

0 0 0 0 4 7 0 - 6 6 0 4

Presented at the Symposium on Enzymes in the
Food Processing Industry, University of California
Cooperative Extension Service, Davis, CA,
January 14, 1976; Also Submitted to Resource
Recovery and Conservation

46-13
LBL-5962

44891

RECEIVED
LAWRENCE
BERKELEY LABORATORY

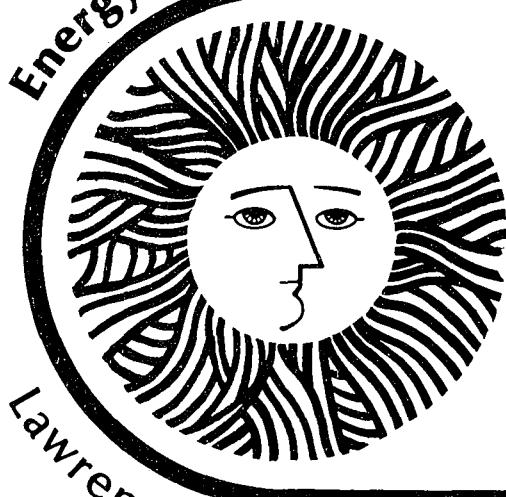
MAR 23 1978

LIBRARY AND
DOCUMENTS SECTION

For Reference

Not to be taken from this room

Energy and Environment Division



Enzymes And Microorganisms In
Food Industry Waste Processing And
Conversion To Useful Products:
A Review Of The Literature

Paul A. Carroad and Charles R. Wilke

December 1976

Lawrence Berkeley Laboratory University of California/Berkeley

Prepared for the U.S. Department of Energy under Contract No. W-7405-ENG-48

44891
JL

LBL-5962
c. 1

— LEGAL NOTICE —

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

1

Title: Enzymes and Microorganisms in Food Industry Waste Processing and
Conversion to Useful Products: A Review of the Literature

Authors: Paul A. Carroad¹ and Charles R. Wilke²

¹Department of Food Science and Technology, University of California,
Davis, California 95616 (U.S.A.)

²Department of Chemical Engineering, University of California, Berkeley,
California 94720 (U.S.A.)

This paper is based on the authors' participation in the Symposium on Enzymes
in the Food Processing Industry, University of California Cooperative Extension
Service, Davis, California, January 14, 1976.

Abstract

Bioconversion of food processing wastes is receiving increased attention with the realization that waste components represent an available and utilizable resource for conversion to useful products. Liquid wastes are characterized as dilute streams containing sugars, starches, proteins, and fats. Solid wastes are generally cellulosic, but may contain other biopolymers. The greatest potential for economic bioconversion is represented by processes to convert cellulose to glucose, glucose to alcohol and protein, starch to invert sugar, and dilute waste streams to methane by anaerobic digestion. Microbial or enzymatic processes to accomplish these conversions are described.

Work supported in part by the U. S. Department of Energy.

Introduction

Waste treatment in the food processing industry is of increasing importance. This is not only a response to environmental restrictions but is also based on the possibility of converting solid and liquid wastes to useful products. A survey by the U.S.D.A.'s Western Regional Research Center was recently undertaken to help define food industry research and development needs as a guide for the future (1). In the area of processing, the need for methods for converting plant effluents into useful chemicals was rated very highly. The bioconversion of food processing and agricultural wastes to useful products is gaining much attention (2). This is due not only to the fact that certain carbohydrates, proteins, and biopolymers occur widely in many different wastes, but also because various microorganisms and purified enzymes can stabilize almost any organic waste and may produce useful products, such as fuel, animal feed, and chemicals.

Chemical nature of waste material from the food processing industry

Most of the information compiled on the nature of liquid food wastes is designed for use by waste treatment engineers. Rather than reporting exact analyses of sugars and starches, proteins, cellulose and other items of value for recovery or utilization, studies usually define liquid waste conditions in terms of biochemical oxygen demand (BOD), chemical oxygen demand (COD), and suspended solids (SS) content. These latter terms may not be sufficiently specific to characterize the liquid waste with respect to its potential for bioconversion to useful products.

There is considerable variation in the characteristics of typical food processing liquid wastes. It is recognized, however, that such products as apples, beets, corn, potatoes, pumpkins, squash, and tomatoes do yield effluents

of high BOD (3).

The high BOD values are probably attributable to the starchy nature and sugar contents of these products. The starch is leached out during processing, particularly when cut kernels or exposed pulp is exposed to water.

In contrast to liquid waste data, there is much information available on solid waste composition, owing to the fact that solid residues from food processing are often marketed as animal feed supplements (4). Successful animal feeding experiments have been conducted with wastes from tomatoes, asparagus, sweet potatoes, potatoes, citrus products, apples, pineapples, beans, and many other agricultural and processing residues (4). Generally, the residues from processing operations which are de-watered can hardly be classified as wastes, since most are sold or hauled away for use as animal feeds (5). These materials should not be overlooked, however, as possible sources of conversion to useful products such as fuels or chemicals which may have higher value.

