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BIOTECHNOLOGICAL RESEARCH AND DEVELOPMENT FOR BIOMASS CONVERSION TO CHEMICALS AND FUELS

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Biotechnological Research and Development for Biomass Conversion to Chemicals and Fuels

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Abstract

It is likely that a growing need to produce chemicals and fuels from renewable resources will stimulate the development of biotechnology as a commerical enterprise of considerable potential.

The purpose of the analysis and the development structure that could lead to establishing this new technology are presented. Two general goals are recommended:

i) in the near term, to revive the older fermentation industry and, by the addition of sophisticated technology, to make it competitive;

ii) in the longer term, to develop a new biotechnology largely based on lignocellulose.

Specific research projects are outlined in these two areas and also for the following: microbial formation of hydrocarbons; methane from anaerobic digestion; lignin; methanol. For cellulose conversion to ethanol the relative merits of using added cellulases or, alternatively, direct fermentation with anaerobic thermophiles, are discussed. In selecting suitable feedstocks for biotechnological processes there is a need to use a production-extraction-conversion system as a basis for evaluation.

An effective research work-force for developing biotechnology must be pluridisciplinary. The strategy adopted at the Solar Energy Research Institute is to design the Biotechnology Branch as an integrated set of three Groups: Biochemistry and Molecular Genetics; Microbiology; Chemical and Biochemical Engineering.

Biotechnology is likely to experience a Renaissance with a commercial potential akin to the computer and microelectronics field several decades ago. This development is being stimulated by a growing demand for establishing a chemicals and fuels industry based on renewable resources.

Some idea can be gleaned of the massive scale on which biomass exists by considering woody biomass alone. The total standing forest inventory is estimated to have an energy content of at least three times the annual energy use of 1800 million tonnes oil equivalent. In commercial forests the total biomass growth is about 250 million tonnes oil equivalent/year. This could be doubled, if not tripled, with intensive management. The use of energy plantations, however, has to be weighed against the potential of grasses and other crops, as discussed later. Germane to the development of biological conversion processes is industrial microbiology and biochemical engineering (Gaden, 1974).

When first introduced, industrial microbiology for producing commodity chemicals, developed vigorously. But, except for the production of pharmaceuticals, the industry largely went into abeyance several decades ago, unable to meet the economic challenge of cheap petroleum and its highly effective cracking technology. As the fraction of U.S. oil supplied by other countries becomes larger, however, hydrocarbon costs are mounting. To seek substitute commodities in response to market forces is the lifeblood of the chemicals industry. It is likely, therefore, that the microbiological production of commodity chemicals will attract entrepreneurs increasingly.

For example, fermentation ethanol can serve as a fuel and as a basic chemical for synthesizing other products. Ethylene, produced from petroleum at present, is a prime chemical feedstock. The supply is becoming tighter, but the demand is unabated and thus price is rising. Ethylene can be produced from ethanol. At some time the economic incentive will be sufficient for the displace-

ment, by fermentation ethanol, of petroleum-derived ethylene. Also, ethanol can be a feedstock for butadiene, acetaldehyde and acetic acid production. Apart from ethanol, there could be a growing interest in fermentation chemicals such as butanol, acetone, butanediol (useful for methyl ethyl ketone production) acetic and lactic acids, glycerol, isopropanol, fumaric, succinic and propionic acids.

In general, the innovation needed for a technological substitution is considerable and expensive. The feasibility of a new technology can be demonstrated on a small scale without undue expenditure and lapse of time. Scale-up to commercial dimensions, however, involves costly engineering research and development and considerable time. The economics of coal- and petroleum-based chemicals processing favors large, capital-intensive plants. Biotechnological processes, however, are an exception. Modestly sized and decentralized plants drawing on biomass resources close at hand (since transportation of biomass is costly) will be preferred. Moreover, smaller fermentor systems are readily controlled and optimized. The net result is that the degree of scaling up required and hence the lead-time and break-even cost for biotechnological development, will be reduced, compared with other energy and chemicals technologies.

The purpose of the analysis presented here is to identify a research and development structure which could lead to establishing, with reasonable efficiency, suitable biotechnological processes for the production of chemicals and fuels from biomass.

