Advances in Biochemical Engineering 20

Edited by A. Fiechter

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Bioenergy

Springer-Verlag Berlin Heidelberg New York

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With Contributions by
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With 58 Figures and 56 Tables

Springer-Verlag
Berlin Heidelberg New York 1981

ISBN 3-540-11018-6 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-11018-6 Springer-Verlag New York Heidelberg Berlin

Library of Congress Catalog Card Number 72-152360
Printed in GDR

2152/3020-543210

20 Advances in Biochemical Engineering

Managing Editor: A. Fiechter

Preface

A. Fiechter, ETH-Hönggerberg, Zürich

Bioenergy obtained an enormous publicity during the past years. It was assumed as a real alternative to the replacement of fossil and nuclear energies. The initial enthusiasm was followed by a certain disillusionment, as besides the lack of economical technologies the feasibility of many well-meant propositions decreased drastically, due to the poor access to the annually synthesized 10^{11} t of biomass. The publication of volume 20 of our series coincides with a stage of development which shows, besides the setbacks, some realistic possibilities for the production of bioenergy from biomass.

This Jubilee Edition shall therefore be dedicated to this topic. A part of the largest integrated research program of the non-medical biology research area is placed into the foreground, e.g. the US program "Fuels from Biomass" with an annually granted sum of currently 100 million dollars.

The most prominent subject of these impressive R + D programs in many countries is the ethanol "gasohol" from sugar and high polymer carbohydrates (starch, cellulose) as well as methane from agricultural waste and sewage sludge. The wide scope of all the investigations is remarkable. This impression becomes evident when studying the reports from different countries also from outside the USA which are included in this selection of the American program. Many other countries which are not listed here have also started programs for gaining bioenergy. A complete list can hardly be made because of the dramatic development of the subject taking place very rapidly. The active scientists from the countries not quoted may forgive the editor for the lack of completeness due to the reasons mentioned. It is impressing that today the R + D for the development of biotechnical methods is highly promoted in all continents. In many cases, the final shape of the process design and the economy are not yet in sight and further efforts of biologists and engineers are required. It can be foreseen with certainty that today's work will result in an enormous support of biotechnology which will lead to significant reactions on biology and economy.

Despite the incompleteness of the selection of topics presented in this volume, it is hoped that the reader may obtain some characteristics of the present-time developments.

Each article has been prepared only recently. No reviewing has been done on them in order to preserve the new and original character of the writing and to allow the inclusion of the most recent results.

Undoubtedly, the reader will esteem the advantages of originality and topicality and overlook the disadvantages of incompleteness and minor insufficiencies in the finish of the writing.

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Biochemical Engineering for Fuel Production in the United States

Henry R. Bungay
Department of Chemical and Environmental Engineering,
Rensselaer Polytechnic Institute, Troy, N.Y., 12181 U.S.A.

Despite confusion, turmoil, and controversy, the United States is rapidly developing firm foundations for large scale production of fuels from biomass. Although methane is produced from manures at relatively large demonstration plants, this gains little attention compared to processes that lead to liquid fuels that could relieve the U.S. dependence on imported oil. Ethanol from corn grain is losing its political appeal, and other, cheaper feedstocks are being sought. There are several distinct processes being developed for lignocellulosic materials with a main difference being the type of pretreatment that permits good hydrolysis to degradable sugars. Byproduct credits and energy-efficient processes make it very likely that fuels from biomass will cost considerably less than imported oil, and it will be profitable to establish a massive new fuels industry.

1 Introduction

The use of energy in the U.S. has paused in its logarithmic growth because of some mild winters, more fuel-efficient automobiles, and conservation. The oil-exporting nations are curtailing production to reduce the glut resulting from the unexpected low demand. The production cost for oil is less than one percent of its selling price, so there is a danger that fuels from biomass could become uneconomic and producers could be bankrupted by a temporary lowering of the cartel's price of oil. Those entrepreneurs who produce fuels from biomass will be doing a great service to all the oil-importing nations of the world by introducing competition to oil, and they deserve protection against the unfair pricing practices of the cartel. Fortunately, there is excellent potential for obtaining fuels from processes which have coproducts that are valuable as food, fiber, or chemicals so that profits are possible even if fuel prices drop precipitously. High-priced ethanol from corn grain is sure to be replaced quickly by fuels from a new generation of biomass processes.