A major agricultural residue which has great potential for conversion to useful products is the cellulose in plant stems, straw, leaves, grasses, bagasse, and husks which are produced every year. It is estimated that 430 billion kilograms of crop wastes were produced in the United States in 1972 (6). At an average content of 40 percent, 170 billion kilograms of cellulose are potentially available. Although much of this material is presently left in the fields to rot, or burned as fuel, it would appear that suitable collection procedures might be devised to recover a portion of this resource. Some wastes such as bagasse, rice and wheat husks, and corn cobs are available at central processing sites.

The cellulose contents of corncobs, bagasse, oat hulls, cottonseed hulls, and flax shives, for example, all range between 34 and 41 percent (7). Cellulose

is also contained in fruits and vegetables, and hence in the residuals of processed foods. Of the 14.6 billion kilograms of fruit harvested in 1974 in the United States, 5.0 billion kilograms could be classified as residual. This residual material would have yielded approximately 356 million kilograms of crude fiber on a dry weight basis, most of which is cellulose. Vegetables would have yielded residual crude fiber of 203 million kilograms dry weight, of a total 5.8 billion kilogram wet weight residual (5). This cellulose is only a small fraction of the total residual of processed fruits and vegetables, 17.4 percent on a dry weight basis, but could be considered as a resource for enzymatic conversion to useful products.

A biopolymer related to cellulose is chitin, which occurs in the solid waste of the processing of shellfish such as shrimp, crab, and lobster. Chitin is a polymer of acetylglucosamine units, with β -1,4 linkages similar to those in cellulose. Recent years' shrimp and crab annual landings have been approximately 0.454 billion kg (1 billion pounds) in the United States (8). Since approximately 75 percent of landed shrimp is waste and approximately half of the dry weight of the waste is chitin, shellfish processing waste represents a significant resource for utilization (9, 10). An advantage of chitin for bioconversion processes is that it is commonly available from shellfish processing in local areas, either from one large processor or in a community with several small processors. Although process research into bioconversion of chitin lags considerably behind that of cellulose, it is expected that process concepts of the same sort should be applicable (11).

In summary, food processing and agricultural liquid wastes are characterized by large water flows of high biochemical oxygen demand. The liquid wastes are a potential, but dilute, source of sugars and starches. The solid wastes are a source of cellulose and protein. Much of the cellulosic waste, however, as

agricultural waste is not harvested, but is left on the field.

Potential for enzyme usage

Given the nature of wastes as described, the potential for enzymatic conversion to useful products can now be considered. The possibilities of converting the starches to invert sugar, other biologically degradable streams to methane via anaerobic digestion, and the cellulose, chitin, and fermentable sugars to alcohol or single-cell protein are excellent in the long run. Enzyme systems are known which can accomplish all these conversions and many more. Moreover, engineering process research and development are progressing in many laboratories around the United States, so that at some time these processes will be economically and technically feasible.

In the short run, however, one may not see rapid implementation of enzymatic processes. The processors of commodities generating much of the residual material may be unwilling to stop providing them to established feed or by-product markets without assurance of a greater alternative return. Most of the solid waste material today is given to farmers or disposed of otherwise. The true waste constitutes only a small fraction, perhaps 20 percent, of the residual material from fruit and vegetable processing (5). The other 80 percent is utilized as animal feed or as by-products.

Characteristics of the industry make many large scale processes unlikely. A 1975 National Canners Association study showed that in the United States, 1600 plants process 35.6 billion kilograms of raw products per 31.5 Ms (year) (12). There are large plant to plant differences. About two-thirds of them are near cities, but one third are located at least 48 kilometers from a similar plant. Thus, the wastes are somewhat disperse.

The plants average 20.5 Ms (7.8 months) per 31.5 Ms (year) in operation, and process 75 percent of their production in 11.0 Ms (4.2 months) (12). It

may not be feasible to establish a waste conversion facility to operate only a few months per year.

Although processors range in size from those handling 203 thousand kilograms to those with 711 million kilograms of raw product per 31.5 Ms (year), the industry is characterized by a large number of small companies. It is competitive and operates with a low profit margin. National Canners Association estimates of average before tax profit on sales for 1972, 1973, and 1974 fiscal years are 3 percent, 3 percent, and 4 percent (12). For this reason, it is unlikely that an individual processing firm will take the initiative in developing new innovative processes for enzymatic waste utilization. It must be left to others to research, and even subsidize, the process until it is of proven value.

The high variability in waste characteristics adds difficulty to enzymatic conversion. Liquid wastes vary widely in composition, depending on product and processing method used. The stream pH may vary from acid in the case of fruits to alkaline when lye peeling is used. BOD values also vary as previously discussed. Certain wastes, such as dairy wastes, may be nitrogen deficient if contemplated as a substrate for microbial growth. Other wastes, such as meat and poultry wastes, may be difficult to treat due to grease. Finally, although treatment may be possible, certain streams may be so dilute in the desired waste that recovery or treatment is unfeasible due to the large volumes of water which must be handled.