General Recommendations:

Two general goals for research and development are recommended:

- The near-term revival and improvement of conventional but, at present, dormant fermentation processes for fuels and commodity chemicals production from sugars and starch.

o The longer-term development of a new biotechnology for producing:

i) fuels and chemicals from lignocellulosic biomass;

ii) hydrocarbons and other chemicals/fuels from algal species and higher plants.

To accomplish these two general goals will require intensive research in microbiology and biochemical engineering. There is much to be gained by integrating these disciplines.

Biosynthetic capabilities of microorganisms need to be modified in order to enhance their commercial potential. Work on biosynthetic pathways, metabolic regulation and on enzyme mechanisms is, therefore, essential. Screening for microorganisms of unusual performance over a range of different environmental conditions is a necessary adjunct of such work.

In yeast research there is a striking contrast between the approach outside and within industry. On the one hand, the tendency outside industry has been to select for characteristics of little commercial value. On the other hand, in industry the selection process for improved strains has been largely empirical. There is a crucial need for basic genetics work to locate genes which control commercially attractive phenotypic character.

An emphasis on recombinant DNA work could be highly beneficial to a research program designed to improve microbial strains. Striking advances in specific fragmentation, cloning and sequencing of DNA are being made. In vitro recombinant DNA experiments for constructing hybrid strains might not yield results immediately applicable, but at the very least, strain compatabilities would be examined for various donor/recipient combinations and gene-banks developed, would add to genetic knowledge of the microbial species involved.

Genetic technology could be used also to accelerate the development of higher plant biomass for extraction and bioconversion. Since plant breeding is

a slow process, many generations are needed to introduce new traits into suitable varieties. The transfer of traits between different species could be of biotechnological advantage. Vector DNA can be transferred using plant viruses or bacteria will tumor-inducing episomes. Accompanying developments in plant cell culture are needed to implement these techniques.

Certain algae and fungi produce relatively large concentrations of chemicals that could be used as chemical feedstocks and fuels. To enhance such biosynthetic capabilities appropriate gene-transfer systems would have to be developed. Marine organisms await thorough analysis for their potential in biotechnology. The existence of genetic recombination in certain phytoplankton species is encouraging in this respect. Mariculture for chemicals/fuels production could reduce pressures on diminishing fresh-water supplies.

In parallel with biological research work, process engineering research and development is crucial. Recovery of fermentation products can be improved by modifying conventional chemical engineering unit operations, e.g., distillation and by developing separation methods which function closer to equilibrium. As novel hybrid microbial strains are constructed, there is a need to introduce high-precision process control in order to take full advantage of increased mass-transfer rates, and the narrowing of optimal ranges of process conditions. Improvements in product recovery and in process control would reduce the cost of production of chemicals in conventional fermentations in the near term.

In the longer term, biochemical engineering research is needed to develop commercial bioreactors for converting insoluble materials such as cellulosic and hemicellulosic biomass. Adequate quantitative information on rheological behavior must be accumulated for design purposes, particularly in regard to time-dependent systems. Flow-control of concentrated two=phase systems requires analysis. There is room for a more adventurous approach in fermenter design;

novel vessel geometries and function as being developed in Europe and Japan, could be worthwhile.

Theprecise evaluation of process engineering systems is an essential component of a research and development program in biotechnology. Process design and evaluation studies would permit distinquishing systems with an economic potential, for example, by using techniques of optimal flow-sheeting. This type of discriminator function, based on conceptual designs could be invaluable in the identification of biotechnologically favorable basic research. Performance thresholds (in terms of yields and rate of production of chemicals) could be established.

Some Specific Recommendations:

I. Improvement of Conventional Fermentation Technology:

These processes are based on easily fermentable substrates: molasses, sugar cane juice, corn, sugar beet, potato starch, and sweet sorghum. The cost of the feedstock, however, constitutes a large proportion of the total cost of production. For ethanol production from maize, for example, the cost of feedstock represents at least 65% of total cost. (Humphrey et al. 1980). Waste products such as whey from the food industry and sulfite waste liquor from the paper pulp industry are cheaper and could be used for fermentations. The potential yield of ethanol from lactose in cheese whey is about 400 million liters/year.