Burning of wood wastes to power lumbering and pulping operations, regional use of wood to heat home and buildings, and burning of bagasse contribute in excess of two quadrillion British Thermal Units (QUADS) of energy annually in the U.S. (1 QUAD = 2.52×10^{14} kilocalories.) This is not an insignificant percentage of the total annual consumption of energy of about 76 QUADS. Extrapolating from existing agricultural practices, it is very optimistic but not unreasonable for the U.S. to aim for 20 QUADS of biomass energy by the year 2000. If total energy usage does not resume its upward spiral, biomass can become a major contributor as the Earth's petroleum runs out.

The U.S. Department of Energy is relatively new but has a gigantic budget that increases each year. In its formative period there was a strong influence from the National Science Foundation because a number of people working on energy programs were transferred to the new agency then known as the Energy Research and Development Administration. Whereas the National Science Foundation has exemplary standards, the Department of Energy has evolved to a highly politicized system. There is some satisfaction in that almost all of the highly meritorious proposals are approved because the available monies are so plentiful. At present, ethanol from corn grain is losing some of its political glamour because the severe heat wave and drought of 1980 greatly elevated the price of corn. Cellulosic feedstocks are receiving attention just as some of the new processes are approaching commercial fruition.

Only recent work, most of it sponsored by the United States government, will be covered, and it is assumed that the reader appreciates the extensive previous research on cellulose hydrolysis and on ethanol formation throughout the world. Other chapters in this volume cover important candidate process or steps that are crucial to one or more processes. A review that ignored these other chapters would lack perspective, thus there is some duplication for purposes of comparison. The judgements expressed should not be taken as definitive because improvements and refinements are occurring at dizzying speed. This review will emphasize bioconversion and will have little to say about hydrogen from engineered photosynthesis. This fascinating, elegant approach will not reach fruition for many decades because there are many fundamental hurdles to overcome. One is the problem of generating hydrogen except at very low redox potentials, and photosynthetic evolution of oxygen nearby is troublesome to say the least. Other obstacles are devising cheap enclosures to capture gaseous products and separation of hydrogen from the other gases.

2 Organization for Biomass Research

The main divisions are:

production of land plants for feedstocks
production of aquatic plants for feedstocks
conversion to fuels by thermochemical processing
bioconversion
hydrogen from engineered photosynthesis

There is some biochemical engineering to growing plants, and culturing of algae demonstrates some sophisticated techniques. Space limits preclude topics other than bioconversion, but a detailed discussion of all the divisions is available [1]. Thermochemical processes are also excluded because there is little biochemical engineering.

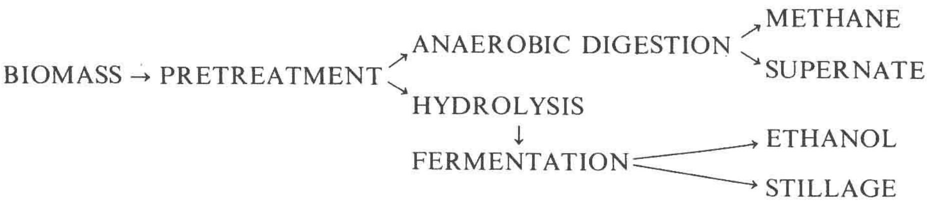

Fig. 1. Bioconversion processes

There is much enthusiasm for fuels from biomass by the thermochemical steps of gasification, pyrolysis, or liquefaction [2]. These are brute-force approaches that work with almost any carbonaceous material and produce simple organic molecules plus oils or tars. The economics of thermochemical conversion may be superior to those of existing bioconversion processes, but better fermentation yields and credits from byproducts are quickly reversing this situation.

The U.S. Department of Energy has had programs with various titles such as "alcohol fuels", "fuels from biomass", and "biomass refining", and administration is by D. O. E. itself or by other branches of government such as the U.S. Department of Agriculture. Portions of various programs are farmed out to branches of D. O. E. such as the Solar Energy Research Institute (SERI), Oak Ridge National Laboratory, and the like. There have been numerous temporary guidelines, but no long-range comprehensive plan for biomass fuels has been adopted.

The bioprocesses to be discussed are shown in Fig. 1.

Pretreatment can be omitted only for finely divided biomass such as tiny algae or loosely structured manures.