Nevertheless, certain processes involving microorganisms or enzymes do exist which have been shown to be practical or are almost at the demonstration stage. These processes, which will now be described, are:

1. Conversion of cellulose to glucose.
2. Conversion of glucose to alcohol
3. Conversion of glucose to protein
4. Conversion of starch to invert sugar

5. Methane production by anaerobic digestion.

Although all the processes involve enzymatic conversions, some are effected with purified enzymes or with enzyme extract solutions, whereas others require the whole microbial cell to be present. Thus, cellulose is converted to glucose and starch to invert sugar by the application of specific enzymes in solution. But, the conversion of glucose to either alcohol or protein and of wastes to methane involve complex metabolic cycles and the presence of whole cells.

Conversion of cellulose to glucose

The enzymatic conversion of cellulose to glucose represents a potential for utilization of agricultural wastes, and possibly of food processing wastes. Cellulosic materials have fuel value in direct burning, but can be converted enzymatically to glucose instead. Glucose, which is itself a food, can be chemically converted to chemical raw materials, microbially converted to single-cell protein, and fermented to produce fuel, solvents, chemicals, antibiotics, and enzymes.

The cellulose hydrolysis reactions are catalyzed by a system of enzymes which are produced by a variety of fungi and bacteria. Perhaps the most active extracellular cellulase complex is that produced by variants of *Trichoderma viride* developed by the United States Army Laboratories at Natick, Massachusetts (13). At least four different types of enzymes have been postulated to act together to break down the cellulose chains and to convert the resulting fragments to glucose (14).

Process development studies for enzymatic hydrolysis are in progress in several laboratories (15, 16, 17). The scheme proposed by the Lawrence Berkeley Laboratory group will be outlined briefly. A cellulosic waste is shredded and milled to allow it to form a slurry which can be pumped and agitated. Chemical

treatment to loosen the fiber structure or to achieve delignification in order to make the cellulose more accessible to the cellulase system may also be appropriate. Approximately seven percent of the waste is diverted from hydrolysis to be used as substrate for the cellulase producing fungus. In a controlled fermentor, the fungus secretes the cellulase enzyme system, using the cellulosic waste and some recycled hydrolysate as carbon and energy source. Application of recycle systems to the enzyme production step itself may alleviate the need to use final hydrolysate product to support fungal growth. The enzyme system so produced is filtered free of fungus cells and unreacted waste and is mixed in a five-stage hydrolyzer, with a residence time of 144 ks (40 hours). Assuming a fifty percent conversion of cellulose and an appropriate recycle configuration to increase the hydrolysis sugar concentration in the hydrolysis product stream, sugar exiting the hydrolyzer at four percent concentration is brought to a 14.3 percent concentration level in a seven effect evaporator. This sugar can serve as a starting point for crystallization and recovery or can be used as a feedstock for microbial conversion to single-cell protein, alcohol, or other chemicals. The undigested waste material can be used to fuel the plant. Residual enzyme in the hydrolysate sugar stream may be recoverable by counter-current adsorption on fresh cellulosic feed solids in a staged mixer-filter (16).

The described process is under continuing development, and although economic projections vary the process appears economically favorable. The studies to date have centered on facilities for treating several million kilograms (hundred thousand tons) of cellulose waste per 31.5 Ms (year), such as would be encountered in municipal waste treatment facilities. This is not applicable to the waste for an individual farmer or food processor, but would require a centralized disposal site. The difficulties in maintaining the fungal cultures and in operating the process would make very small scale applications impractical. The potential

for large scale application, however is considered promising (18).

Conversion of glucose to alcohol

One has the opportunity to convert microbially a glucose source, such as would be provided by a process for enzymatic cellulose degradation, to any number of useful products, such as ethanol, acetone, butanol, acetic acid, lactic acid, and protein. Given the energy crisis and the possibility of protein shortages, two conversions which are particularly significant are the conversions of sugar to alcohol and to protein.

The microbial conversion of sugar to ethanol has been used in winemaking for thousands of years. During and following World War II, industrial alcohol production from molasses and grain depended on microbial action to meet the consumer demands for alcohol. With the rise of the petrochemical industry, however, microbial ethanol production was replaced by chemical conversion. Now, with pending shortages of oil feed stocks, fermentation is receiving serious reconsideration. Ethanol can be used as a motor fuel, and was in fact blended with gasoline for automobile use in the middlewest United States in the late 1930's (19). More recently, the use of ethyl alcohol in a 20 percent blend with gasoline is being projected as an answer to the energy crisis in Brazil (20). A 20 percent alcohol blend can be handled without retuning cars, and it is possible to design an engine to run on 95 percent ethanol. Ethanol has a fuel value of 24 kJ per cm^3 (8500 BTU per gallon). Gasoline, for comparison, yields about 33 kJ per cm^3 (11800 BTU per gallon).

