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BIOPROCESSING RESEARCH FOR ENERGY APPLICATIONS

Results of a Workshop Organized by the Center for Bioprocess/Product Development, University of Virginia, for the Council for Energy Engineering Research held at Alexandria, Virginia, on November 2-4, 1988

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PREFACE

The Council for Energy Engineering Research (CEER) reviews, on a continuing basis, a broad scope of engineering research efforts that have a potential impact on energy production and conservation and makes recommendations for future emphasis and direction. As each new area is identified, the CEER usually arranges for a workshop in order to receive input from the research community.

The Council chose "Bioprocessing Research for Energy Applications" as an important new initiative early in 1987 and organized a planning committee that included Dr. Charles D. Scott of Oak Ridge National Laboratory (chairman), Prof. Elmer L. Gaden, Jr., of the University of Virginia, and Prof. Arthur E. Humphrey of Lehigh University. Subsequently, the Center for Bioprocess/Product Development of the University of Virginia was commissioned to organize and manage the workshop. Dr. Gaden was selected as the chairman of the organizing committee, which also included the other members of the planning committee as well as Profs. Giorgio Carta and Donald J. Kirwan of the University of Virginia. The meeting was held during November 2-4, 1988, in Alexandria, Virginia, with approximately 60 participants. This Proceedings summarizes the results of the workshop.

I would like to express my sincere appreciation to all of the participants for their constructive input. Special thanks go to my colleagues on the organizing committee, who planned the activities, helped manage the meeting, and then assisted in compiling and writing the Proceedings of the workshop. I am particularly indebted to Dr. Richard J. Goldstein, Chairman of CEER, and to Drs. Oscar P. Manley and James C. Coleman of the U.S. Department of Energy for their valuable help and suggestions.

Charles D. Scott
Chairman of Planning Committee
Workshop on Bioprocessing Research for Energy Applications

1. EXECUTIVE SUMMARY

The new biotechnology that is emerging could have a major impact on many of the industries important to our country, especially those associated with energy production and conservation. Advances in bioprocessing systems will provide important alternatives for the future utilization of various energy resources and for the control of environmental hazards that can result from energy generation. Although research in the fundamental biological sciences has helped set the scene for a "new biotechnology," the major impediment to rapid commercialization for energy applications is the lack of a firm understanding of the necessary engineering concepts. Engineering research is now the essential "bridge" that will allow the development of a wide range of energy-related bioprocessing systems.

A Workshop entitled "Bioprocessing Research for Energy Applications" was held to address this technological area, to define the engineering research needs, and to identify those opportunities which would encourage rapid implementation of advanced bioprocessing concepts.

1.1 POTENTIAL IMPACT OF ENERGY-RELATED BIOPROCESSING

Bioprocessing has been, or is currently being, utilized in several energy-related applications, and there is an even greater potential for future work in this area. New bioprocessing approaches are now being considered for application to fossil fuels. These include the beneficiation and biological conversion of coal to liquids and gaseous fuels as well as microbial systems for enhanced recovery of petroleum and other sources of hydrocarbon fuels. Liquid product upgrading by biological removal of detrimental or hazardous components may be possible, and it may even be possible to remove or recycle a portion of the generated CO_2 by biocatalytic systems that are driven by solar energy. The majority of these concepts are still in the formative state, and little is known about the underlying biological and engineering principles.

In a sense, the conversion of renewable feed materials (biomass) to fuels and chemicals is the most mature area of bioprocessing development since some of these processing concepts are now commercially used. Grains with a high starch content are

currently used for the production of fuel-grade ethanol and other commodity chemicals, and some waste materials are being used to produce methane. However, the production of these commodity products and other organic chemicals is expected to expand significantly, especially as the price of petroleum increases. There are potentially hundreds of millions of tons of other biomass materials (e.g., woody biomass, agricultural and forest product wastes, herbaceous crops, etc.) that can be considered as feedstocks for such bioprocesses. The concept of a "biomass refinery" could result in an integrated approach to the use of such materials with a large product and by-product slate. Most bioprocessing systems currently used in this area are based on technology that was developed many years ago. More efficient bioreactors, feed material fractionation, and product purification concepts could have important impacts on the expansion of this industry.

