heichel (117) |
| Soybeans (1970) | 14 | | " |

* Feed grade processes, additional processing and hence energy expenditure required to produce food grade products.

† Insufficient data available to make calculation (55), (74).

a food (either directly as protein in the sludge or indirectly exploiting its fertilizer potential) it is an attractive process. As reported previously, in 1970, the energy required to produce fertilizers for farm use in the United States was 0.6% of the total energy consumption, i.e. 94 X $10^{12}$K cals (118). The potential of the sludge as a fertilizer should be examined in depth with an emphasis on the survival of pathogens, the heavy metal content and pesticide build up in the sludge. Several universities are examining the safety factors involved in land-spreading of the undigested solids. The feed potential of the sludge is also under investigation (*vide infra*).

In summary, while the figures presented in this analysis are by no means definitive, they do look encouraging and do not eliminate SCP production from cellulosic wastes from consideration as an effective method for stabilizing wastes with a financial subsidy (i.e. selling SCP) at a comparatively low energy expenditure.

TABLE 30

ENERGY PRODUCTION PER TON INPUT FOR ANAEROBIC DIGESTION TO METHANE

| Waste | Capacity TPD | net Btu out / ton input | Reference |
|---|---|---|---|
| Feedlot waste | 200 | $8.05 \times 10^6$ | Weisberg & Krishnan (107) |
| Feedlot waste | 9120 | $6.37 \times 10^6$ | Christopher (122) |
| MSW | 1000 | $3.25 \times 10^6$ | Kispert *et al* (112) |
| MSW | 1000 | $3.15 \times 10^6$ | Pfeffer (111) |

Environmental Impact

There is no doubt that many current methods of disposal of cellulosic wastes have a negative effect on the environment. Incineration produces polluting emissions and may not destroy pathogens which survive in incompletely burned residues (caused by overcharging, compaction or spontaneous water production during burning). The costs of complying with Federal standards for stack emissions have placed an impossible economic burden on small-scale incineration operations. The burning of crop residues in situ is now limited by enforcement of environmental legislations.

Land disposal of wastes is dependent upon suitable and available land which is increasing in cost as it decreases in supply. Leachate problems have occured even with sanitary land fills. Fecal coliforms, fecal streptococci, salmonellae and enteroviruses have been found in leachates from land fills. Animal wastes eroded from feedlots have been identified as the cause of fish kills and eutrophication.

Single cell protein growth on cellulosic wastes may produce waste effluents at several stages in the process:

(a) washings from pretreatment to improve digestibility of the cellulose;

(b) supernatant liquor after harvesting and drying of the protein;

(c) effluents from washing the product;

(d) solids remaining after fermentation.

The washings described in (a) may or may not present a disposal problem depending on the nature of the pretreatment. For example, if the cellulosic substrate undergoes alkaline heat treatment, the alkali must be either neutralized or removed prior to fermentation. If the latter, wash waters generated at this stage will contain relatively high concentrations of solubilized lignin, hemi-celluloses and hydrolyzed sugar monomers as well as caustic soda (123).

It might be possible to subject the washings to a chemical recovery process to regenerate the alkali and separate the lignin for burning, but this would require a large plant. Another alternative might be to burn the organics in an oxidation furnace to recover the energy.

If only crude SCP for a feed is required, the alkali could be neutralized with hydrochloric acid and the sodium chloride permitted to pass through the fermentation to come out in the centrifuge wash. For a high quality product, the lignin must be removed prior to degradation.

The polluting potential of the treatment liquor depends on the composition

of the wastes. For example, Black Clawson fibers produce a much cleaner stream than do agricultural wastes (123).

It is also possible to choose a pretreatment which is less likely to produce a waste with a high chemical oxygen demand (COD). For example, physical or gas-phase treatments might be chosen instead of alkaline and other liquid chemical treatments.

With alkaline pretreatment of the wastes, the effluents generated at stages (b) and (c) above, contain almost no suspended solids and therefore have a low BOD. These liquors are, however, relatively high in inorganic nutrients, and so some fraction would be recycled to the fermenter for better utilization. Remaining effluents would require some treatment in a waste stabilization pond.

The amount and composition of any sludge remaining depends on the substrate, the method of pretreatment, harvesting techniques and further processing of the product. The residue may be small if undigested fiber is included with SCP as part of the feed mixture (50). It has been reported that for MSW, overall waste reduction is small, though of course the wastes are stabilized.

Enzyme hydrolysis does not produce volumes of polluting effluents or solids as the process design includes recycle phases. After filtering, the enzyme broth goes to the hydrolysis reactor. The filter cake consists mainly of protein with some undigested cellulose. It may be sold as a feed cake. Effluents from the saccharification vessel are filtered to produce glucose syrup. The unhydrolyzed cellulosic waste is recycled to the hydrolysis vessel after drying and regrinding to improve susceptibility of the cellulose to enzyme action. Solid wastes from the reactor are mainly lignin which could be used as a fuel (61). The percentage of solids remaining after hydrolysis presumably depends on the composition of the waste. Temperature control during the process results in hot water which must be cooled prior to disposal to prevent thermal pollution.

Though the main product of anaerobic digestion of agricultural wastes is energy (methane), the sludge remaining has potential as a feed or fertilizer. If the sludge does prove a useful feed or fertilizer, this is obviously an extremely environmentally acceptable process in that it recovers both energy and nutrients while reducing the BOD of the wastes. If the potential of marketing the sludge is not realized, there is still a 50% decrease in mass for manures (81). Liquid effluents from the process will require further treatment to stabilize them.

All of the above systems result in biodegradation of materials with a high BOD, so that they are comparatively odor free, and do not attract insects and vermin. All result in a greater or lesser degree of solid waste reduction. Less land is therefore required for ultimate disposal of the solids remaining after reaction. Also, biodegraded wastes are less likely to cause settling problems in land fills, so that the land may be used for building purposes soon after the closing of the fill.

All the three processes mentioned above result in conservation of resources by reclaiming either nutrients and/or energy. The enzyme hydrolysis process is more flexible in this respect, in that the glucose produced could be fermented to ethanol, and hence to other chemicals normally derived from fossil fuels; or else used as a substrate for SCP growth. Alternatively, glucose could be directly used as an energy source in human food and animal feed. Anaerobic digestion could also produce both food and fuel. Aerobic fermentation to SCP produces either a food or a feed, and consumes energy. However, it is not as energy intensive as modern farming to produce animal protein *vide infra*.

## NON-CELLULOSIC CARBOHYDRATES

### Spent Sulfite Liquor

Spent sulfite liquor is a waste product of the pulping process in paper making. The composition of waste sulfite liquors varies, depending on the species of wood used and the nature of the pulping process (10). Hardwoods tend to yield a liquor with up to 70% pentose sugars (mainly xylose), while soft woods may contain up to 75% hexose (principally mannose). Table 31 illustrates the composition of spruce wood liquors.

In 1948, the first U.S. plant to grow yeast on spent sulfite liquor started production. Today, the firms of Boise Cascade and St. Regis Paper have production facilities of capacity 6,000 tons/year and 5,000 tons/year respectively.

Though yeasts contain around 45 - 50% protein, the original market in the United States was as a source of B-complex vitamins and other minerals. Growth of yeasts on sulfite liquor is a satisfactory means of pollution control. Yeast production removes 90 - 96% of reducing sugars and acetic acid present in waste liquors, reducing the $BOD_5$ more than 60% (10). The exact reduction in polluting capabilities of the liquor depends on their composition.

The liquors usually require treatment to remove excess sulfur dioxide prior to use as a medium for microbial growth. Stream stripping is the favored method in the United States. The amount and distribution of oxygen in the fermenter is crucial for growth of yeasts. Nitrogen, phosphorus, potassium and other nutrients must be added to the culture. As the reaction is exothermic, heat removal is required. *Candida utilis* is a favored species, as it can utilize both pentose and hexose sugars (124). *Saccharomyces cerevisiae* can only ferment hexoses.

The "Pekilo" process under development by the Finnish Pulp and Paper Research Institute involves submerged cultivation of microfungi (notably *Paecilomyces varioti*) on sulfite waste liquor and molasses. The chemical composition of "Pekilo" protein is shown in Table 32. Note that the protein content is slightly higher than Torula yeast grown on sulfite liquor (45 - 50%).