To improve processing costs for ethanol and other fermentation chemicals the following research is needed:

- Increase the energy efficiency of product recovery. Conventional chemical engineering unit operations such as distillation can be made more efficient by operation nearer pinch conditions (lower reflux ratios) (Coulson and Richardson, 1962). Vapor recompression systems can be incorporated (Danziger 1979). It is interesting that an estimated 100,000 barrels/day of crude oil could be saved if the energy efficiency of distillation in the petroleum in-

dustry were improved by 10% (Mix et al. 1978).

There is room for developing novel separation processes operating closer to equilibrium membrane separation (Matsuura et al. 1977) adsorption (Barrer and Fender 1961); crystallization (Izergin 1958); extraction (Humphrey et al. 1980).

- o Develop concentration techniques for cheese whey lactose to give higher concentrations of fermentation products.
- o Develop yeast strains with higher tolerance to ethanol. This would make vacuum fermentation (Ramalingham and Finn 1977) more feasible. Thermophilic strains could permit fermentations of higher rates and conserve cooling. Yeast strains capable of fermenting 50% (weight/volume) sugar solutions would be useful.
- o Zymomonas (formerly Pseudomonas lindneri) is capable of producing ethanol at three times the rates with yeast and with almost the same yield of ethanol. Product- and thermo-tolerant strains are needed (Swings and DeLey 1977).
- o Develop continous fermentation. Rates of throughput not limited by the maximum specific growth rate of the cells could be achieved by immobilization in gels as biocatalyst pellets (Villet et al. 1979) or by using suitable flocculating strains in tower fermenters (Prokop et al. 1969).
- o Incorporate on-line computer and microprocessor control. Suitable algorithms are to be formulated for new instrumentation devised for carrying out optimal strategy (Blachere et al. 1978).
- o Develop fermentation process optimization techniques. (Atkinson and Kossen 1978).
- o Investigate novel fermenter geometries (Hines et al. 1975).
- o Develop agronomical methods which are low-cost in terms of energy utilization (e.g., low-tillage; drip irrigation; genetic manipulation of crops for drought-

stress resistance) (Wittmuss et al. 1975)

- o Study the economic impact of by-products such as distillers grains on feed markets and agricultural patterns (Hertzmark et al. 1980)
- o Investigate inexpensive engineering materials for process design.
- o Develop conventional anaerobic bacterial strains, for example, Clostridium acetobutylicum for acetone-butanol (Underkofler et al., 1937) and C. butyricum for isopropanol (Langlykke et al. 1937).

II. Development of a New Biotechnology for Processing Lignocellulosic Biomass:

For converting woody biomass to sugars the Scholler/Madison acid hydrolysis is the only process to have been operated on a fully commercial scale (Harris and Belinger 1946). The yield of fermentable sugars is poor, sugar concentration is low, reactor cost is high and batch operation is inefficient. Short residence-time reaction improves yields (Grethlein 1978). Extruder-type technology has advantages for acid hydrolysis. A continous-flow, 1 tonne cellulose/day pilot plant using dilute sulfuric acid has been developed. (New York University Process 1979). A sugar yield of 60% (w/w) is obtained at 240 C and 34.5 bar. There is a need to bring this process to a full commercial scale while continuing, however, to rate it against biological conversion systems.

The latter are promising alternatives to acid hydrolysis. Two chief research directions are:

i) the use of fungal extracellular cellulases to produce fermentable sugars; (Mandels and Andreotti 1978).

ii) the development of anaerobic and thermophilic bacteria such as Clostridium thermocellum which produce ethanol and acetic acid directly from cellulose (Wang et al. 1978).

The multienzyme complex of cellulose appears to comprise at least three different types of enzymes (Wood and McCrae 1972); the overall mechanism of action is synergistic and not fully understood. The possible control mutants

such as constitutive cellulase producers are being investigated.

These would allow the enzyme to be produced in the presence of glucose; eliminating the need for an insoluble inducer, cellulose, would be of advantage in the process design. Relieving the inhibition of β-glucosidase activity caused by glucose and of endo-glucanase and cellobiohydrolase activities due to cellobiose would improve the efficiency of hydrolysis.