Future bioprocessing options may directly utilize solar energy or take advantage of some of the unique properties of biological materials. These could include microbial or enzymatic processes such as those being studied for the light-induced hydrolysis of water to hydrogen and oxygen, or isolation of dissolved metals by bioadsorbents for the economic and energy-savings recovery of strategic materials from low-grade resources or treatment of waste streams.

All energy technologies require control of the release of hazardous materials in waste streams and residues. Bioprocessing schemes may be the most efficient approach for the treatment of some nuclear and chemically hazardous wastes. Biodegradation processes are among the most effective approaches for the treatment of many types of organic pollutants either in aqueous effluents or for the clean-up of contaminated soil and the water supply. Off-gas processing utilizing an immobilized biocatalyst represents an entirely new concept that can be considered for a variety of energy-related processes. The necessary engineering and scientific principles are still not complete for many of these potential bioprocessing concepts.

1.2 EXISTING RESEARCH SUPPORT IN THE U.S. DEPARTMENT OF ENERGY

The primary research support within the U.S. Department of Energy (DOE) that has an impact on bioprocessing includes relatively fundamental investigations of microbiology and plant biology being carried out in the Division of Energy Biosciences of the Office

of Basic Energy Sciences (BES). Also, various applied programs, including those sponsored by the Division of Biofuels and Municipal Waste Technology and the Office of Fossil Energy, provide support that is primarily directed toward process development. The Energy Conversion and Utilization Technologies Program within the conservation area has established a broad program on biocatalysis. This program includes some support for engineering research, especially for investigating bioprocessing concepts that could have an industrial impact.

The Division of Engineering and Geosciences within BES is investigating the initiation of a program in bioprocessing research. The primary goal would be to provide the necessary long-term, generic engineering research that would help provide the underlying fundamentals for future, energy-related bioprocesses.

Other governmental agencies are active in supporting bioprocessing research. Foremost among them are the Engineering Directorate of the National Science Foundation, which provides support for relatively fundamental research, and the Department of Agriculture, which provides support of more applied research, especially as it impacts the agricultural sector. Two trade organizations, the Electric Power Research Institute and the Gas Research Institute, also support bioprocessing research, most of which is directed toward specific applications affecting the Institute members.

1.3 RESEARCH NEEDS AND OPPORTUNITIES

Many issues and concerns either favor or impede the future development of bioprocessing for energy applications; however, they are also the basis for establishing future needs and opportunities within appropriate research areas. Advanced bioprocesses will include necessary biological reagents which are contained within advanced bioreactors that have effective process control and are well integrated with the other processing steps. The resulting product, usually in a dilute aqueous mixture, must be separated and purified for final use. Research needs and opportunities are summarized in each of these areas.

1.3.1 Biological Reagents

Bioprocessing systems require necessary biological reagents to effect a physical or chemical change on a feed material (substrate). Biocatalysts or bioadsorbents will be the

two most important classes of these reagents. The biocatalyst itself (microorganisms, enzymes, or other biological fraction) can be considered to be a series of small microreactors that are integral parts of a larger bioreactor system. In some cases, these microreactors may simply consist of biocatalysts immobilized into, or onto, a suitable solid matrix that will allow the material ready access to the chemical environment while preventing it from washing out of the reactor.

We must develop techniques that will allow a quantitative exploration of the internal behavior of these microreactors, as well as determination of the impact of the external reaction environment. Quantitative measurements of cellular and enzymatic rates in both the immobilized and free-suspension states are critically needed, and these will surely require advanced analytical techniques such as NMR spectroscopy, fluorometry, FTIR, etc. The development of predictive models of cellular and enzymatic processes will be greatly beneficial, especially if useful generic techniques based on fundamental mechanisms can be developed.

Immobilization techniques and the effects of immobilization on intrinsic biokinetics and on mass transport must also be established. Again, predictive models of these mechanisms should be established using generic techniques that will have broad utility.