TABLE 31

COMPOSITION OF A SPENT SPRUCE SULFITE LIQUOR (125)

| Component | % | % |
|---|---|---|
| Lignosulfonic acids | —— | 43 |
| Hemilignin compounds | —— | 12 |
| Incompletely hydrolyzed 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 | 22 |
| Acetic acid | —— | 6 |
| Aldonic acids and substances not investigated | —— | 10 |

TABLE 32

CHEMICAL COMPOSITION OF PEKILO PROTEIN (69)

| Item | % of Total |
|---|---|
| Dry matter | 95 |
| Crude protein | 55-60 |
| Nucleic acids | 10-11 |
| Crude fat | 1 |
| Ash | 5 |

TABLE 33

$BOD_7$ OF A SPENT SULFITE LIQUOR BEFORE AND AFTER CONTINUOUS PEKILO FERMENTATION IN A 450 LITRE FERMENTOR (69)

| $BOD_7$ | Before fermentation | After fermentation |
|---|---|---|
| $BOD_7$, g $O_2$/l SSL | 46 | 6 |
| $BOD_7$, kg $O_2$ of pulp | 370 | 48 |

* Note: The $BOD_7$ readings show higher oxygen demand reductions than would conventional $BOD_5$ values. They are quoted because $BOD_5$ values were not found in the literature.

The hexoses, pentoses, aldonic acids and acetic acid in the liquors are readily decomposed by the fungi (69). The lignosulfonic acids and hemilignin sulfonic acids are not utilized in the "Pekilo" process. One advantage of the process is the ease with which the fungi may be harvested by filtration and mechanically dewatered.

Costs of production of "Pekilo" protein are estimated at $110 per ton (for a 10,000 ton per year plant). The market price should therefore be very competitive in the European market for feed-grade SCP (c.f. ICI figures for "Pruteen"). For the same size plant, energy consumption of the process is estimated at 1,250 kilowatt hours/ton (126).

The composition of the product is found in Table 32. The protein will be marketed as a feed for calves, pigs and poultry. Feeding trials have confirmed the value of the protein as a feed for pigs and poultry. Mixed with whey it is suitable for calves. It is reportedly 87% digestible (126).

Fungi and yeast grown on sulfite waste liquors have been found to contain small percentages of lignosulfonic acids adhering to the cell wall (127). In a study conducted in Norway, large concentrations of lignosulfonic acids (to 13%) fed to pigs resulted in diarrhea, poor weight gain and low feed conversion (127). It was felt that the very low level of lignosulfonic acids in the SCP products studies ("Pekilo" protein 0.1; *C. utilis* 0.15; *S. cerevisiae* 0.6) would probably not prevent consumption of SCP even as the sole protein source.

## Food Industry Wastes

As indicated in Table 2, food industry wastes are highly polluting and cause immense environmental damage when discharged untreated. The industry is well aware of the problem and has investigated a number of treatment and disposal methods (see Table 34).

Though each food processing facility has its own particular problems, certain procedures are found helpful throughout the industry. For example, for all wastes, separation of the solid or liquid components of the waste stream facilitates handling. The solid waste may be burned, landfilled, anaerobically digested or composted.

The Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, has investigated the growth of fungal protein on starchy substrates. The processing of potatoes results in the generation of from 2.6 to 5.3 million tons of high BOD wastes annually. Rogers and Coleman (128) have reported that growth of *Aspergillus niger* (NRRL 5474) on homogenized and pre-sterilized starchy wastes results in the complete utilization of the substrate within 20 to 48 hours. In addition to potato wastes, their process is applicable to whey, citrus and other fruit wastes.

Homogenization and sterilization of the wastes prior to fermentation was found most effective in reducing the time requirements. Homogenization

increases the surface available for microbial attack, and heat sterilization swells the fibers. The concentration of the wastes which can be successfully utilized is described in a recent patent as from 6 to 10% by weight/100 mls. of culture medium (128). The fungus grows aerobically at temperatures from 25° C to 50° C at pH values from 2.0 to 5.0. After 20 to 48 hours, the culture is sterilized and the fungus removed by filtration. The protein content of the mycelia is from 35 to 40%(by amino acid assay) and compares very favorably with the FAO reference protein.

Church *et al* have investigated the growth of fungi imperfecti as a means of treating corn and soy processing wastes (129). Twenty one strains of fungi were evaluated for their ability: to reduce the $BOD_5$ of the variable composition carbohydrate wastes; to produce protein mass; to compete with naturally occuring organisms. *Trichoderma viride, Giocladium deliquescens* and *aspergillus oryzae* were favored organisms.

The fermentations were carried to the pilot stage at the Green Giant Company canning plant at Glencoe, Minnesota. Both continuous fermentation in a 25 litre carboy and treatment in an aereated ditch were investigated. Continuous fermentations were successfully operated for several weeks duration. Except for the initial inoculation with fungi, no attempt was made to maintain aseptic conditions. Addition of sulfuric acid to pH levels 3 - 4 with steady addition of fresh substrate, stimulated steady growth of the fungi and kept bacterial and yeast contamination to a minimum.

The aereated ditch at Glencoe operated at 11,000 gallons capacity. A second pilot unit in Cedar Rapids, Iowa, used a plastic lined concrete tank of 50,000 gallons (130). At full capacity, after thirty-six hours, the fermentation operated continuously with a feed rate increased from an initial 20 gallons/minute to 35 gallons/minute.

At the Cedar Rapids plant, underflow from the fermenter was tapped into a settling tank. The underflow passed either to a vibratory screen or a filter. Overflow was pumped to a rotary vacuum filter where the fungi was received and dried.

The aereation requirements of the fungi and the degree of mixing in the fermenter were important considerations.

The amino acid analyses of *T. viride* and *G. deliquescens* indicated that the fungi contained relatively high percentages of lysine, threonine and tryptophan. Methionine percentage was above soybean meal (1.20 and 1.1 g/16 g nitrogen) but less than hoped for. Feeding trials (23% SCP + methionine) with rats showed that the protein was non-toxic and highly palatable. An estimate of net protein utilization (NPU) of 50% was considered a low value because no correction was made for non-protein nitrogen in the fungi. The study recommended more detailed feeding trials with chicks and ruminants, as well as feeding at lower percentages (129).

Table 35 summarizes the general efficiency of the process for stabilization of corn and pea-canning wastes and silage juice. These results represent

TABLE 34

COMPARISON OF VARIOUS TREATMENT AND DISPOSAL METHODS FOR FOOD INDUSTRY WASTES (2)

| Method | BOD reduction % | Relative costs | Special requirements and limitations |
|---|---|---|---|
| Primary | | | |
| Screening | 10 to 20 | Low | Provisions must be made for cleaning to prevent binding of screens. |
| Grit removal | 10 to 20 | Low | Must have continuous scrapes & removal system to prevent accumulation decomposable organic materials. |
| Oil & grease | 10 to 40 | Low | Mechanical skimming desirable, flotation may be required. |
| Sedimentation | 10 to 50 | Low | Detention times must be short enough to avoid anaerobic composition of settled solids. |
| Secondary | | | |
| Chemical treatment | 35 to 60 | Moderate | Useful in seasonal operations. Large volume of sludge produced requires extensive drying bed area. |
| Lagooning | 80 to 95 | Low | Low cost land and distance from residential areas important. Major portion of a year required for total treatment. |
| Irrigation ridge and furrow | 80 | Low | Limited to rural areas because of odor nuisances. Only a limited number of crops can be grown on land so irrigated, may create ground pollution problems. Land must be located within economic pumping distance. Only cover crops can be grown on spray irrigated land. |
| spray | 90+ | Low | |
| Trickling filter | 90+ | Moderate | Suitable for year-round operations. Difficult to operate with seasonal and variable waste flows. |
| Activated sludge | 90+ | High | Effective for high BOD wastes; difficult to use in seasonal operations. |