3 Anaerobic Digestion

Most work on anaerobic digestion features methane, but a few projects are aimed at recovering valuable organic acids. The great advantages for methane are: non-aseptic technique employing elective mixed cultures; production in relatively low-cost crude equipment; ease of recovering the insoluble product gas from water; and compatibility of the product with pipeline gas. Disadvantages are the low selling price of methane and $^1/_3$ to $^1/_2$ of the gas is carbon dioxide which lowers the fuel value. Only very inexpensive feedstocks can be digested economically. The demonstration plants and some commercial installations for manufacturing methane from biomass use cattle manure at very large feedlots. Much of the profit is derived by supplementing the cattle feed with digester residue that is high in protein. The value of the protein for refeeding is about twice that of the methane. The economies of scale are illustrated by the troubles of a skilled team of investigators who have been trying to show that a digestion operation at a small farm is an attractive investment [3,4]. However, several groups are perfecting packed anaerobic reactors which seem to have high efficiency [3,5].

All agricultural residues and trees are decomposed by anaerobic cultures, but yields vary [6]. Treatment of the biomass can improve digestibility, but it probably is not cost effective because of purchasing chemicals, equipment, operating the process, and introduction of sludges or salts from neutralization of the reagents [7]. Marine plants such as kelp digest well in saline systems or in fresh water after washing, but the cost of ocean-grown plants is presently far too high [8,9]. Most of the organic matter in land plants is cellulose, hemicellulose, and lignin. While the carbohydrates digest rapidly and well, lignin is little effected. Some side chains are reacted or removed, but neither the ether linkages nor aromatic rings of lignin are attacked under anaerobic conditions.

Organic acids from anaerobic digestion may be recovered by a membrane process [10] or by solvent extraction [11]. The acids are valuable chemicals and could be used as fuels, especially if converted to hydrocarbons similar to gasoline.

Jones [12] has analyzed the economics of methane from anaerobic digestion of various materials and concluded that it is unprofitable unless the feedstock is priced well below the present costs of crops and agricultural residues, but others [13] are optimistic about digestion, particularly when manure is used.

4 Ethanol Processes

The most important processes for producing ethanol from biomass are shown in Table 1. The grain alcohol process is very popular presently because of the high subsidy provided by the Federal government and by several states for ethanol blended with gasoline. This program was intended to prop up the price of corn by creating more demand, but the drought of 1980 was of a serious nature and caused major price perturbations so that there is much less margin for profit at the prevailing price of corn in the Fall of 1980. Sugarcane juices and molasses are being processed to ethanol in Brazil at a large scale, and there are factories in other countries. With excess bagasses to fuel the factories and with low labor costs, the production of fuel alcohol is a good way to reduce requirements for imported oil. There have been several small technological advances, but the process relies on rather old technology. The wide distribution of cellulose and its relatively low price make it likely to become the main alcohol feedstock displacing corn and sugarcane.

The Natick process was the first significant advance in using cellulose to produce ethanol. Pretreatment by various types of grinding has proven too consumptive of energy. The molds which produce cellulase have been studied intensively by Reese, Mandels, and coworkers [15], and these efforts plus contributions of other groups (especially at Rutgers University) have led to excellent strains in terms of producing high titers of enzymes. The Berkeley process is derived from the Natick process and has contributed engineering solutions to most of the problems and has explored several alternative pretreatments. The economic prospects are good if uses can be developed for lignin and hemicellulose.

The Purdue group headed by Tsao showed great ingenuity in devising pretreatments and thus achieved nearly theoretical yields of glucose from cellulose. There are now several competing schemes at other institutions, but most resulted from the stimulus of the Purdue work. Other accomplishments are better dehydration methods for ethanol, various processes for the sugars from hemicellulose, different fermenter designs, and improvement of the solvent pretreatment to the point where good yields are