Bioadsorbent materials from microbial, plant, and animal tissues have just recently been recognized as important biological reagents, especially for heavy-metal removal. The sorption mechanisms of such materials must be established, and the optimum physical form must be determined. Predictive models based on sorption mechanisms and transport properties will be critical for the future use of these materials.

1.3.2 Advanced Bioreactor Concepts

There must also be a better understanding of the "macroreactor," especially for the nontraditional continuous systems that may also include columnar configurations capable of operating essentially at "plug flow." Such bioreactors are multiphase systems that utilize a liquid and/or gaseous feed stream normally containing biocatalyst or bioadsorbent particulates. New studies are needed to elucidate the hydrodynamics and transport processes in these complicated bioreactors so that phase distribution and interactions can be understood. A better understanding will allow the bioreactor parameters to be coupled with the biocatalyst kinetics to allow the development of predictive mathematical models.

Much of the approach should be on a generic basis so that the resulting techniques will have general applicability.

Bioadsorbers or contactors that utilize bioadsorbent particulates will require a similar treatment in order to allow the prediction of the overall sorption kinetics and system optimization. Experimental and modeling techniques used for the study of advanced bioreactor systems will also be appropriate for this area.

1.3.3 Bioseparations

Our knowledge base and experience with separation science and technology are extensive because this area is also important in many of the chemical process technologies. Even so, bioprocessing presents a range of novel problems and situations for which our current understanding is not sufficient. Paramount among them is that many of the products are available as relatively dilute aqueous solutions. Therefore, both concentration and purification are essential steps.

A fundamental concern is the lack of adequate thermodynamic knowledge in certain key areas. For example, the effect of "water structure" on solution biochemistry and phase equilibria is not well understood, and predictive models for solution properties and multiphase equilibrium are badly needed.

Although nontraditional separation techniques are beginning to be used in bioprocesses, most are not well understood for this application. Extraction processes appear very promising as do the uses of adsorption and membrane separations. The study of separation dynamics and the development of predictive models that can be used with the more complex mixtures usually found in bioprocesses are of highest priority.

New and more effective separation reagents and materials are also important, especially those which are adapted to the specific needs of bioprocessing. These include membranes, adsorbents, and solvents that are compatible with biological systems. Of particular interest is the use of certain biological material as adsorbents (bioadsorbents) for the isolation and concentration of dissolved metals and low-molecular-weight bioproducts.

1.3.4 Process Control and Integration

Integration of the various processing steps and consideration of the entire bioprocess offer significant advantages but will require a "systems" approach that can accommodate the various process interactions. There is an intriguing potential for combining the bioconversion step (e.g., fermentation) with the necessary separation steps (product concentration and purification). This will probably necessitate the inclusion of another (separation) phase in the bioreactor, thus complicating the understanding of the system. However, the early removal of some bioconversion products eliminates biocatalytic inhibitions and makes the conversion process more efficient while requiring one fewer processing vessel.

Optimization and control of such systems will require effective on-line sensing methods that are coupled with control instrumentation and kinetic algorithms. Measurements of substrate, product, and biocatalyst concentrations are of particular interest. Devices to achieve such measurements must be stable, rugged, and relatively inexpensive. Biocatalysts or other biological reagents may ultimately prove very useful as sensing components, but various photometric and fluorometric approaches may have even greater application. Estimation techniques based on proven models and adequate understanding of process biochemistry are an alternative to direct on-line measurements.

Mathematical models of the bioprocessing systems will contribute to design and scale-up, but they can also be the basis for process control and optimization. Perhaps a hierarchy of models -- complex models to improve understanding and permit process simulation and somewhat simpler models for process control and optimization -- would be the most effective approach. In addition, control techniques that are adaptive in nature and make use of artificial intelligence (AI) should have extensive applications.