TABLE 35

GENERAL EFFICIENCY OF FUNGI IMPERFECTI PROCESS (129)

| Component | Corn Canning Wastes | Pre-Canning Wastes | Silage Wastes |
|---|---|---|---|
| $BOD_5$ removal (%) | 96 | 95 | 80 |
| COD removal (%) | 88 | 81 | 83 |
| TOC removal (%) | 93 | 87 | 85 |
| Mycelium produced per unit $BOD_5$ removed | 0.5 | 0.6 | 0.3 |
| Sulfuric acid use (lb/1000 gallons) | 4.0 | 6.5 | 8.1 |
| Retention time (hours) | 22 | 18 | 18 |

TABLE 36

EFFICIENCY OF REDUCING SUGAR UTILIZATION BY *Morchella hortensis* (131)

| | Yield Grams of Mycelium per 100 g Reducing Sugar | | Protein Content of Mycelium | Protein Efficiency grams per 100 g Reducing Sugar | |
|---|---|---|---|---|---|
| Substrate | Supplied | Consumed | % | Supplied | Consumed |
| Whey | 23.5 | 43.6 | 34.5 | 8.12 | 14.6 |
| Pumpkin Extractor Waste | 27.5 | 48.1 | 35.2 | 9.69 | 16.9 |
| Corn Canning Waste | 22.3 | 33.5 | 32.7 | 7.29 | 11.3 |

an average for the pilot plant ditch. More complete BOD and COD removal was accomplished in the laboratory ($BOD_5$ reduction above 99%, COD reduction about 96%) (129). The reasons given for this discrepancy were; the finer fungal mass produced in the pilot plant, lower temperatures of operation and the variable composition of the wastes stabilized in the pilot study.

A 1973 economic analysis of the process based on an annual $BOD_5$ of 1.08 x 106 lb indicated that with sale of the protein, the wastes could be treated with profit. A net surplus of over 1¢/lb of $BOD_5$ would be realized for a flow of 3,000,000 gal/day and an initial investment of $1.15 million (130).

The Battelle Memorial Institute has investigated submerged cultural growth of three *Morchella* species of mushroom on various food processing wastes, including whey, pumpkin and corn, and canning wastes. Of the species grown, *Morchella hortensis* gave highest yields of protein, while corn canning wastes were the most poorly utilized (131).

The carbon to nitrogen ratio was identified as key to efficient utilization of the substrate by the fungi. An 8:1 ratio gave better yields than higher ratios. Addition of ammonium salts and inorganic phosphates increased production of mycelial mass. Protein content of *Morchella hortensis* on the different media is indicated in Table 36.

Marketing of the product as a flavoring ingredient to compete with dried mushrooms was considered, but abandoned for economic reasons.

Battelle with the Fats and Proteins Research Foundation have investigated enzyme hydrolysis of rendering plant raw materials. A slurry of shop and fat bone was agitated for two hours at 120° F with a fungal or bacterial proteolytic enzyme. Centrifuging of the products gave hydrolyzed protein, bone and tallow and a sludge for recycle (132). The product typically contained 74% protein. A satisfactory amino acid profile could be produced by blending raw materials of different composition. An economic analysis concluded that the break even capacity of plant was probably 150 tons/day.

## Brewery Wastes

Brewery wastes have an extremely high polluting potential. The spent grain liquor may have a BOD of from 56,000 to 132,000 mg/litre (133). In the past, the waste stream has been treated by municipal facilities. Higher costs of treatment and enforcement of pollution standards have stimulated research into protein growth on brewery wastes. Shannon and Stevenson reported that growth of four species of yeast and four types of fungi on brewery spent liquors produced reasonable yields. The quantities ranged from a low of 2.45 g/l for *M. esculenta* to a high of 6.46 g/l for *C. Steatolytica* grown on fermentation sludge liquor (134). Yields on trub press liquor (TPL) were higher, ranging from 8.94 g/l for *S. cerivisae* to 20.05 g/l for *P. ostreatus*. Table 37 summarizes their results.

BOD reduction for growth of yeasts or fungi on grain press liquor (GPL),

TABLE 37

REDUCING SUGAR UTILIZATION AND YIELDS OF ORGANISMS GROWN ON SELECTED BREWERY WASTE (134)

| Organism | GPL | | | TPL | | | FSL | | |
|---|---|---|---|---|---|---|---|---|---|
| | Red. sugar usage % | (1) | (2) | Red. sugar usage % | (1) | (2) | Red. sugar usage % | (1) | (2) |
| *S. cerevisiae* | 88.5 | 5.02 | 0.189 | 66.4 | 8.94 | 0.245 | 83.3 | 5.21 | 0.631 |
| *S. uvarum* | 89.4 | 4.94 | 0.177 | 73.5 | 8.95 | 0.221 | 84.5 | 4.02 | 0.480 |
| *C. utilis* | 73.5 | 5.09 | 0.231 | 58.1 | 9.86 | 0.308 | 78.7 | 3.65 | 0.468 |
| *C. steatolytica* | 74.2 | 6.28 | 0.282 | 63.6 | 10.56 | 0.302 | 81.0 | 6.46 | 0.805 |
| *P. ostreatus* | 69.2 | 3.81 | 0.184 | 52.7 | 20.05 | 0.691 | 74.7 | 3.42 | 0.462 |
| *M. esculenta* | 78.3 | 3.28 | 0.139 | 53.6 | 16.88 | 0.570 | 72.2 | 2.45 | 0.343 |
| *A. bisporus* | 83.2 | 3.45 | 0.138 | 54.5 | 11.32 | 0.377 | 87.1 | 3.38 | 0.392 |

(1) - g/litre (dry wt basis)
(2) - g/g sugar (dry wt basis)

trub press liquor(TPL), fermented sludge was in the range of 20 - 45% (134).

In a related study, Shannon and Stevenson found that addition of ammonium sulfate and fermented sludge to GPL and TPL improved BOD reduction (133). The highest BOD reduction for growth of C. *gigantea* on augmented GPL was 75%. The same yeast reduced the BOD of enriched TPL by 65%.

Hang *et al* at Cornell University have investigated growth of *Aspergillus niger* on spent grain liquor (135). They have reported a 97% conversion of sugar to fungal mass and yields of approximately 57% (based on sugar usage). BOD reduction approached 96% (decreasing from 22,500 mg/litre to 900 mg/litre).

## Acid Food Wastes

The stabilization of acid food wastes has also been studied at Cornell. Wastes such as sauerkraut brine, pickled beets, and olive brine have a very high biochemical oxygen demand, a large concentration of sodium chloride and low pH values. Table 38 gives the typical composition of acid brine obtained from a sauerkraut factory. Hang *et al* have investigated both growth of fungi and yeasts on sauerkraut brine. They have reported that the fungi *Geotrichum candidum*, completely neutralized the brine and reduced the $BOD_5$ by 88% (72). The mycelium was easily harvested by filtering at a yield of around 13 g/l of brine (or a 62% yield based on BOD removal). The fungi contained approximately 39% protein and could be sold as a feed supplement.

Aeration requirements for yeast production on sauerkraut waste were found to limit the growth of the organisms and impede removal of BOD and lactic acid (71).

TABLE 38

TYPICAL COMPOSITION OF SAUERKRAUT BRINE (72)*

| Component | mg/l |
|---|---|
| BOD | 24,000 |
| Lactic acid | 19,900 |
| Kjeldahl nitrogen | 1,100 |
| Total phosphorus | 192 |
| Sodium chloride | 26,500 |

* pH value of 3.4

*C. utilis* neutralized the brine and reduced BOD by 90%. Pilot plant studies in commercial factories have so far indicated that *C. utilis* is a promising organism for biograding sauerkraut brine (136).

Growth of fungi on beet brine has also been investigated at Cornell (137). *Aspergillus niger* reduced COD by 93.4% in 72 hours. Nitrogen reduction was 75% and phosphorus removal 72%. The pigments in the beet juice were almost entirely degraded (up to 96%). The yield of *A. niger* was approximately 52 gms/100 gms of COD removed.

Many food processing wastes of differing compositions have been investigated as possible substrates for microbial growth. Carbohydrate wastes and acid brines have high polluting potential and must be stabilized prior to disposal.

The biodegradation of these wastes by yeasts or fungi is technically feasible. The limited economic analyses performed are encouraging. If the micro-organisms grown do find a market as an animal feed, this type of management technique will possibly operate with profit. It is emphasized that toxicological and nutritional studies must establish the safety, digestibility and palatability of the SCP product for each category of waste.