An analysis of the total cost of producing ethanol from cellulose, using Trichoderma veesei cellulase, for hydrolysis, followed by yeast fermentation, indicates that the cost of enzyme production and the hydrolysis cost together comprise about 50% of the overall production cost (Moreira et al. 1980). This could suggest that vigourous research activity be supported to develop hyper-producing mutants. It is important, however, to consider that limits in transcription, translation (t-RNA pool availability) or protein secretion may have been reached. To settle such a question there is a need for an analysis based on regulation of fungal protein synthesis. The limit to the cell's ingenuity to produce and secrete active cellulase may well be close.

The Gulf Oil/University of Arkansas Process (Emert and Katzen 1979) for ethanol production from municipal solid waste, is based on a hydrolysis with added cellulase followed immediately by yeast fermentation in the same vessel. It is known as the Simultaneous Saccharification and Fermentation process. About 300 liters/day of 95% (v/v) ethanol are produced from one tonne/day of feedstock comprising 55% cellulose. Reducing the cost of producing cellulase would cause substantial improvement in the total cost of ethanol production.

This relatively expensive step of cellulase production can be eliminated by following the second option namely the direct microbial conversion of cellulose using thermophilic anaerobes. Clostridium thermocellum (Wang, et al. 1978) converts cellulose heterofermentatively producing acetic and lactic acids as well

as ethanol. There is active work to increase the ratio of ethanol to acetic acid. Clostridium thermohydrosulfuricum (NG et al. 1979) metabolizes a wide range of carbon sources including pentoses and starch. The stoichiometry of ethanol production is 1.9 moles per mole of glucose. Thermoanaerobicum ethanolicum is extremely thermophilic (Wiegel and Ljungdahl 1979). A problem with all these organisms is a low tolerance to fermentation products. Intensive research work is needed to improve this tolerance since efficient recovery processing will depend on such advances (Herrero et al. 1979).

Selection of the best crops for producing fuels and chemicals must be done within the framework of a production-extraction-conversion system. Rates of biomass production per unit land area are important. But so too is the ease of biological hydrolyzability. This can be enhanced by choosing plants with lower lignification or cropping at early vegetative stages (Linden et al. 1979). There could be considerable biotechnological advantage in extracting fermentable sugars, plant oils and protein before conversion of cellulose and hemicellulose.

The potential in using unconventional crops is not to be ignored. Forage crops, for example, have lower lignification and biopolymer hydrolysis is enhanced. There is experimental evidence that sudan grass and the brown midrib mutants of sorghum can produce 2,600 and 2,300 liters/hectare/year respectively of 95% (v/v) ethanol (Moreira et al. 1980). Ensiling as a pretreatment can be advantageous. In fact, optimal storage is an important consideration.

Ecological niches such as semi-arid zones can be exploited using unconventional forage- and tree-crop species. Leucaena fixes nitrogen, produces biomass at a rate of 50 tonnes/hectare/year and forms pods rich in oils and protein (Brewbaker et al. 1972). There is much to be gained in planning for agricultural diversity.

Some specific research topics for developing lignocellulose conversion processes are recommended as follows:

- o Weigh the relative merits of various pretreatment processes for lignocellulose, including comparison of enzymic methods with chemical methods. For example, examination of the effects of redox catalysts could be worthwhile. The thermodynamic efficiency of exploding wood with steam pressure (Iotech process) should be determined. A comprehensive economic evaluation of pretreatment techniques (biotechnological, chemical, and physical), based on sound experimental results, is needed.
- o Develop microbial strains for hydrolyzing cellulose and hemicellulose.
- o Improve techniques for selecting strains of microorganisms that produce large quantities of cellulases.
- o Set up standard procedures for the preparation of cellulose and hemicellulose so that experimental comparisons can be precise.
- o Extend the number of species of cellulolytic microorganisms being investigated.
- o Study the molecular mechanisms of cellulase production and action. Stabilization and activation are important requirements for process efficiency.
- o Elucidate the mechanism of action of xylanases. Efficient ways of converting hemicellulose to ethanol need to be developed. Hemicellulose comprises about 25% by weight of lignocellulosic biomass.
- o Investigate more fully the biochemistry and genetics of phytopathogenic fungi; for example, the isoenzymes of cell-wall-degrading enzymes. How mechanisms of phytopathogenicity involve extrachromosomal elements (plasmids) requires elucidation.
- o Determine at which stage of development biomass should be harvested for a maximum production of fermentable sugars. At an early vegetable stage there would be less lignification.
- o Assess unconventional crops of high photosynthetic efficiency in regard to