Ethanol is produced from glucose by yeasts, using the biochemical pathways of glycolysis and conversion of pyruvate to ethyl alcohol. The overall reaction converts one mole of glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) to two moles of carbon dioxide (CO_2) and two moles of ethanol ($\text{C}_2\text{H}_5\text{OH}$). Theoretically, 0.454 kg (1.0 pound) of glucose

can be converted into 0.232 kg (0.511 pounds) of ethanol, but in fact the practical limit is about 0.218 kg (0.48 pounds) of ethanol since some microbial cell mass is also produced.

Conversion of food processing wastes containing sugars to ethanol has been recognized for years as a possibility. Pear, apple, cherry, and citrus wastes have all been investigated as sources of fermentable sugars (21). Also whey has been found to be a suitable substrate (22). Food processing wastes contain many fermentable sugars such as glucose, mannose, maltose, fructose, and lactose, but the degradation of cellulosic wastes yields some cellobiose, which has been found not readily fermentable to alcohol.

Several drawbacks exist to the use of fruit juice wastes for alcoholic fermentation. One is that the wastes are often deficient in a nitrogen source, making it necessary to add nutrient salts at some expense. A second is that the sugar concentrations in the wastes are commonly only 4 percent, which yield an alcohol solution too dilute for economic recovery. Required is an evaporative concentration step to bring the solutions into the economic range of 14 percent sugar (21, 23).

Alcohol has been produced by batch and continuous fermentation. Fermentors are customarily equipped with both pH and temperature control. The substrate solution is sterilized prior to inoculation, but the acid condition of the broth helps to make contamination less of a problem in this process than in many others. A limitation to the batch system is that accumulation of alcohol to the 15% level would inhibit the organism. Continuous fermentation also suffers the same disadvantage of alcohol inhibition, but can be run at twice the productivity of a batch system, by avoiding frequent start-up and shut-down periods. Following the alcohol fermentation, the ethanol is concentrated to 95 weight percent by distillation.

The Lawrence Berkeley Laboratory process previously described has ethanol as its main end product from cellulose bioconversion. Assuming no cost for the cellulosic feed as waste, a municipally operated process could produce 95 percent ethanol for 0.000161 United States dollars per cm^3 (\$0.61 U.S. per U.S. gallon). The cost increases for 3 percent taxes, 12 percent interest on invested capital, and a charge of \$0.0197 U.S. per kilogram for cellulosics (\$20 U.S. per ton) would be \$0.0000237 U.S. per cm^3 (\$0.09 U.S. per U.S. gallon), \$0.0000475 U.S. per cm^3 (\$0.18 U.S. per U.S. gallon), and \$0.0002097 U.S. per cm^3 (\$0.794 U.S. per U.S. gallon), respectively (16).

Conversion of glucose to protein

In almost any situation where one could produce alcohol from food processing wastes, one could instead produce single-cell protein. Hundreds of organisms have been investigated for protein production (24), and of these yeast-based processes are the farthest advanced toward commercialization (25). Yeasts and other microorganisms double their mass quickly compared to mammals and so single-cell protein production can be rapid. Additional advantages are that since fermentors are used, climatic conditions are of no effect and much less land is required than for animal or vegetable farming (26).

The most important aspect of conversion of sugar wastes to protein is the quality of the protein itself. Although somewhat deficient in methionine, dry *Torula* yeast contains all the essential amino acids in a proportion not unlike that of beef or milk. Yeast can be considered as a food ingredient for its B-vitamin content and its value as an extender of other protein products, but its use as a primary protein source has been limited by flavor and acceptability problems. A major problem also, is the high nucleic acid content of yeast single-cell protein. Ingestion of over 3 grams per day of yeast nucleic acid over a prolonged period may cause elevation of serum uric acid levels, resulting

in significant risk of kidney stone formation and gout (25). This high nucleic acid content can be removed in processing, and its reduction is the subject of much research (27). Alternatively, yeast can be used as an animal feed. This avoids the problems of human acceptance and requires much less processing. For poultry feeding, the lysine/arginine ratio must be adjusted closer to 1, but in general single-cell protein could find application in poultry, swine, and cattle feeding and fish farming (25).

The production of single-cell protein is similar to ethanol production, but does differ in two important aspects. Firstly, production of cell mass is an aerobic process requiring addition of oxygen. Since transfer of oxygen into solution is crucial, high power inputs in the form of agitation are necessary. Secondly, much heat is generated due to yeast metabolism. The excess heat must be removed to maintain temperature control. Again, the acid conditions and the fact that yeast can grow faster than most other organisms reduce the danger of contamination. Harvesting of cells is usually by centrifugation, followed by spray drying for animal feed. For human consumption, additional steps such as cell lysis nucleic and hydrolysis, protein extraction and purification, and drying are required (28). This additional processing for human consumption could add \$0.55 U.S. to \$0.66 U.S. per kg (25 to 30 cents U.S. per pound) to the cost of single-cell protein. As described for the ethanol system, single-cell protein production suffers economically from dilute feed streams and so recycle configurations to build up cell mass concentrations are common.