## WHEY

Though whey was a popular beverage in seventeenth and eighteenth century Europe, it presents a serious disposal problem in twentieth century America. In 1975, in this country alone, approximately 30 billion poinds of whey were produced as a by-product of the cheese-making industry (138). Only half this quantity is currently used as a food or feed (139); the remainder presents an ever more costly treatment problem, as cheese-making factories struggle to comply with Federal water pollution standards.

Liquid whey has a $BOD_5$ of from 32,000 parts/million (ppm) (140) to 60,000 ppm. Thirty billion pounds of whey are equivalent to a sewage demand of 21 million people (138). The cost of building waste treatment plants to handle this quantity is estimated at $1 billion with annual operating and maintenance costs of approximately $39 million (138).

### Composition

Liquid whey from a cheese plant is 93 - 94% water (139). Sweet and acid wheys produced from cheddar and cottage cheeses respectively, differ slightly in total solids composition (see Table 39). The main solid ingredient of whey is lactose (approximately 66 - 74% by weight). High quality protein represents some 12 - 13% total solids, together with smaller quantities of ash, fat and lactic acid. Cottage cheese whey has a higher lactic acid content than cheddar whey. The cost of concentrating and drying the highly perishable liquid has in the past limited utilization of the solids. For some uses of whey (e.g. baby food), it is also necessary to remove the ash content of the whey which may constitute 11% of solids. Technical improvements stimulated by enforcement of pollution control legislation, make utilization of the whey preferrable to disposal.

Thirty billion pounds of whey could provide 1.3 billion pounds of lactose and 0.25 billion pounds of protein (138). Whey protein has an excellent amino acid pattern, and with a protein efficiency ratio of 3.0 to 3.2 (c.f. casein at 2.5) is second only to the protein of egg white in quality (141). The energy value of whey solids is slightly higher than most feed grains and is equivalent to the energy value of shelled corn (141). Lactose is more than just an energy source. It increases protein digestibility and nitrogen retention when fed to nonruminants, aids mineral balance in the blood stream and is the source of galactose, a sugar needed for repair of delicate brain and nerve tissue (138). It must be noted at this point that some individuals with a low lactose enzyme activity experience intense gastro-intestinal discomfort on consumption of lactose.

## Utilization of Whole Whey

Because of the expense of transportation, in relation to its solids content, and problems of spoilage, liquid whey has been used as a feed for swine and cattle only on farms close to the site of production. For swine, growth rates were acceptable for consumption of whey up to 20% dry matter intake (141). Both sweet and acid wheys have been successfully fed to cattle. The few problems encountered include excessive urination at high intakes, and some scouring and off-feed if high levels of whey consumption were introduced too suddenly. Teeth erosion was noted when whey was stored for some time and mixed with molasses prior to feeding (141).

Many possible whey utilization systems have been proposed (see Figure 8). It is possible to concentrate and dry whey liquid to produce a powder for food/feed consumption. Kraft spray dries sweet whey from its cheese-making facilities to produce a food-grade powder primarily utilized in-house as a substitute for nonfat milk solids (142) although the lower protein content of dry whey limits this substitution for some uses. Whey powder reportedly has special properties which are not demonstrated by nonfat dry milk (NDM). Whey powder is a flavor enhancer and according to Kraft, adds shortness and tenderness to bakery products.

Condensed whey has been fed to cattle and pigs with satisfactory results. Addition of molasses improves the palatability to cattle. Addition of urea and/or silage to whey increases the nutritive value but reduces the palatability (141). Anaerobic fermentation of whey to lactic acid followed by addition of anhydrous ammonia produces a feed with digestibility approximately equal to soybean meal. The ammonium lactate together with unseparated bacterial cells can be fed to cattle to provide up to 85% of required nitrogen (14).

Addition of dry whole whey to alfalfa silage, urea-treated corn silage and other grass or legume silages improved the fermentation characteristics of the silage. Feeding trials with whey-treated silages have generally resulted

TABLE 39

DRY SOLIDS IN CHEESE WHEY (138)

| Component | Cottage | Cheddar |
|---|---|---|
| | % | % |
| Protein | 13.0 | 12.9 |
| Lactose | 66.5 | 73.5 |
| Ash | 10.2 | 8.0 |
| Fat | 0.1 | 0.9 |
| Lactic Acid | 8.6 | 2.3 |

in higher digestibility than the control. Though nutritional trials with lactating animals have been limited, cows fed urea-treated corn silage with a 1% addition of dry whey ad libitum showed a 6.5% increase in milk production over the control (urea-treated corn silage) (143).

Whey liquid can be concentrated, sterilized and flavored (particularly with citrus fruits) to produce nutritious beverages. Many recipes for flat and carbonated, alcoholic and non-alcoholic beverages have been tested. Perhaps the most commercially successful product to date is a fermented beverage prepared from deproteinized whey and sold in Western Europe under the trade name of Rivella. Twenty to thirty million litres of Rivella are sold annually (144).

## Utilization of Whey Components

In addition to recovering and utilizing the whey solids intact, it is possible to separate the protein and lactose components and to remove the mineral salts. In the United States, mineral salts which adversely affect palatability, functionality, etc., are removed by electrodialysis. Electrolytes pass through ion-selective, semi-permeable membranes under the influence of an electric potential (138). Removal of salts on ion-exchange columns presents a greater problem of sanitation, uses large volumes of water, and thus produces copious quantities of waste water.