their suitability in a biomass production-extraction-conversion system. Crops should be screened on the basis of their hydrolyzability as well as their photosynthetic efficiency. Already in the Philippines nitrogen-fixing Leucaena is used as an energy crop. Weeds (for example, the common thistle) require evaluation. Information accumulated regarding biological control of undesirable plants (using fungi, for example) would be useful.

- o Investigate the transfer of genes for growth at high temperature from obligate thermophiles (for example, B. caldolyticus) to mesophiles of biotechnological merit. The genetic control of thermophily needs to be understood.
- o Study the role of cyclic nucleotides in catabolite repression. Biochemical manipulation, as an alternative to genetic methods, might be used to achieve catabolite repression resistance.
- o Investigate and optimize novel fermenter geometries, including immobilized-cell bioreactors. Study microbial attachment to solid surfaces. Work is needed on cell-surface chemical topography.
- o Direct biosynthetic pathways toward a homofermentative mode for increasing yield of a desired product. Considerable support for work on microbial physiology would be required.
- o Investigate microbiological and chemical engineering aspects of mixed-culture processing, including possibilities with photosynthetic bacteria.
- o Construct hybrid microbial strains for metabolizing pentoses as well as hexoses.
- o Investigate respiration-deficient strains of yeast for increased production of ethanol.
- o Extend work on membrane biochemistry in relation to ethanol- and thermotolerance.
- o Develop a chemical engineering methodology for novel bioreactors to accomodate insoluble substrates.

- o Examine preferred substrates (the polyauxie phenomenon) for mixtures of sugars from biomass hydrolysis.
- o Study quantitatively the rheological behavior of cellulosic slurries in high concentration. Develop mathematical models.
- o Set up and maintain a bank of cultures and plasmids important to biotechnological processing of biomass.

Decisions will have to be made as to which avenues of R&D should be emphasized. This is not an easy task. Hasty action founded on inadequate information could be detrimental to the progress of a biotechnological energy development program. For example, the question could arise whether to pursue mixed-culture processing or microbial hybrid construction. Mixed-culture processing poses engineering problems in bioreactor design, control, and stability. In the construction of hybrids, however, there is no guarantee of compatability in experiments on gene transposition. It is therefore advisable to exert a reasonably high level of R&D effort in both directions until solid ground for discrimination is gained.

III. Microbial and Plant Production of Hydrocarbons and Other Petrochemical Substitutes:

Certain algal species synthesize high concentrations of hydrocarbons from fatty acids (Tornabene 1977). Squalenes and hydrosqualenes are synthesized by the extremely halophilic organism, Halobacterium. The amounts of various polyisoprenoid components in cultures can be varied by altering geration rates. It has been shown that Dunaliela, a salt-tolerant algal species not only produces glycerol, but with suitable physiological triggering, can synthesize hydrocarbons (Tornabene, in the press).

The oil-producing capabilities of certain arid-zone plants (such as Euphorbia) already were exploited by the French and the Italians before World War II: yields of about 3 tonnes/hectare were obtained in North Africa (Calvin 1978).

The potential for producing chemicals and chemical feedstocks needs to be developed. For example, squalene could be a feedstock for terephthalic acid production; the U.S. demand for this acid is two million tonnes/year.

Some recommendations for research and development are:

- o Screen organisms to identify those producing chemicals of commerical interest.
- o Evaluate the chemicals as potential fuels and/or chemical feedstocks.
- o Measure rates of metabolite production.
- o Investigate the physiology of the organisms and methods of increasing yields of biomass.
- o Perform a preliminary process economic evaluation and establish thresholds of economic feasibility.
- o Analyze biosynthetic pathways and their regulation. Identify enzymes and manipulate pathways.
- o Evaluate the possible conversion of metabolites by microbial or synthetic means to commerically attractive compounds.
- o Perform genetic analysis.
- o Apply genetic engineering technology to enhance rates of production or to couple biosynthetic features.
- o Perform chemical engineering conceptual flow-sheeting.
- o Initiate biochemical engineering design and development.