2. INTRODUCTION

Biotechnology can be defined as the directed and controlled use of living organisms or their products to bring about desired chemical and/or physical changes. In the process industries, such technology can be further defined as processes, or processing steps, in which biologically derived feed materials are used or in which a biological agent, such as a biocatalyst or a bioadsorbent, is an important component. A new biotechnology is

emerging that could have a major impact on many of the industries important to our country, particularly those associated with energy production and conservation. The genesis of this new biotechnology is undoubtedly the very broad research effort in the life sciences that has resulted in a better understanding and control of biological systems on a molecular level.

A study by the Office of Technology Assessment[1] indicates that the impact of biotechnology should be significant and should be felt over the relatively short term, especially if the necessary bridging research (i.e., bioprocessing research) is carried out expeditiously. It has been suggested that bioprocessing systems should have important roles in several energy-related areas.[2] There also appears to be an increasing interest by the research community.[3] Although some federal support is available for bioprocess development in those areas, there is little fundamental engineering research that would provide the necessary basis for broad use of these exciting new approaches. An expanded DOE research role in this area has recently been recommended by the Basic Energy Sciences Advisory Committee.[4]

The Council for Energy Engineering Research commissioned a workshop entitled "Bioprocessing Research for Energy Applications," which was held in Alexandria, Virginia, on November 2-4, 1988 (see Appendixes 6.1 and 6.2 for organization of and participation in the workshop). The function of this workshop was to distill from the several formal presentations and extensive discussions the issues, concerns, and engineering research opportunities that will expedite further development of bioprocessing systems for energy applications. More specifically, it was desired to outline those areas of engineering research which would (1) improve and advance the fundamental understanding of the mechanisms underlying energy-related bioengineering practices; (2) develop advanced methods for analysis, control, and diagnostics for bioprocessing concepts; and (3) broaden the technological and conceptual bases for future energy-related biotechnologies.

The Proceedings of this workshop provides a summary of the results of these deliberations and identifies research opportunities in this exciting basic engineering area.

2.1 STRUCTURE OF RESEARCH

Although a wide range of research will be important in the ultimate development of new biotechnology concepts, the focus of this workshop was "engineering research." In order to put engineering research into a proper perspective, terms like "basic," "fundamental," and "applied" are commonly used, but they are subject to so many different interpretations that misunderstandings are almost certain to arise. To avoid this difficulty and to ensure the clearest possible exposition of the discussions and conclusions of the workshop, a conceptual framework was proposed and used to generally define research activities. This framework encompasses four types, or levels, of research: (1) expanding the knowledge base, (2) generating technologically useful knowledge, (3) developing generic technologies, and (4) bioprocess development (Table 1).

2.1.1 Expanding the Knowledge Base

Activities that have no apparent practical objective, but serve to expand the knowledge base, have been referred to as "pure science" or, in some instances, as "basic research." Efforts of this type contribute to a more complete understanding of the physical universe and are usually not based on technological considerations. An example of this type of research would be the study of the metabolic patterns that occur in microorganisms, which could be useful for many different applications.

2.1.2 Generating Technologically Useful Knowledge

Relatively fundamental research can be carried out, but in a broad, generic sense. In this case, the research is expected to be more readily applicable to technological needs even though a specific application is not envisioned. For example, the study of the effects of microbial immobilization on the intrinsic biochemical kinetics could be useful in many technological areas, including several advanced bioreactor concepts.

Table 1. Structure of research

Research areas	Examples of bioprocessing systems: Bioconversion of sugar to ethanol	Examples of bioprocessing systems: Ethanol recovery by distillation
Expanding the knowledge base	Elucidation of microbial metabolic patterns	Thermodynamics of mixtures
Developing generic technologies	Effects of immobilization on microbial kinetics	Separation methods for dilute aqueous solutions
Generating technologically useful knowledge	Development of advanced concepts for bioreactors	Phase equilibrium of oxychemicals in aqueous solutions
Bioprocess development	Predictive system model of ethanol fermentation	Improved design of sieve-tray columns

2.1.3 Developing Generic Technologies

There is a class of problems affecting ultimate technological development that has common features. Generalized solutions can be sought; for example, several advanced bioreactor concepts will have important impacts on a number of potential technologies.