Several processes have recently appeared for combining single-cell protein production with waste disposal. This includes cellulase and protein production from mixed cultures of a cellulase producing organism and a yeast (29), from two symbiotic bacteria of which one is cellulolytic with good protein characteristics (30), and the production of protein from starch by yeasts which can themselves

convert starch to sugars (31). It may be mentioned that recently the fungus *Sporotrichum pulverulentum* has been shown to be able to convert a cellulosic waste to a quality protein by producing a cellulase and assimilating the resulting sugar (32).

The market for single-cell protein would probably be the same as for soy protein or fish meal. As an animal feed, single-cell protein would have to sell for between \$0.30 U.S. and \$.49 U.S. per kg (\$300 U.S. and \$500 U.S. per ton) (25). Since much solid waste from food processing already finds value as an animal feed, the market for protein produced from dissolved sugar in liquid waste should be well-established and easily reached.

Conversion of starch to invert sugar

Perhaps the best example of a practical enzyme process is the production of invert sugar from starch. Invert sugar, a mixture of glucose (dextrose) and fructose (levulose), can either be produced by hydrolysis of sucrose or by saccharification of starch and isomerization of glucose. Since waste streams have been identified as a starch solution, the latter alternative is of interest here. Waste starch streams are fairly dilute, which is a disadvantage. To employ the following technology, some concentration step, such as evaporation, would be required.

Conversion of starch to glucose was established in the 1930's as an acid hydrolysis process. In the 1960's, enzymatic conversion became important with the application of glucoamylase to effect saccharification. The addition of the enzyme glucose isomerase, in the 1970's, allowed for the production of invert sugar (33). Invert sugar is used commercially, with a composition of 42 percent fructose, 50 percent glucose, and 8 percent other saccharides. The processing advantages of using invert sugar in food formulations include more rapid dissolution than glucose and more rapid crystallization than fructose. Invert

sugar has higher osmotic pressure and greater protection against microbial growth than equally sweet sucrose. From a taste perspective, invert sugar is sweeter than glucose and as sweet as sucrose at 15 percent solids solution levels, and has a synergistic sweetness effect when added to synthetic sweeteners (34).

Industrial production of amylases which hydrolyze starch has mainly involved bacteria, although fungi also supply an enzyme. An advantage of bacterial enzyme is its ability to operate at higher temperatures. The amyloglucosidases of fungi, however, do permit greater direct conversion of starch to single glucose units (19,35). There are also many microbial sources of glucose isomerases, to convert glucose to fructose (36).

Several patents and reviews have described the overall process from starch to invert sugar. Initial breakdown of starch is done by bacterial amylase (37, 38). As an example, starch is slurried, amylase enzyme is added, and the solution heated to 105 - 110⁰C for 480 s to 600 s (8 minutes to 10 minutes) to accomplish the liquefaction. The high temperature does not destroy the saccharifying activity of the enzyme. The solution is cooled to 85 - 95⁰C, another enzyme added, and held for 3.6 ks (one hour) to allow dextrinization to occur, resulting in many polysaccharide units in solution. Saccharification to glucose units proceeds with an amyloglucosidase at 60⁰C, in 173 ks to 346 ks (two days to four days).

The conversion of starch to glucose is one of the systems being researched for application of immobilized enzyme technology, as the largest single industrial application of enzymes today (39). A plant to produce 454 kg (1000 pounds) of dextrose daily from cornstarch, which is not itself a waste however, has been installed by Corning at Iowa State University. The several day reaction time can be reduced to minutes in such a system.

The glucose solution which is so prepared by starch saccharification is then

isomerized by contact with glucose isomerase enzyme. The enzyme is produced intracellularly, usually by growth of a *Streptomyces* organism (40). Because the enzyme is so expensive to produce and would be costly to recover if allowed to mix in solution, the enzyme is immobilized on an inert carrier. Clinton Corn Processing Company, which has done much work in this field, has processes for immobilizing the enzyme on diethylamino-ethylcellulose and for entrapping and stabilizing the enzyme within the cell by heating (41,42, 43). Reactor configuration can be either a stirred tank or a packed column, although Clinton uses a shallow bed reactor. The extent of reaction is controlled by temperature and by the feed rate, or time of reaction. Typically, conversion of glucose to a suitable invert sugar solution takes less than 11 ks (three hours) in a column at 60 - 70°C (38).

Methane production by anaerobic digestion

An excellent opportunity for conversion of food processing wastes to methane exists with the anaerobic digestion process. Viable microorganisms play an important role in waste treatment because of their ability to decompose most organic materials and to assimilate them (31). The important reactions can be represented as the biological conversion of complex soluble organic material to volatile acids and the subsequent conversion of these acids to methane.