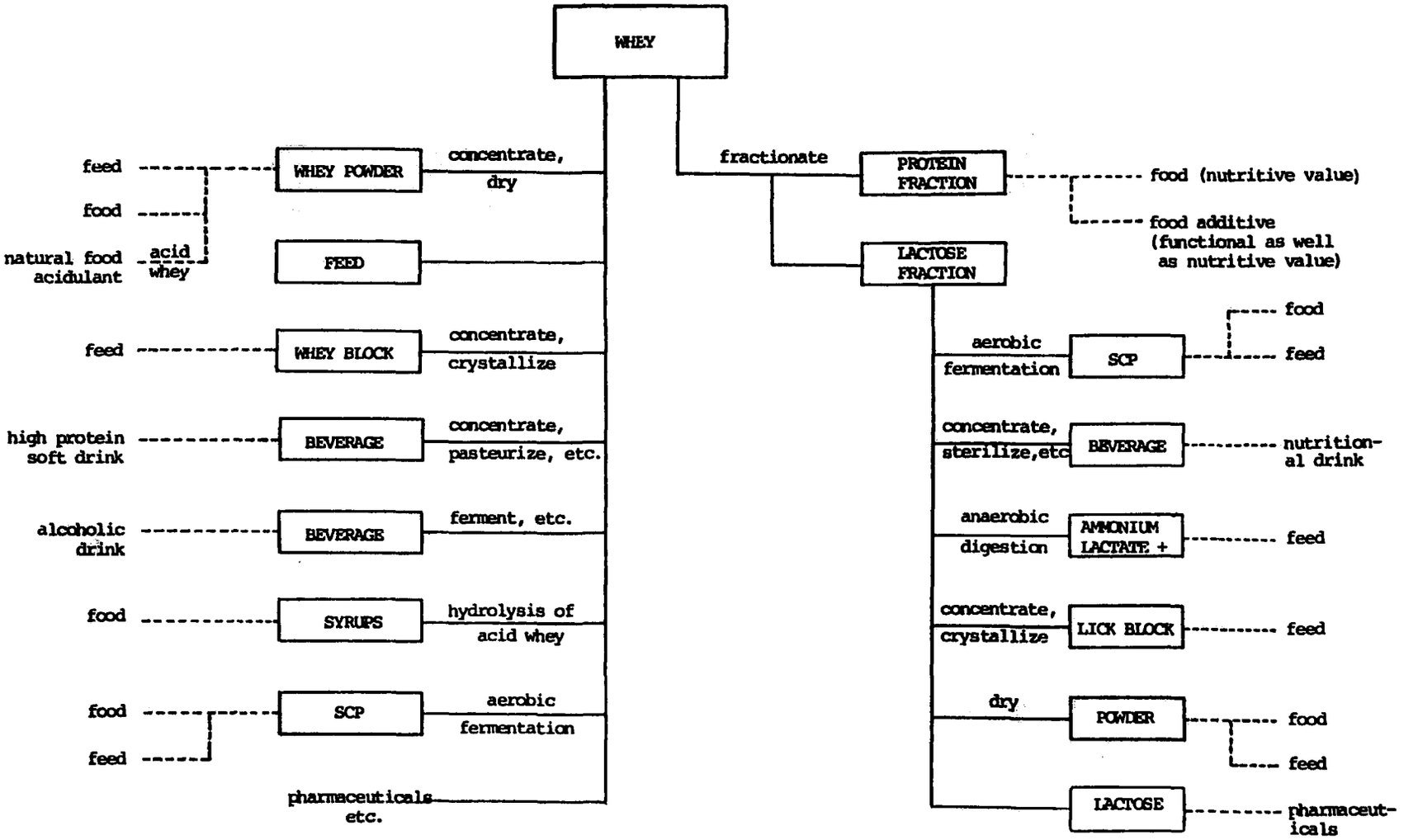


Figure 8. Possible Whey Utilization and Salvage Systems

Several methods are in commercial production for either crystallization of the lactose or precipitation of the protein. Lactose may be crystallized from cooling concentrated whey with or without chemical solubilization of the protein, though chemical solubilization produces a higher yield (138).

Protein may be precipitated from the whey by addition of various chemicals (including ferric chloride, aluminum chloride, carboxymethyl cellulose, etc.) or by heat coagulation. Most chemical additives produce denatured protein with poor functional properties, though addition of long chain polyphosphates followed by centrifugation results in undenatured protein.

Most recently, techniques to concentrate and/or separate the solid components have been developed. In reverse osmosis, water, ionizable salts and lactic acid are removed from the whey by applying a pressure in excess of the osmotic pressure, to force small molecules through a semi-permeable cellulose acetate membrane about 1/3 micron thick (145). A backing of porous material supports the membrane yet allows water to pass through. Several designs are available including: the spiral wound module, the membrane stack and the most popular tubular model. The tubular model consists of a hollow support tube lined with a continuous membrane through which the whey is circulated. The water passes through the tubes and collects outside. The extent of water, salts and lactic acid removal depends on the pressure used. Too great a pressure will destroy the membrane or shorten its life considerably (145).

Ultrafiltration uses hydraulic pressure with a special membrane to separate the smaller sized molecules including lactose, from the larger protein molecules and fat globules. Because of the nature of the membrane, lower pressures are required for ultrafiltration than for reverse osmosis. Protein concentrations ranging up to 60% of solids may be obtained. Ultrafiltration produces a pure, undenatured protein concentrate with a composition equivalent to skim milk, and a watery fraction containing lactose (138).

The cost of the membrane accounts for around 30% of the total cost of the process and the membrane has a life time of about one year (146). The development of membranes with a longer lifetime would reduce the cost of ultrafiltration. Also needed are membranes which can be easily sterilized by heat and/or chemicals without damage to them.

Whey protein concentrate produced by ultrafiltration costs around $1/lb. for a 50% protein product (dry weight). The cost of nonfat dry milk is around 73¢/lb with a 36% protein content on a dry weight basis (146).

Gel filtration is another process to fractionate whey solids. Liquid whey passes along a column containing sephadex beads and the components of the whey are eluted at varying rates depending on the size of the molecule (138).

Whey protein concentrate (WPC) has been evaluated as a replacement for nonfat dry milk by the Agricultural Research Service, U.S. Department of

Agriculture. The essential amino acids are all present in concentrations exceeding the FAO reference. Addition of 40% WPC to nonfat dry milk raised the protein efficiency ratio of the milk from 2.51 to 2.83 (147). The digestibility of the protein, as measured in feeding trials with rats, was superior to casein. Whey protein concentrate demonstrated very acceptable functional properties; it retained water well, and formed stable whips by manipulation of heat and pH, but was denatured (about 20%) at normal pasteurization temperatures (148).

Human taste panels more easily detected the flavor of acid WPC than sweet WPC in a blend with skim milk. Even when whey was detected, the majority of tasters rated a 40% sweet WPC concentration and a 20% acid WPC concentration at least "satisfactory" in flavor (149).

While there are many possible markets for the high quality WPC, uses for the immense volumes of permeate are perhaps more limited. The volume of the lactose fraction is 90% of that of the original whey and has only a slightly reduced BOD (150). The U.S. Department of Agriculture, Beltsville, Maryland, has investigated the preparation of solid feed blocks from the permeate. The deproteinized whey was evaporated to a total solids concentration of about 30% and the pH was adjusted to 6.2 - 6.4 with anhydrous ammonia. When the total solids concentration reached 65 - 70%, the concentrate was pumped into forms and air-dried for 1 to 2 days. A 45 - 50 lb. dry cake could be prepared from an initial volume of 90 gallons of permeate.

The cake was evaluated as a lick block for cattle. The ammonia increased the protein equivalent of the block and improved palatability, especially of the acid whey permeate blocks (150). Other materials which could be added to the block include urea, soybean meal, molasses, potato peelings, etc. The initial phase of controlled feeding studies at the Ruminant Nutrition Laboratory, U.S. Department of Agriculture at Beltsville, is substantially complete. Holstein calves fed pelleted alfalfa and corn ad libitum voluntarily consumed from 15 to 25% total dry feed from the blocks. Weight gains equivalent to the control were maintained. Nutritional trials are continuing and will eventually include feeding the lick block to lactating animals. A number of companies are moving to commercialize this process.

It is easier to spray dry the permeate of acid whey produced through ultrafiltration than it is to dry the whole whey. The deproteinized whey powder can be used in foods, feed mixtures and in bacterial media (151).

Another possible use of the permeate is as a substrate for growth of *Saccharomyces fragilis*. A recent evaluation indicated that the economics of combined ultrafiltration to produce WPC and fermentation of the permeate to produce yeast are very attractive. It is reported that for plants of varying capacities (1/4 million to 1 million lbs. of lactose permeate/day) the sale of WPC would cover costs, while the marketing of the yeast would represent profit (121).

Yeast can, of course, be grown on the lactose in whole whey without prior fractionation. Knudsen Wheast* (*Saccharomyces fragilis*) was grown on fresh cottage cheese whey and contained 54 - 56% protein. The firm has now been taken over by the Stauffer Chemical Company which has just built a new combined ultrafiltration/gel-filtration plant in California (146).

Growth of the fungus, *Aspergillus niger*, on the lactose of acid whey has been studied at Cornell University. Beta-galactosidase manufactured by the fungus hydrolyzed the lactose to mono-saccharides. The syrupy product was concentrated to 70% total solids and either heat-treated to precipitate the protein, or deproteinized and demineralized to produce a non-salty neutral syrup. Both types of syrup were satisfactorily added to a variety of foods (152).

## Summary

It is clear that whey is changing its status from an unwanted polluting by-product of the cheese-making industry to a valuable and versatile food/feed additive with a high nutritional value and unique properties. The technology exists either to concentrate and dry whole whey, or to fractionate it into two potentially valuable components.

While whey consumption will probably never have the social and medical status achieved in the seventeenth and eighteenth centuries, the future for whey salvage looks very promising.

* Trade name

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# APPENDICES

## APPENDIX I, A Note on Cost Updating

Costs and prices presented in text tables were adjusted to March, 1976 figures using specific cost series, when available. The Bureau of Labor Statistics indexes (Monthly Labor Review, 99:5, May, 1976) with their implied yearly cost changes were applied. Specific adjustments used the following series:

| Product (series base = 1967) | Average yearly price increase |
|---|---|
| Soy bean oil | .07 |
| Crude fuel | .21 |
| Food stuffs | .10 |
| Iron and steel | .12 |
| Hay | .09 |
| Plant fibers | .10 |
| Sugar and confectionary products | .11 |
| Coal | .30 |
| Electric power | .11 |
| Refined petroleum products | .19 |

In the absence of specific indexes, costs were adjusted by an annual .10 inflation factor. Capital, as well as product costs, were adjusted in this manner.