2.1.4 Bioprocess Development

The results of all the previous levels of research plus experience can be used ultimately to address specific process development or design problems. This could involve an entirely new processing concept or the utilization of more fundamental approaches to "sweep out empiricism" previously used in existing bioprocesses. In the latter case, there may be little underlying conceptual foundation for the process, and it would be impossible to extrapolate beyond previous experience. Therefore, engineers especially seek to improve understanding sufficiently to decrease reliance on prior experience alone. An example of this type of research would be the development of a predictive mathematical model for ethanol fermentation using conventional stirred-tank bioreactors.

2.1.5 Engineering Research

Although engineers and scientists can contribute to expanding the knowledge base, this "pure science" is primarily the domain of the scientist. Generating technologically useful knowledge is part of the more applied science in which both engineers and scientists participate, while engineers are predominantly involved in developing generic technologies. All three of these research levels are the proper domain of engineering research; however, during the workshop deliberations, the major emphasis was placed on the second and third areas. While specific bioprocessing development activities were acknowledged to be of great prime importance, they were not considered to be in the province of engineering research.

2.2 STRUCTURE OF THIS REPORT

The results of the Workshop on Bioprocessing Research for Energy Applications are presented in this report, with an emphasis on the needs, challenges, and opportunities for engineering research. Section 3 presents a summary of the potential impacts of bioprocessing on energy production and conservation. This is followed in Sect. 4 with existing research and development support for biotechnology, especially in the area of DOE-funded projects. Perhaps the most important results of the workshop are presented in Sect. 5, which is a summary of perceived engineering research needs and opportunities.

Various backup information, including details of the organization and management of the meeting and a complete list of workshop participants, is summarized in Sect. 6. Copies of the various position papers are also included, as are visual aids used by several DOE staff members to outline current R&D support.

2.3 REFERENCES

1. Staff of the OTA, Commercial Biotechnology: An International Assessment, OTA-BA-218, Congress of the United States, Office of Technology Assessment, Washington, D.C., 1984.

2. Energy Research Advisory Board, The Federal Role of Energy R&D, U.S. Department of Energy, Washington, D.C., 1983.

3. Charles D. Scott, Ed., "Proceedings of the Ninth Symposium on Biotechnology for Fuels and Chemicals," Appl. Biochem. Biotechnol., 17-18, (1988).

4. Basic Energy Sciences Advisory Committee, 1988 Review of the Basic Energy Sciences Program of the Department of Energy, U.S. DOE Report DOE/ER-0395, 1988.

3. POTENTIAL OF ENERGY-RELATED BIOPROCESSING

Engineering research on bioprocessing concepts is relevant to many problems and needs where technological barriers impede progress. This is particularly true in several energy-related fields, including fossil energy, renewable energy resources, energy conservation, and environmental control technology for nuclear and chemical waste management. Processing requirements are identified, and potential opportunities that can be created by bioprocessing research in each of these areas are discussed below and presented in more detail in the formal papers from this portion of the workshop that are included in Sect. 6.3.

3.1 BIOPROCESSING FOR FOSSIL ENERGY

Current and potential bioprocessing concepts for application to fossil energy include enhancing the extraction and recovery of petroleum and other fluid fuel resources, beneficiation of coal, conversion of coal to fluid fuels, upgrading product quality, and environmental control technology for effluents and residues (see Sect. 6.3.1 for additional details).

3.1.1 Enhanced Resource Recovery

Microbial enhanced oil recovery (MEOR) represents an innovative bioprocessing approach to the recovery of additional oil from exhausted reservoirs. It is felt that the microbial populations can either be used in surface facilities or injected underground to produce surfactants, polymers, solvents, and/or carbon dioxide that can interact with entrapped oil to enhance release. Similarly, microbial systems may be able to interact with the structures that tie up the hydrocarbons in oil shale and tar sands, thus enhancing the recovery of the included oil.

Only a limited amount of information is available on these concepts at present, although MEOR looks particularly attractive. To fully exploit these approaches, our understanding of the interactions between cells/enzymes and the interfaces between phases must be improved and entirely new approaches to bioreactor configuration and predictive process models must be developed. For example, it might be appropriate to consider processing oil shale in heaps as is now done in the mineral industry, thereby obviating the need for enclosed reactors.