More specifically, the first step of acid formation involves the oxidation of wastes, usually comprised of carbon, hydrogen, oxygen, nitrogen, and sulfur as primary elements, to organic acids such as acetic acid. This is done by common anaerobic facultative bacteria, and proceeds at a rapid rate. Typical times for this first phase may be several hours to a day. Ammonia, carbon dioxide, and hydrogen sulfide are also generated. The second step involves the reduction of the carbon dioxide and cleavage of the acetic acid, forming methane (44, 45).

Several methanobacteria have been identified in this second conversion to methane all acting symbiotically (46). The methane fermentation is significantly slower than the first phase and is considered the rate limiting step, since the bacteria associated with it grow slowly and are quite sensitive to environmental conditions. Even at optimized conditions, this second step may require residence times of 430 ks to 1300 ks (5 to 15 days) for municipal waste treatment.

Because of their high BOD, food processing wastes containing sugars, starch, proteins and organic acids are particularly well suited to anaerobic digestion. These materials can be fermented by anaerobic digestion. A second advantage of food processing wastes is the absence of materials such as metals, glass, stones, rags, and other substances which require expensive pretreatment steps such as shredding and milling or separation (47). Such materials not only increase capital costs for preparation equipment, but consume much power in their treatment. Experiments on a synthetic waste of bread, potatoes, apples, ground beef, citrus fruit, carrots, cabbage, celery, coffee grounds and paper showed it to be well suited to rapid anaerobic digestion (45).

The gas yields from anaerobic digestion range from $0.75 \text{ m}^3/\text{kg}$ (12 cubic feet/pound) of waste decomposed for protein to about $1.25 \text{ m}^3/\text{kg}$ (20 cubic feet/pound) for carbohydrate (46). Since the heat of combustion of methane is about 39900 kJ/m^3 (1070 BTU/cubic foot), the gas from the process which consists of roughly 50 percent methane and 50 percent carbon dioxide has a fuel value of about 19950 kJ/m^3 (540 BTU per cubic foot) (48). The carbon dioxide can be absorbed out of the gas to increase the fuel value considerably. Total gas production is in the range of $0.94 \text{ m}^3/\text{kg}$ to $1.12 \text{ m}^3/\text{kg}$ (15 to 18 cubic feet/pound) of volatile solids consumed. 0.31 to 0.44 m^3 of methane per kg of COD consumed (5 to 7 cubic feet of methane/pound of COD consumed).

In the treatment of dairy waste which removed 95 percent of the pollution

load, the adding of an anaerobic digestion step to a trickling filter scheme could reduce costs by a factor of ten (46). Anaerobic digestion has been applied to the full scale treatment of wastes from a meat packing operation (44). BOD reduction was 91 percent, from an inlet value of 1400 mgm per liter. The system was loaded to the extent of $0.029 \text{ kg BOD}/\text{kg} \cdot \text{m}^3$ (156 pounds BOD/day/1000 cubic feet) of reactor volume. Retention time for treatment was only half a day, due to the easily fermentable substrate.

Recent research has centered on the investigation of organisms which can operate at higher temperatures, perhaps 65°C , rather than the 30°C to 40°C range currently employed (49). At the expense of adding more heat to the process, the advantages include increased rates of digestion, decreased fluid viscosity, decreased biomass formation, increased conversion of waste to gas, and decreased chance of bacterial and virus pathogen accumulation. This involves the utilization of new organisms which also convert organic wastes to methane. Of significance is that the production of methane from volatile solids can be increased from $0.47 \text{ m}^3/\text{kg}$ to $0.69 \text{ m}^3/\text{kg}$ (7.5 cubic feet per pound to 11 cubic feet per pound), at a 58 percent conversion level.

Summary

The nature of food processing and agricultural wastes allows for conversion to useful products. Microorganisms and individual enzymes can be employed to carry out the conversions efficiently and under gentle conditions. Although technically feasible, implementation is hindered by economic considerations. Except in certain situations; such as grain mills, shellfish processing plants, or vegetable and fruit processing operations which utilize water recycle and achieve high concentrations of effluent wastes; the dilute and dispersed characteristics of food industry wastes require either concentration and associated energy costs or centralization and concomitant transportation costs.

Nevertheless, considerable process development is underway to reduce bioconversion costs for the day when recycling and concentration of wastes become common and when the values of the final products sufficiently offset process costs. Major programs concerning cellulose bioconversion have involved both microorganism strain selection and engineering process analysis. It is estimated that even under private operation ethanol could be produced from cellulosic waste for \$0.000442 U.S. per cm^3 (\$1.67 U.S./gallon). Although this is somewhat above current market price, process improvements which reduce costs and market factors which increase the price from competitive sources should narrow the difference. Similarly as single-cell protein, invert sugar, and methane become more valuable commodities, the bioconversion processes which yield these products will become more economically competitive.