IV. Methane and Other Products from Anaerobic Digestion:

Anaerobic digestion to produce methane is not yet an efficient process. Greater yields and lower retention times are necessary. Little is known about the interactions among fermentative, acetogenic and methanogenic microbial species. There is no genetic information available as yet. An intriguing research effort is concerned with the conversion of acids from anaerobic diges-

tion to hydrocarbons electrochemically. (Sanderson et al. (1978). An extensive review of the technology, in general, for anaerobic conversion of wastes is not appropriate here. A few highlights recommended for research and development, however, are as follows:

- o Study microbial ecology of anaerobic processes and investigate interspecies hydrogen transfer. Little is known about the interactions among fermentative, acetogenic, and methanogenic species.
- o Investigate possibilities for using genetic techniques to accelerate methane production; there is a pressing need for genetic information on methanogens.
- o Attempt to increase yields by suitable pretreatment.
- o Investigate the feasibility of producing chemical feedstocks such as acetic aicd and propionic acid.
- o Examine the possibility of developing a continuous feed for anaerobic digestion plants. Currently, feedstock is added intermittently and could contribute to process instabilities.
- o Study the application of on-line computer data logging, analysis, and control.
- o Obtain quantitative data on the use of marine inocula for digesting macroalgae.
- o Develop thermophilic strains of anaerobes.
- o Examine strains of methanogens for oxygen tolerance.

V. Lignin:

The biological conversion of lignin is attractive because of its advantages of specificity of action. The degradation and utilization of lignin by microorganisms is being investigated actively (Drew et al. 1979).

There is a need to improve rates of conversion before biotechnological feasibility can be assessed. Some areas of research on lignin which could advance

the mission of developing viable bioconversion processes are:

- o Set up a standard procedure for lignin preparation so that experimental results can be compared.
- o Perform a process evaluation study to select the best procedure for isolating lignin.
- o Identify the most promising microorganisms for lignin conversion.
- o Produce reliable quantitative kinetic data from controlled experiments.
- o Perform chemical engineering flow-sheeting analyses and establish thresholds for process economic feasibility.
- o Develop and genetically engineer strains of promising microbial species.
- o Elucidate the enzymic mechanisms of lignin conversion.
- o Study the biogenesis of lignin. Investigate biochemical or genetic interruption of biosynthesis, with the objective of producing commerically attractive intermediates.
- o Develop biotechnological processing of Kraft wastes from the paper-pulp industry.

VI. Methanol:

There is no biotechnological process as yet for producing methanol. Some possibilities are to investigate the use of methane-oxidizing microorganisms with methanol produced as an intermediate. These organisms also have an interesting cometabolism in which hydrocarbons other than methane can be converted, at the same time, to chemical products; for example, alkenes can be epoxidated (Hou et al. 1979).

In developing an efficient biotechnology for the production of chemicals and fuels from biomass the basis for research and development must be pluridisciplinary. The strategy which has been adopted at the Solar Energy Research Institute is to design the Biotechnology Branch as an integrated set of Groups and Tasks as follows:

BIOTECHNOLOGY BRANCH

Group	Biochemistry & Molecular Genetics	Microbiology	Chemical & Biochemical Engineering
Task	Lignocellulose Bioconversion Processing	Higher Alcohols and Hydrocarbons Biotechnology	Fermentation Technology and Engineering

Basic and applied research work is performed by all three groups. A particular Task is aligned with that group having a major responsibility for it. All three groups, however, are necessarily concerned with each Task. Research results are transferred to the Engineering Group for scaling up and for process design. An importnat feature of this group is its discrimination function: process engineering economic evaluation are made using steady-state optimization methods. By means of process sensitivity analyses conclusions can be drawn as to biotechnological feasibility. Recommendations are made about required microbial rates and yields, for example. In this way, guidelines for commerically fruitful research paths can be established. In no way, however, does the Engineering Group dominate: a well-integrated ensemble is the prime objective.

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