Table 1. Processes for manufacturing ethanol

Process	Description	Remarks
Grain alcohol	Corn grain is malted to hydrolyze the starch. Yeast produce ethanol and stillage is concentrated for cattle feed	Profitability can be destroyed by high corn prices or collapse of cattle feed market
Sugarcane	Juices or molasses are converted directly by yeast which are washed and recycled	Stillage too high in salts for cattle feeding. Credits for cane fiber could be high
Natick Process	Cellulosic materials treated with Trichoderma enzymes to get degradable sugars	Pretreatment by grinding too expensive. Has not focussed on using hemicellulose
Berkeley Process	Derived from Natick process and also uses hemicellulose	Strong candidate for large-scale operations
Purdue Process	Removal of cellulose and hemicellulose permits excellent hydrolysis with acid or enzymes	Regeneration of solvent may be costly, but this is a very high yielding process
Gulf Process	Enzymes added for simultaneous saccharification and fermentation	Hydrolysis yields not outstanding and good use of hemicellulose undeveloped
Pennsylvania/ General Electric	Solvent extraction of lignin gives excellent hydrolysis	Costly recovery of organic solvents
Iotech Process	Steam explosion fractures biomass for good hydrolysis	Very valuable lignin byproduct
M.I.T. Process	Mixed mold cultures hydrolyze biomass and produce ethanol	Simple but effective; highly promising

obtained by acid hydrolysis. Enzymatic hydrolysis is more expensive thus acid hydrolysis is presently featured at Purdue although yields are somewhat lower. Corn stover is probably the best cellulosic feedstock in the midwestern farm states.

The Gulf process appeared to be in the technological forefront just a few years ago, but newer processes have demonstrated superior yields. The concept of simultaneous hydrolysis and fermentation to relieve glucose inhibition of the hydrolysis of cellobiose has much merit, but the separate steps have different pH and temperature optima, thus process conditions require a compromise. Nevertheless, the simultaneous process deserves further research, and improvements such as better pretreatment of the biomass could revitalize its prospects.

A team effort of groups at the University of Pennsylvania and the General Electric Company has led to a process based on solvent extraction of lignin for better hydrolysis of cellulose and new thermophillic cultures to supply the cellulases. This is another highly promising process, and there are plans to get significant credits for byproduct lignin by such measures as using solutions in alcohols as diesel fuels.

The Iotech process uses steam explosion for pretreatment. High pressure steam permeates the biomass, and sudden release through a die shreds and disintegrates the structure. Hydrolysis of cellulose and conversion to ethanol proceed nicely. The biggest advantage, however, is development of high-value uses for lignin as a wood binder or specialty chemical. When there are many factories for fuel alcohol, the coproduct lignin will greatly overwhelm the foreseeable markets, but the first few factories selling lignin will be highly profitable. The search for new applications for lignin should be very rewarding because enormous quantities of material with superior properties compared to lignin from paper pulping will be available.

The M.I.T. process has more simultaneous steps than does the Gulf process. Carefully selected mixed cultures are added directly to coarsely ground biomass. Enzymes hydrolyze both the cellulose and the hemicellulose while the organisms convert the resulting sugars to ethanol. The organism which ferments the sugars from hemicellulose may be added later after the first organism has nearly completed the hydrolysis and has consumed most of the glucose. The really clever feature of this approach is investing very little in feedstock preparation and not being overly concerned with a high efficiency of feedstock utilization. This means that much of the feedstock is unreacted, but the residue does not represent much money. It would be burned to supply energy for the factory. Some improvement in efficiency of feedstock utilization would be desirable, however, because the fuel content of the residue exceeds the needs of the factory; steam or electricity would be products of about equal importance to the ethanol. There does not appear to be an opportunity to recover valuable lignin from the residue although it is enriched with respect to the other polymers. There are other problems such as inability of the present strains to reach high concentrations of ethanol, but the rate of accomplishment by the M.I.T. group has been outstanding.

Kelsey and Shafizadeh [14] have still another simultaneous operation whereby the grinding of the feedstock is performed in the presence of cellulases. The rate of hydrolysis and the concentration of glucose were both improved.

5 Project Descriptions

Selected projects related to the U.S. program are shown in Table 2. Not all are currently active; some achieved their goals and were terminated while others are awaiting renewal of financial support before continuing. Those that are identified with processes listed in Table 1 are, of course, featuring further process development. Each project will be reviewed briefly.

The Natick group has performed excellent research despite rather erratic financial support. They selected grinding as a pretreatment step and other methods have proven superior. Lowest grinding cost results from wet milling between two rollers, but it is expensive compared to extraction or explosion techniques. As would be expected from reports of other groups, converting the Natick pilot plant to computer interfacing and control was very time consuming, but better operations and better analysis should repay the investment. Development of improved cultures for cellulase production has progressed well. Activity of beta-glucosidase is subject to biological controls different than those for cellulases, and optimum conditions are being defined. Pilot plant runs with *Aspergillus phoenicus* have shown good yields by either batch or continuous cultivation, thus supplementation with its beta-glucosidase should not be prohibitively expensive.