3.1.2 Coal Beneficiation

Bioprocesses can also be used to enhance coal quality by the removal of heteroatom components such as sulfur and by modifying the coal surface to make it more reactive. Most of the research on biological beneficiation has been directed toward microbial removal of pyritic sulfur; however, the removal of metals, organic sulfur, oxygen, and nitrogen may also be possible. The optimum microbial population for these applications is not known, and the most efficient bioreactor configuration has not been studied.

3.1.3 Conversion of Coal to Liquids and Gases

It has been shown that some types of microorganisms and various enzyme systems can actually interact with coal to form a liquid product. Concepts to convert coal to gaseous fuels have also been developed, and demonstrations of the microbial conversion of fuel gases such as methane and syngas to liquid fuels have been conducted. These potential biological processes can be carried out at essentially ambient conditions, making them very attractive alternatives to the existing thermal/chemical processing approaches.

The use of biocatalysts in organic solvents is one intriguing approach that may revolutionize the bioprocessing of coal, although very little is known about the activity of biocatalysts in organics. Information is also needed on the bioprocessing chemistry, proper form of the biocatalyst, and probable bioreactor concepts for these various possible bioprocesses.

3.1.4 Product Upgrading

The liquid hydrocarbons that can be, or possibly could be, used for fuel may include undesirable heteroatoms and/or hazardous organic constituents. These materials are

conventionally removed by severe hydrotreating at high temperatures and high pressures; however, we now know that several types of microorganisms will interact with such constituents, possibly removing them from the liquid fuel at mild operating conditions. An emulsion-type bioreactor will probably be required for applications of this type; however, very little is known about this, including the proper form of the biocatalyst.

3.1.5 Waste Treatment

Advanced bioprocessing concepts have already been shown to be important in the cleanup of aqueous waste streams from processing facilities for fossil fuels, although predictive processing models of these systems are not yet available. It may even be possible to use certain bioadsorbents (microbial, plant, or animal biomass) to accumulate and remove dissolved heavy metals from such waste streams. Some evidence indicates that bioprocessing systems could also be used for treating product spills and solid residues, perhaps with in situ applications of the biocatalyst. Microbiological processing may prove useful in removing nitrogen and sulfur oxides from combustion off-gas systems, an approach which would require a bioreactor that can accommodate a biocatalyst in contact with a moist gaseous stream.

3.1.6 CO_2 Recycle

Energy production from fossil fuels always results in large quantities of CO_2 in the off-gas waste streams that are released to the environment. Since there is increasing concern over the continued release of large quantities of CO_2, it is appropriate to consider techniques for recovering this material, especially if recycle to a fuel material is possible. A potential approach is the photoreduction of CO_2 in conjunction with other substrates to hydrocarbons and oxychemicals by catalytic agents that are driven by solar energy. Biocatalysts, heterogeneous metal catalysts, or combinations of both types should be studied for this application.

3.2 FUELS AND CHEMICALS FROM RENEWABLE FEEDSTOCKS

The primary focus of bioprocessing development for energy applications in recent years has been the direct use of renewable feedstocks as a fuel or conversion of this

material to more useful fuels and energy-rich chemicals (see Sects. 6.3.2 and 6.3.3 for additional details). Substantial quantities of both liquid and gaseous fuels are already being produced from these materials, and even greater quantities are expected in the future. Perhaps the most important trend is the concept of a "biomass refinery" in which the renewable feed material is fractionated into several process streams having a variety of products and by-products that are optimized for efficiency and economic return.[1] Wet-corn milling is an example of this approach and could serve as a model for other types of biomass.

3.2.1 Renewable Feedstocks

The primary renewable feedstocks of interest include agricultural crops that produce sugar (sugar cane or beets), starch (grains), or biologically produced oils, as well as a large supply of lignocellulosic materials (wood, etc.) that is not yet fully utilized and potential herbaceous crops specifically grown for energy use. Much agricultural waste, forest product wastes, and municipal solid waste (MSW) can also be considered as valuable feedstocks for this application.