Widespread implementation of bioconversion processes thus depends upon the revision of agricultural and food processing practices to yield centralized wastes in as concentrated a form as possible, the engineering development of the processes themselves, and the necessary increase in the demand for the conversion products. While market factors and government policy will largely determine the first and third factors, continued research and development can increase the chances for commercial success.

REFERENCES

1. Krochta, J. M., Rumsey, T. R., and Farkas, D. F., 1975. Defining food R and D needs as a guide for the future. *Food Technology*, 29(10): 74.
2. Tsao, G. T., 1976. Enzymes in disposal and utilization of food wastes. In: F. W. Cramer (Editor), *Enzymes: Use and control in foods*. Institute of Food Technologists Short Course. Chicago, Illinois, pp. 12-1 to 12-12.
3. Eckenfelder, W. W., 1970. *Water Quality Engineering for Practicing Engineers*, Barnes and Noble, New York.
4. Ben-Gera, I. and Kramer, A., 1969. The utilization of food industries wastes. In: C. O. Chichester, E. M. Mrak, and G. F. Stewart (Editors), *Advances in Food Research*, Academic Press, New York, pp. 77-152.
5. Cooper, J., 1975. The potential of food processing solid wastes as a source of cellulose for enzymatic conversion. *Symposium on enzymatic conversion of cellulosic materials: technology of applications*. Boston.
6. Stephens, G. R. and Heichel, G. H., 1975. Agricultural and forest products as sources of cellulose. In: C. R. Wilke (Editor), *Cellulose as a chemical and energy resource*. Biotechnology and Bioengineering Symposium Series No. 5, Wiley-Interscience, New York, pp. 27-42.
7. Dunning, J. W. and Lathrop, E. C., 1945. The saccharification of agricultural residues. *Industrial and Engineering Chemistry*, 37(1):24.
8. Wheeland, H. A. and Thompson, B. G., 1975. *Fisheries of the United States*, 1974. Current fishery statistics No. 6700. U. S. Government Printing Office, Washington, D. C.
9. Kreag, R. and Smith, F., 1973. Seafood solid waste in Oregon: disposal or recovery. Oregon State University Extension, Marine Advisory Program Special Report 395. Corvallis, Oregon.
10. Mauldin, A. F. and Szabo, A. J., 1974. Shrimp canning waste treatment study. Environmental Protection Agency Study EPA-660/2 74-061. Office of Research

and Development. U. S. Environmental Protection Agency, Washington, D.C.

11. Carroad, P. A. and Tom, R. A., 1977. Bioconversion of shellfish chitin wastes: process conception and selection of microorganisms. Paper Number 139, presented at the Institute of Food Technologists Annual Meeting, Philadelphia.
12. National Canners Association, 1975. Liquid wastes from processing fruits, vegetables, and specialties. NCA Western Research Laboratory Report 1671 0-2756, Berkeley, California.
13. Mandels, M., Weber, J., and Parizek, R., 1971. Enhanced cellulose production by a mutant of *Trichoderma viride*. Applied Microbiology, 21:152.
14. v. Hofsten, B., 1975. Topological effects in enzymatic and microbial degradation of highly ordered polysaccharides. In: M. Bailey, T.-M. Enari, and M. Linko (Editors), Symposium on Enzymatic Hydrolysis of Cellulose, Helsinki, pp. 281-295.
15. Wilke, C. R., Yang, R. D., and von Stockar, U., 1975. Preliminary cost analysis for enzymatic hydrolysis of newsprint. Lawrence Berkeley Laboratory Report LBL-4408, University of California, Berkeley, California.
16. Wilke, C. R., Cysewski, G. R., Yang, R. D., and von Stockar, U., 1976. Utilization of cellulosic materials through enzymatic hydrolysis. II. Preliminary assessment of an integrated processing scheme. Biotechnology and Bioengineering, 18: 1315.
17. Spano, L. A., Medeiros, J., and Mandels, M., 1975. Enzymatic hydrolysis of cellulosic wastes to glucose. U. S. Army Natick Laboratories Bulletin, Natick, Massachusetts.
18. Crocco, S. C., 1975. Tomorrow's promise: enzymatic systems at work. Food Engineering, 47(7): 54.
19. Underkofler, L. A. and Hickey, R. J., 1954. Introduction. In: L. A. Underkofler and R. J. Hickey (Editors), Industrial Fermentations, 1:1.