Engineering refinements of the Natick scheme and new departures are featured at Berkeley. A simple vacuum fermentation for ethanol has been superseded by a vacuum flash pot arrangement which allows escape of most of the carbon dioxide in

Table 2. Selected projects in the U.S. Biomass Program

Institution	Principal investigator	Description	Typical Reference
Natick Labs	Spano	Production of enzymes, hydrolysis ethanol formation	[15]
Berkeley	Wilke	Engineering aspects of complete process from cellulose to ethanol	
Pennsylvania/ General Electric	Pye	Complete process based on pretreatment by extraction of lignin	[17]
M.I.T.	Wang	Direct conversion of cellulose to ethanol. Several other processes	[18]
Purdue	Tsao	Complete process based on solvents for cellulose	[19]
Dartmouth	Grethlein	Kinetics of continuous acid treatment	[20]
Connecticut	Klei	Beta-glucosidase to improve cellulose hydrolysis	[21]
Auburn	Chambers	Extraction and fermentation of sugars from hemicellulose	[22]
Columbia	Gregor	Membrane steps in product recovery	[23]
N.Y.U.	Rugg	Acid hydrolysis in an extruder	[24]
Rutgers	Eveleigh	Genetics and selection of improved cultures	[25]
General Electric	Brooks	Pretreatment by chemically augmented steam explosion	[26]
Arkansas	Emert	Simultaneous hydrolysis and fermentation	[27]
Georgia Tech	O'Neil	Pilot plant for process comparisons	[28]
Battelle	Lipinsky	Sequenced process	[29]
Dynatech	Wise	Mixed-cultures for acids, then electrochemical conversion to hydrocarbons	[30]
Solar Energy Research Inst.	Villet	Complete process	[31]
Argonne Natl. Laboratory	Antonopolous	Cellulases from Fusarium	[32]
Mississippi State	McGinnis	Pretreatment by oxidation	[33]
Washington	Sarkanen	Solvent pulping with catalyst	[34]
Colorado State	Moreira	Organisms with alcohol tolerance	[35]

the main fermenter so that boiling in the flash pot gives a vapor rich in ethanol. Several different feedstocks have been tested for hydrolysis to sugars with enzymes from new and old strains of *Trichoderma reesei*.

Results have been good with a new process using hydrolysis with high pressure hydrogen chloride gas. Milled poplar wood dried to about 6% moisture absorbs the gas with a heat of solution. Yield is 75% reducing sugars. Although the yield is better with enzymes, acid hydrolyis is much cheaper and can be cost effective just as long as yields are reasonable. As with other acid hydrolyisis processes, the key is economical recovery and reuse of the acid. There is research on hydrochloric acid for hydrolysis at several institutions throughout the world, and acid recovery does not yet appear to have an economical answer.

The Berkeley group has also cooperated very effectively with other contractors and has integrated some efforts with the pilot plant at Gulf Oil Chemical Company at Pittsburg, Kansas. There have been economic evaluations of alternative processes and alternative feedstocks. One conclusion is that ethanol from cellulose is very likely to be uneconomic unless there are sizeable credits from products derived from hemicellulose and lignin.

A major realignment has taken place at the University of Pennsylvania because A. E. Humphrey, one of the preeminent biochemical engineers, has moved to Lehigh University. However, the logistics are good for cooperation between the universities and the General Electric Company. Work is also performed at Hahnemann Medical College in support of culture improvement. One similarity to the Berkeley group is the interest in vacuum fermentation. Thermophillic organisms permit reactions at a significantly higher temperature that gives a much higher vapor pressure thus milder vacuum means more attractive economics. Great attention has been paid to feedstock costs because they represent a major fraction of total cost, and tree nurseries in Pennsylvania have demonstrated high yields of poplar with short harvest cycles such that feedstock cost should be under $ 15 per dry ton. It is particularly interesting that the material produced from harvesting young trees two or three years old has about 20% of fines which can be separated easily by air classification. This fraction has 24–27% protein and an estimated price of $ 150 to $ 200 per t for animal feed.