The reader should remember that many products covered in text analysis are subject to severe seasonal and cyclical price fluctuations. This is particularly the case with agricultural products of all kinds.

## APPENDIX II, A Discussion of Cost Estimates

Methods of generating cost estimates, based almost entirely on computer simulation and/or a patching together of opinion and fact about equipment and

performance, differed widely among studies reviewed. As was mentioned in the text, it is impossible to be confident about "optimum" plant size and cost configuration without production and marketing data. But some areas of estimation that were treated quite casually by most researchers deserve special mention:

- Labor cost: techniques of estimation varied here from selecting plausible hourly wage averages to taking labor cost as a per-cent of total investment. It is impossible to check these estimates with reasonable figures from existing processing plants.

- Capital: in some cases, researchers in this field confused start-up costs with long-run capital costs. Average total costs will fall over time, once start-up costs have been paid and experience with the technology gained. Long-run costs reflect the level of equipment and other capital needed to support an operating plant.

- Ownership: some researchers make the distinction between private ownership (which implies tax payments as part of costs) and public ownership (which does not), though most ignore the difference.

In general, studies reviewed seemed to have equipment specifications and costs fairly well in hand. Other costs, however, tended to reflect informed judgment or unsupported guesses.

Unit costs used in text tables for this report were computed from data provided or recorded in published studies, if usable figures were available. For most of the studies used, computations were required:

- costs/input: in food and fuel studies, costs/input were obtained by computing average daily costs and dividing by rated daily capacity. In some cases where the tonnage of product out was given, it was necessary to calculate the tons input from the mass balance sheet.

APPENDIX III, Treatment of Capital in Various Studies

1. Brownstein and Constantinides Petro-Chemical-Based SCP Processes, (1975)

Total capital costs (301.37 tons/day capacity)
SCP growth on:

| | |
|---|---|
| paraffins | $ 90.5 million |
| gas oil | 106.7 million |
| methanol | 66.0 million |
| methane | 72.2 million |

Break-down
Not broken down by type, module

2. Inman An Evaluation of the Use of Agricultural Residues as Energy Feedstock, (1975)

Total capital costs

Pyrolysis

| | |
|---|---|
| 29% moisture content manure, low Btu gas 161.1 tons/day capacity | $6.9 million |
| High Btu gas, methanation of 29% MC gas additional -- same capacity | 2.0 million |
| 50% moisture content manure, low Btu gas 229.6 tons/day | 7.4 million |
| 35% moisture content crop residue, 300 Btu gas 133.6 tons/day capacity | 6.9 million |

Break-down

Not broken down by type, module

Amortization: 10%/year

3. IR&T Problems and Opportunities in Management of Combustible Solid Wastes, (1972)

Total capital costs

Waste co-fired with coal

| | |
|---|---|
| 900 tons/day capacity | $5.2 million |
| 1960 tons/day capacity | 8.8 million |

Break-down

| Units | 900t | 1960t |
|---|---|---|
| Front-end | $811,000 | $1,288,000 |
| Processing | 3,427,000 | 6,432,000 |
| Firing | 973,000 | 1,060,000 |

Start-up costs not included

4. Dynatech Fuel and Gas Production from Solid Waste, (1974)

Total capital costs (1000 tons/day capacity)

Anaerobic digestion $22.2 million

Break-down

| Units | capital |
|---|---|
| Front-end | $ 111,500 |
| Processing and upgrading | 11,995,746 |
| Buildings | 1,000,000 |
| Contingency, design and working capital | 9,084,914 |

Land not included
Amortization: 20 years, straight line

5. Pfeffer Reclamation of Energy from Organic Refuse: Anaerobic Digestion Processes, (1974)

Total capital costs (1000 tons/day capacity)
$14.3 million

Break-down

| Units | capital |
|---|---|
| Front-end | $2,917,000 |
| Processing and incineration | 9,340,000 |
| Upgrading | 2,032,000 |

No start-up costs, no specific building and land costs
Amortization: 10 years base, sensitivity test on various figures from 10 to 25 years

6. Rosenbluth and Wilke Enzymatic Hydrolysis of Cellulose, (1970)

Total capital costs (10 tons/day capacity)
$2.2 million

Break-down

| Units | capital |
|---|---|
| Plant | $1,375,810 |
| Start-up contingency | 860,000 |

Amortization: 10 year, straight line

7. Tate and Lyle Production of Single Cell Protein from Agricultural and Food Processing Wastes, (May, 1975)

Total capital costs

| | |
|---|---|
| .33 tons/day capacity | $ 56,000 |
| 1.5 tons/day capacity | 156,000 |

Break-down

| Units | .33t | 1.5t |
|---|---|---|
| Front-end | $ 7,000 | $ 12,000 |
| Processing | 44,000 | 139,000 |
| Buildings | 5,000 | 14,000 |

No start-up costs
No land costs

Amortization: 5 years, straight line

8. Weisberg and Krishnan Engineering Design and Economic Feasibility of a Feedlot Waste Bio-Conversion System, (1975)

Total capital costs (200 tons/day capacity)

| | |
|---|---|
| low pressure, low purity methane | $1.7 million (lplp) |
| low pressure, high purity methane | 2.8 million (lphp) |
| high pressure, high purity methane | 2.9 million (hphp) |

Break-down

| Units | lplp | lphp | hphp |
|---|---|---|---|
| Front-end | $ 168,000 | $ 168,000 | $ 168,000 |
| Processing | 1,148,000 | 1,148,000 | 1,148,000 |
| Upgrading | 130,000 | 1,030,000 | 1,130,000 |
| "Engineering contingency" | 289,000 | 470,000 | 489,000 |

Amortization: 15 years, straight line

APPENDIX IV, Energy Calculations

A. Recalculation of Data

1. Energy Consumption Corn Production based on Pimental *et al* (116)

In 1945, for corn production in the U.S.,

$$\frac{\text{K cal return}}{\text{K cal input}} = 3.70$$

Corn contains 9% protein and 1 lb. of corn returns 1800 K cals, therefore, to convert to gms of protein,

$$\text{Protein in corn} = \frac{1 \text{ lb corn}}{1800 \frac{\text{K cals}}{\text{lb corn}}} \times 454 \frac{\text{g corn}}{\text{lb corn}} \times 0.09 \frac{\text{protein}}{\text{lb}}$$

$$= 0.0227 \text{ gm protein in corn/K cal return}$$

To convert to Btu's, multiply by $\frac{1 \quad \text{Btu}}{0.252 \text{ K cal}}$,

$$\text{Energy consumption} = \frac{\text{K cal input}}{\text{g protein in corn}} \times \frac{1 \quad \text{Btu}}{0.252 \text{ K cal}}$$

or,

$$\text{Energy consumption} = \frac{1}{\frac{\text{K cal return}}{\text{K cal input}} \times \frac{0.0227 \text{ g protein}}{\text{K cal return}} \times \frac{0.252 \text{ K cal}}{\text{Btu}}}$$

$$= \frac{1}{3.70 \times 0.0227 \times 0.252 \frac{\text{Btu}}{\text{g protein}}}$$

$$= 47.2 \text{ Btu input/g protein}$$

Note the following ratios were used for $\frac{\text{K cal return}}{\text{K cal input}}$,

| 1945 | 1950 | 1954 | 1959 | 1964 | 1970 |
|---|---|---|---|---|---|
| 3.7 | 3.18 | 2.67 | 2.88 | 3.06 | 2.82 |

2. Corn production, USA 1970, based on Leach (120)

$$\frac{\text{Energy in}}{\text{protein}} = 62.1 \times 10^6 \frac{\text{joules}}{\text{Kg protein}}$$

1 Btu is approximately equivalent to 1055.79 joules

$$\text{therefore, } \frac{\text{energy in}}{\text{protein}} = \frac{62.1 \times 10^6 \text{ joules}}{\text{Kg protein}} \times \frac{1 \text{ Btu}}{1.05579 \times 10^3 \text{ joules}}$$

$$= \frac{58.8 \text{ Btu} \times 10^3}{\text{Kg protein}}$$

$$= 58.8 \text{ Btu input/g protein}$$

3. Soybean Production, USA 1970, based on Heichel (117)

$$\text{For soybeans, } \frac{\text{protein}}{\text{energy input}} = \frac{0.28 \text{ K protein}}{\text{M cal input}}$$

$$\text{For corn, } \frac{\text{protein}}{\text{energy input}} = \frac{0.09 \text{ Kg protein}}{\text{M cal input}}$$

$$\text{for corn, therefore, } \frac{\text{energy in}}{\text{protein}} = 44.09 \text{ Btu/g protein}$$