20. Hammond, A. L., 1977. Alcohol: a Brazilian answer to the energy crisis. *Science*, 195: 564.
21. Nolte, A. J., von Loescke, H. W., and Pulley, G. W., 1942. Feed yeast and industrial alcohol from citrus-waste press juice. *Industrial and Engineering Chemistry*, 34(6): 670.
22. Prescott, S. C. and Dunn, C. G., 1959. *Industrial Microbiology*. Third Edition, McGraw-Hill, New York.
23. Cysewski, G. R. and Wilke, C. R., 1976. Utilization of cellulosic materials through enzymatic hydrolysis. I. Fermentation of hydrolysate to ethanol and single-cell protein. *Biotechnology and Bioengineering*, 18: 1297.
24. Bunker, H. J., 1968. Sources of single-cell protein: perspective and prospect. In: R. I. Mateles and S. R. Tannenbaum (Editors), *Single-Cell Protein*, M.I.T Press, Cambridge, Massachusetts, pp. 67-68.
25. Lipinsky, E. S. and Litchfield, J. H., 1974. Single-cell protein in perspective. *Food Technology*, 28:16.
26. Vilenchich, R. and Akhtar, W., 1971. Microbiological synthesis of proteins. *Process Biochemistry*, 6(2):41.
27. Hedenskog, G. and Mogren, H., 1973. Some methods for processing single-cell protein. *Biotechnology and Bioengineering*, 15: 129.
28. MacLaren, D. D., 1975. Single-cell protein--an overview. *Chemtech*, 5(10): 594.
29. Peitersen, N., 1975. Cellulose and protein production from mixed cultures of *Trichoderma viride* and a yeast. *Biotechnology and Bioengineering*, 17: 1291.
30. Callihan, C. D. and Dunlap, L. E., 1971. Construction of a chemical-microbial pilot plant for production of single-cell protein from cellulosic wastes. U. S. Environmental Protection Agency Report SW-24C. U. S. Environmental Protection Agency, Washington, D. C.
31. McLoughlin, A. J., 1972. Fermentation of pollutants. *Process Biochemistry*, 7(1): 27.

32. Eriksson, K. E. and Larsson, K., 1975. Fermentation of waste mechanical fibers from a newsprint mill by the rot fungus *Sporotrichum pulverulentum*. *Biotech. Bioeng.* 17:327-348.
33. Allen W. G. and Dawson, H. G., 1975. Technology and used of debranching enzymes. *Food Technology*, 29:70.
34. Wardrip, E. K., 1971. High fructose corn syrup. *Food Technology*, 25:47.
35. Fogarty, W. M., Griffin, P. J., and Joyce, A. M., 1974. Enzymes of *Bacillus* species--Part I. *Process Biochemistry*, 9:11.
36. Fogarty, W. M., Griffin, P. J., and Joyce, A. M., 1974. Enzymes of *Bacillus* species--Part II. *Process Biochemistry*, 9:27.
37. Wieland, H., 1972. Starch and sugar conversion process. In: *Enzymes in food processing and products*. Noyes Data Corporation, Park Ridge, New Jersey, pp. 38-56.
38. Aschengreen, N. H., 1975. Production of glucose/fructose syrup. *Process Biochemistry*, 9(4): 17.
39. Weetall, H. H., 1975. Immobilized enzymes and their application in the food and beverage industry. *Process Biochemistry*, 10(6):3.
40. Kooi, E. R. and Smith, R. J., 1972. Dextrose-levulose syrup from dextrose. *Food Technology*, 26(9):57.
41. Mermelstein, N. H., 1975. Immobilized enzymes produced high-fructose corn syrup. *Food Technology*, 29(6):20.
42. Thompson, K. N., Johnson, R. A., and Lloyd, N. E., 1970. Process for isomerizing glucose to fructose. U. S. Patent Number 3,788,945.
43. Lloyd, N. E., Lewis, L. T., Logan, R. M., and Patel, D. M., 1970. Process for isomerizing glucose to fructose. U. S. Patent Number 3,694,314.
44. Pfeffer, J. T., 1966. Application of the anaerobic process to waste water treatment. In: E. J. Kirsch (Editor), *Symposium: Methanogenesis and anaerobic treatment of waste*. *Developments in Industrial Microbiology* 7:134.
45. Pohland, F. G. and Ghosh, S., 1971. Developments in anaerobic treatment

processes. In: Raymond P. Canak (Editor), *Biological Waste Treatment. Biotechnology and Bioengineering Symposium Series No. 2*, pp. 85-106.

46. Buswell, A. M., 1954. Fermentations in waste treatment. In: L. A. Underkofler and R. J. Hickey (Editors), *Industrial Fermentations. Volume II*. Chemical Publishing Company, New York, pp. 518-555.

47. Wise, D. L., Sadek, S. E., and Kispert, R. G., 1974. Fuel gas production from solid waste. Dynatech Report No. 1151, Dynatech Corporation, Boston, Massachusetts.

48. Kaplovsky, A. J., 1957. Activity of microorganism in organic waste disposal. *Applied microbiology*, 5: 175.

49. Cooney, C. L. and Wise, D. L., 1975. Thermophilic anaerobic digestion of solid waste for fuel gas production. *Biotechnology and Bioengineering*, 17: 1119.

This report was done with support from the United States Energy Research and Development Administration. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the United States Energy Research and Development Administration.