Massachusetts Institute of Technology (M.I.T.) has one of the outstanding bioengineering groups in the world because of synergism of engineers, biochemists, geneticists, and microbiologists. Impressive results have come from developing microbial cultures with improved performance, as for example, higher titers of ethanol because of better ethanol tolerance. The old acetone/butanol process has been studied with more modern techniques such as pH control and nutrient feeding. Higher titers of butanol seem particularly difficult to reach because this alcohol is severely inhibitory at about 10 g l^{-1}. One promising approach is continuous removal of the butanol by extraction with a water-immiscible solvent during the reaction. Another example of innovative research is finding the bases for a commercial process to produce acrylic acid which is an important intermediate in manufacturing plastics and resins [35].

Current preoccupation with alcohol fuels has curtailed most of the financial support from D.O.E. except for the M.I.T. direct conversion of cellulosic materials to ethanol. Strains of clostridia have been developed that are approaching economically practical ethanol concentrations. Whereas the parent strains reached only a few grams per liter of ethanol, the selected strains achieve about 40 g l^{-1}. The parents had considerable lactic acid and roughly the same amount of acetate and ethanol, but the new strains have little lactate and 10 times as much ethanol as acetate. The best strain for hydrolysis of both cellulose and hemicellulose can degrade the resulting glucose to ethanol but does not use the sugars from hemicellulose. However, a second strain of clostridium does a fairly good job of degrading mixed sugars to ethanol. A brief review cannot do justice to the broad range of investigations by this group.

Other chapters in this volume describe the achievements of the Purdue group in defining the mechanisms of cellulose hydrolysis and the effects of various pretreatments. There has been both excellent theoretical research and practical engineering.

Much of their financial support has come from the State of Indiana which would like to assume a leadership role in alcohol fuels. The Purdue process employing solvents for destroying the crystallinity of cellulose can achieve yields approaching theoretical; the main problem is economical recovery and reuse of the solvents. This process can be characterized as high technology compared to the M.I.T. process which could be a very simple technology. It will be interesting to follow the various processes to see which becomes most successful.

The Dartmouth group has devised a continuous reactor for acid hydrolysis and determination of kinetic coefficients for the reactions occurring during hydrolysis and decomposition has been useful to all the other groups interested in acid hydrolysis. The small reactor used for several years has been replaced by a larger unit that permits higher pressures and provides data of greater reliability for scale-up. A particularly important discovery was pretreament by slightly less severe conditions of acid strength and temperature to remove hemicellulose hydrolytically and enable cellulases to achieve high yields of glucose in a subsequent step.

Some organisms produce a mix of cellulase enzyme activities that is deficient in beta-glucosidase. This enzyme converts cellobiose to glucose, but the rate declines as glucose accumulates. To maintain a high rate, excess beta-glucosidase is desirable. Several groups in the U.S. and other countries have shown that beta-glucosidase can be immobilized and used to supplement cellulase. Without immobilization, the cost is too high because the expensive enzyme is lost with each batch. A project at the University of Connecticut has found a good method for immobilization, and systems with the immobilized enzyme in an external column or right in the hydrolysis reactor have been demonstrated.

The group at Auburn University features hydrolysis of hemicellulose and fermentation of the resulting sugars. Red oak hardwood which is available locally has been hydrolyzed under a variety of temperatures and acid strengths to determine the kinetic coefficients. The maximum yield of xylose was 83% of theoretical when wood chips were treated; about 8% of the chip weight appeared as furfural. A trickling reactor minimizes dilution and produces fairly concentrated sugar solutions. The fermentation is also carried out in a packed reactor. Raschig rings or wood shavings are used to support and retain the microbial culture. Several different processes are being investigated, and results are encouraging for butanol production with little acetone and for the butanediol production.

Gregor at Columbia University has produced semipermeable membranes of a charged polymeric structure which resists fouling. Samples from projects in the U.S. and other countries have been subjected to various membrane treatments. Results with stillage from grain alcohol or from sugarcane alcohol have been excellent in terms of high flux rates, long membrane life, and low projected costs. Reverse osmosis instead of evaporation to concentrate stillage would reduce operating costs and also produce a more favorable overall energy balance. Recovery of ethanol by distillation is very easy in the range of the liquid-vapor equilibrium diagram where the curves are widely separated but difficult as the curves pinch together. It is attractive to distill to about 85% ethanol, and to complete the dehydration by a different method. Whereas the Purdue group is studying drying agents, the Columbia group is devising a membrane process based on polymers which have large flux differences for water and ethanol. It is too early to comment on the possibilities for success.