(converting M cals to Btu's and Kg's to g)

If we assume the same calorific value for 1 lb of soybeans as 1 lb of corn, i.e. 1800 K cals per pound of crop, using the protein ratio 0.09/0.28, it is possible to estimate the energy input/g protein for soybeans

$$\text{For soybeans, } \frac{\text{Energy in}}{\text{protein}} = \frac{0.09}{0.28} \times \frac{44.09 \text{ Btu}}{\text{g protein}} = 14.17 \text{ Btu input/g protein}$$

B. More detailed Analyses

1. SCP from Whey based on Pace and Goldstein (121)

(a) raw materials:

| Material | lbs. amount/day | k cals to manufacture (118) | K cal/day total |
|---|---|---|---|
| ammonia | 2544 | 5000 | $12.72 \times 10^6$ |
| phophoric acid | 1152 | 540 | $0.62 \times 10^6$ |
| potassium chloride | 1224 | 530 | $0.65 \times 10^6$ |
| sulfuric acid | 456 | nil | -0- |
| u.f. cleaning solution | 200 | 1000 | $0.20 \times 10^6$ |

Raw material energy input = 14.19 K cal/day

$$= 14.19 \text{ K cal/day} \times \frac{\text{Btu}}{0.252}$$

$$= \underline{56.31 \text{ Btu input/day}}$$

(b) Utilities

Electricity

$$\text{electrical power} = 1400 \frac{\text{Kwhr}}{\text{day}} \times 3000 \frac{\text{K cal}}{\text{Kwhr}} \times \frac{\text{Btu}}{0.252 \text{ K cal}}$$

$$= 16.67 \times 10^6 \text{ Btu } \frac{\text{input}}{\text{day}}$$

Steam

$$\text{steam input} = 216 \times 10^3 \text{ lbs} \times \frac{361 \text{ K cal}}{\text{lb}} \times \frac{\text{Btu}}{0.252 \text{ K cals}}$$

$$= 309.0 \times 10^6 \frac{\text{Btu input}}{\text{day}}$$

Water

Note that the heat input is calculated without consideration of the heat generated in the fermentor which must be removed by the process water. The raw data considered did not make any mention of a heat recycle.though it should be noted that an increased awareness of energy conservation would lower the energy needs of the system.

$$MC_p \Delta T = (168{,}000 \frac{gal}{day}) (8.34 \frac{lbs}{gal}) (0.32 \frac{Btu}{16°F}) (94° - 40° F)$$

$$= 24.2 \times 10^6 \frac{\text{Btu input}}{day}$$

Totals

| Item | Btu input/day x $10^6$ |
|---|---|
| Raw material | 56.3 |
| Electricity | 16.67 |
| Steam | 309.0 |
| Water | 24.2 |
| Bldg, supplies, packaging, etc. | 16.67 (same as electricity, rule of thumb) |

Total = $422.84 \times 10^6 \frac{\text{Btu input}}{day}$

Note: Not counting labor energy or material preparation

From 24,800 lbs SCP daily in bulk production if we assume the yeast is 50% protein, then total single cell protein (approximation) = 12,400 lbs/day.

Combined ultrafiltration and fermentation produces whey protein concentrate yeast protein.

For every 100 lb SCP produced there is also an additional 28.6 lb of whey protein concentrate.

Hence 24,800 lbs SCP will also be associated with $\frac{24{,}800 \times 28.6}{100}$ lbs of WPC

$$= 7092.8 \text{ lbs/day}$$

Total protein (WPC + SPC) = 19,492.8 lbs/day

$$= 19{,}492.8 \text{ lbs/day} \times \frac{454 \text{ g}}{1 \text{ lb}}$$

$$= 8.8 \times 10^6 \text{ g/day}$$

Total Btu input = $\frac{422.84 \times 10^6 \frac{\text{Btu input}}{day}}{8.8 \times 10^6 \text{g/day}}$ = 48.05 Btu input/ day

2. SCP from Carob based on Imrie and Vlitos (51)

Power consumption of plant = 3,000 K cal/Kg dry SCP

$$= \frac{3{,}000 \text{ K cal}}{\text{Kg SCP}} \times \frac{\text{Btu}}{252 \text{ cal}}$$

= 11.9 Btu input/g protein

| Inputs | Protein from Petroleum (Btu input/g protein) | Tate & Lyle (Btu input/g protein |
|---|---|---|
| Paraffins (calorific value) | 58.537 | 0 |
| Electricity | 10.9 | 11.9 |
| Fuel | 34.0 | 0(no cooling or refrigeration) |
| Steam | 51.095 | 51.1 |
| Chemicals | 10.44 | 5.00 (economy of scale) |
| Water | 16.32 | 16.32 |
| General Supplies | 0.43 | 0.43 |
| Bldg. Equipment | 2.58 | 2.58 |
| Packaging | 0.143 | 0 |
| General Overhead | 0 | 0 |
| Labor | 0 | 0 |
| Total | 184.63 | 87.33 (based on best estimates) |

3. Enzymatic Hydrolysis of Cellulose to Glucose based on Rosenbluth and Wilke (78)

Use charts show that the process begins with 20,000 lbs/day of cellulose and ends with 700 lbs of protein and 18,300 lbs/day of glucose

Utilities

(a) Electrical Power = 380 Kw = 9120 Kwhr (24 hour day)

Electrical input = $9120 \frac{\text{Kwhr}}{\text{day}} \times 3000 \frac{\text{K cal}}{\text{Kwhr}} \times \frac{\text{Btu}}{0.252 \text{ K cal}}$

$= 108.6 \times 10^6$ Btu/day

(b) Steam = 5,542 lbs/hr

$= \frac{5{,}542 \text{ lbs}}{\text{hr}} \times \frac{24 \text{ hr}}{\text{day}} \times 361 \frac{\text{K cal}}{\text{lb}} \times \frac{\text{Btu}}{0.252 \text{ K cal}}$

$= 190.5 \times 10^6 \frac{\text{Btu input}}{\text{day}}$

(c) Gas = $193 \frac{\text{scf}}{\text{hr}}$

$= 193 \frac{\text{scf}}{\text{hr}} \times 24 \frac{\text{hr}}{\text{day}} \times 0.0448^* \frac{\text{lbs}}{\text{scf}} \times 6100 \frac{\text{K cal}}{\text{lb}} \times \frac{\text{Btu}}{0.252 \text{ K cal}}$

Gas input = $5.02 \times 10^6$ Btu/day

*at STP

(d) Process Water

Note: The heat input to the preheat section of the process was inventoried. Since the microbial reaction is exothermic it is assumed that this reaction heat is used to heat up the reaction vessel and contents to proper temperature while the remaining heat is dissipated without contribution to the system. It should be noted that an increased awareness of energy conservation and heat recycle to the system should lower the energy needs of the system.

$$Q = M \ C_p \ \Delta T$$

energy input = $(42{,}000 \frac{\text{gal}}{\text{day}})(8.34 \frac{\text{lb}}{\text{gal}})(0.32 \frac{\text{Btu}}{\text{lb°F}})(392° - 84°\text{F})$

$= 34.5 \times 10^6 \frac{\text{Btu input}}{\text{day}}$

Raw materials, plant supplies, buildings, etc. estimated at around $100 \times 10^6 \frac{\text{Btu}}{\text{day}}$

Total energy input = $438.6 \times 10^6 \frac{\text{Btu}}{\text{day}}$

Products are 700 lbs protein and 18,300 lbs glucose.

According to Humphrey (25):

$$Y_s = \frac{\text{g cell}}{\text{g substrate}} = 0.51$$

Thus the protein potential of the glucose is approximately equal to 9,150 obs.

Total protein potential/day = 9850 lbs.

$$9850 \frac{\text{lbs protein}}{\text{day}} = 9850 \frac{\text{lbs protein}}{\text{day}} \times 454 \frac{\text{g protein}}{1 \text{ lb protein}}$$

$$= 4.47 \times 10^6 \text{ g protein/day}$$

$$\frac{\text{energy input}}{\text{protein production}} = 98.12 \frac{\text{Btu input}}{\text{g potential protein*}}$$

*Note: These calculations do not include energy needed to convert glucose to protein. The conversion to potential protein was made simply to facilitate comparison.

4. Anaerobic Digestion based on Singh (153)

In general for various wastes, the amount of volatile solids available for digestion varies from 33% to 57% (154).

For example:

Assuming that of 3.5 lbs of solids of chicken manure that 1 lb is converted to biogas leaving 2.5 mass.

gas production/day = 5 $ft^3$/lb.v.s. (w. 59% methane)

Assuming a volume reduction of 33% and a 30% heat recycle to digesters

$$900 \frac{\text{Btu}}{ft^3} \times \frac{60}{100} \times \frac{5 \text{ ft}^3}{\text{lb v.s.}} = 540 \frac{\text{Btu}}{5 \text{ ft}^3} \times 5 \text{ ft}^3$$

Sludge remaining = 2.5 lbs (containing 5.3 to 9% N)

Assume 7% N by weight

(a) fertilizer

This constitutes almost 40% of the non-solar input into agriculture.

energy to produce N/lb = 8400 $\frac{\text{K cal}}{\text{lb. N.}}$
(for commercial fertilizer)

$$= 3.33 \times 10^4 \frac{\text{Btu}}{\text{lb}}$$

Fertilizer value

2.5 lbs x 0.07 nitrogen = 0.175 lbs nitrogen

energy savings for fertilizer substitute = 0.175 lbs x $3.33 \times 10^4 \frac{\text{Btu}}{\text{lb}}$

$$= 5827.5 \frac{\text{Btu}}{\text{lb v.s.}}$$

Total energy savings = 8527.5 Btu less 30% to heat reactors

= 7,717.5 Btu/lb v.s.

= 2205 $\frac{\text{Btu}}{\text{lb manure}}$

(b) Missed opportunity protein

Assume 25% protein in remaining solids (81)

Protein = 0.25 x 2.5 $\frac{\text{lbs protein}}{\text{3.5 lb. manure}}$

= 0.625 lbs $\frac{\text{protein}}{\text{3.5 lb. manure}}$

= 283 g/3.5 manure

The system will either produce gas plus fertilizer of total energy production/savings of 2205 Btu/pound manure or gas at a rate of 568 Btu/pound manure and 80.8 gm of protein/pound manure.

Anaerobic digestion is a bonus system in that not only does it produce energy but food (either directly as increased protein or indirectly as a fertilizer).

Note: Gas production from vegetable matter would be about 5 times the amount given for chicken manure.

## GLOSSARY

acid detergent fibre: The residue remaining after a cellulosic waste has been treated with acid detergent to remove hemi-celluloses.

actinomycete: A member of an order of filamentous and rod-shaped bacteria.

aerobic (organism): An organism which requires air or free oxygen in order to survive.

algae: Generally aquatic non vascular plants with chlorophyll.

amino acid: One of a group of organic acids containing an amino group ($-NH_2$); amino acids are the building blocks for protein molecules.

anaerobic (organism): An organism which does not require air or free oxygen in order to survive.

bacteria: Microscopic plant in the same class as the fungi with a round, rod-like, spiral or filamentous body.

bagasse: The pulp remaining after the juice has been extracted from sugar cane.

biochemical oxygen demand: A measure of the amount of oxygen used by micro-organisms to break down organic wastes.

bioconversion: The biological conversion of waste materials to useful products.

biodegradable: Material which is capable of being biologically broken down to simpler compounds.

cellulase: One of a group of enzymes manufactured by micro-organisms to turn cellulose into soluble sugars readily consumed by the microbes.

cellulose: A polymer of glucose with chains of from 2,000-4,000 units.

chemical oxygen demand: A measure of the amount of oxygen required to completely oxidize wastes.

chlorophytes: A class of green algae.

digestibility: A measure of how well the food/feed is utilized by the digestive system; the ratio of absorbed nitrogen to total nitrogen intake.

digestion: Fermentation, especially anaerobic fermentation.

electrodialysis: A method for removing unwanted particles from solution by passing through an electrically charged membrane.

ensiling: The anaerobic fermentation of crops to preserve and enrich the fodder.

enzyme: One of a group of proteins produced by living organisms which hasten metabolic reactions and are not destroyed in the process; a "living" catalyst.

enzyme hydrolysis: A process in which a cellulose containing material is broken down to glucose by the action of enzymes.

essential amino acid: One of eight amino acids which cannot be manufactured by the human body and which are essential for nitrogen balance.

fermentation: An enzymatic reaction generally accompanied by the evolution of gas and with growth of micro-organisms.

fungi: A group of saprophytic spore-bearing plants lacking chlorophyll.

glucose: A simple sugar.

hammer milling: Crushing or pulverizing material in a hammer mill.

hemi-cellulose: A polysaccharide (type of sugar) found in plant cell walls.

ion exchange: A technique for separating materials by the reversible exchange of ions of the same charge.

leachate: A liquid draining out of land-fills and containing decomposed waste, bacteria, chemicals, etc.

lignin: A three dimensional aromatic polymer found in wood -- closely associated with cellulose as lignocellulose.

limiting amino acid: The amino acid the furthest below the FAO standard which lowers the quality of the protein source.

mesophilic (organism): An organism which grows most successfully at temperatures between 20° - 40° C.

methionine: A sulfur containing essential amino acid, often deficient in yeast SCP.

monosaccharide: A simple sugar.

mycelium: A mass of fungal filaments which compose the vegetative body of a fungus.

net protein utilization: A measure of how well a protein source is utilized by the body; defined as

$$\frac{\text{retained N}}{\text{intake of N}} \times 100, \text{ the perfect score is } 100$$

petroprotein: Single cell protein grown on a petroleum-based substrate.

phytoplankton: Floating microalgae.

protein efficiency ratio: A measure of how well a protein is utilized by the body expressed as grams gain in body weight per 100 grams of protein consumed (adjusted to an assumed value of 2.5 for casein).

pyrolysis: Thermal decomposition in the absence of air.

reverse osmosis: A method of concentrating a solution by applying hydraulic pressure to force water through a membrane.

single cell protein: The non-viable dried cells of micro-organisms grown on a variety of carbon sources.

substrate: The material which the SCP utilizes for growth.

ultrafiltration: A technique permitting solids separation from a solution using pressure to force water and small molecules through a membrane.

vascular (plant): A plant with tissues capable of conducting water and nutrients from the roots to the leaves (xylem) and food from the leaves to the roots (phloem).

whey: The watery part of milk separated from the curds (semi-solid) in cheese making.

yeast: A species of fungi.

**TECHNICAL REPORT DATA**
*(Please read Instructions on the reverse before completing)*

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**15. SUPPLEMENTARY NOTES**

**16. ABSTRACT**

Current research into methods of solid waste management is focusing on formation of marketable products to defray the costs of treatment prior to land disposal. Some wastes are already being commercially exploited for their energy value. It is also possible to produce a food or feed through a number of technologies including single cell protein production, enzyme hydrolysis, anaerobic digestion, and various methods to improve the digestibility and acceptability of cellulose wastes.

This report examines the technological, economic and environmental feasibility of the above processes. Single cell protein production from wastes is compared to SCP production on other substrates(alcohols, alkanes, etc.) and to conventional methods of farming.

**17. KEY WORDS AND DOCUMENT ANALYSIS**

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| Proteins, Cellulose, Regenerated cellulose, Waste, Waste disposal, Cost comparison, Economic factors, Waste treatment, Nutritive value, Fermentation, Substrates, Biomass, Anaerobic processes | Single cell protein production, Cellulosic wastes, Enzyme hydrolysis, Anaerobic digestion, Food recovery, Recycling, Sociological acceptable, Solid waste management, Bioconversion, Municipal solid waste, Solid waste disposal, Resource recovery, Cellulose degradation | 6A |

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