An important fraction of the 200 million tons of corn per year is used for ethanol production, and increasing amounts of MSW are used for methane production. However, the potential from all sources represents several million tons of valuable and renewable biomass feedstocks that can be used for energy applications.

3.2.2 Liquid Products

Sugars derived from biomass can be converted by bioprocessing systems into a wide range of liquid products, including various alcohols, organic solvents, organic acids, and many other oxychemicals that are useful for fuels or the chemical commodity market. Other components of renewable feedstocks (e.g., lignin) can also be used to produce phenols, furfural, furans, and various aromatics. The potential market for biologically produced ethanol, other fuel enhancers, neutral solvents, and organic acids could be greater than 100 million tons per year. In many cases, use of the biomass feed materials represents the direct replacement of fossil feedstocks.

The fermentation industry currently produces ethanol and organic acids (primarily from sugar extracted from grain) and would be the basis for a significant expansion into the

production of much larger quantities of these and other chemicals. However, the technology used in this industry is evolving very slowly, and advanced concepts are just beginning to be considered. The primary fractionation of the biomass feedstock into different processing streams will become even more important as other feedstocks are considered. Significant advances in the bioconversion step should be possible with better forms of the biocatalyst and advanced bioreactor concepts. More efficient methods for downstream processing for product concentration and purification will also be critical to ensure the economic viability of the bioprocesses.

3.2.3 Gaseous Products

The components of biomass are primarily hemicellulose, cellulose, and lignin, while available carbonaceous wastes may contain these materials and other fermentable constituents. These materials can be biologically converted into a variety of gaseous products, including hydrogen, methane, and possibly ethylene (see Sect. 6.3.3 for additional details). Methane is produced by anaerobic digestion of the feed material, which is typically composed of various types of municipal wastes, including sanitary wastes. Alternatively, biomass may be gasified to produce synthesis gas, a low-Btu mixture of H_2 and CO, that may then be reacted biologically to yield additional H_2, acetate, or CH_4.

Although some operating facilities utilize microbial processes for the anaerobic production of methane from municipal wastes, these bioprocessing concepts are based on very conventional technology and are largely designed by various empiricisms. Forward movement of this technology will require a much more extensive understanding of the biocatalyst (microbial population or enzymes) and its efficient formulation, as well as advanced bioreactor concepts that effectively accommodate the gaseous feed materials and products.

3.3 OTHER ENERGY-RELATED AREAS

There are many other potential technological areas where bioprocessing systems may provide alternatives that would affect energy production or the conservation of energy. For example, microbial and enzymatic processing systems can directly use solar energy to hydrolyze water to H_2 and O_2. A similar concept would allow biocatalysts to provide

electrical current from solar energy. As already mentioned in Sect. 3.1, biocatalytic systems could also utilize solar energy to reduce CO_2 to various energy-rich chemicals.

Biocatalysts may allow the more efficient conversion of some intermediate organic feedstocks to energy-intensive products. One of the most attractive approaches would be to utilize enzymes in organic media, a technique that was unknown a decade ago. Another innovative consideration is to use bioadsorbents for the economic and energy-saving recovery of strategic materials in waste streams and residues.

Most of these types of bioprocessing options are in the conceptual stage, and much additional research would be required to study the biological reagent itself and to determine the most effective bioreactor system.

3.4 ENVIRONMENTAL CONTROL TECHNOLOGY

Bioprocessing systems may be especially well-suited for the treatment of nuclear and chemically hazardous wastes with high specificity and energy economy (see Sect. 6.3.4 for additional details). The ability of such processes to effectively operate with dilute solutions of the pollutant is of paramount importance.

Many of the future processing requirements in this field are driven by federal and state statutes that are expected to be especially severe for some of the energy production areas. Process-derived effluents and residues must be accommodated; however, the environmental restoration of severely contaminated sites must also be addressed.

3.4.1 Treatment of Effluents and Residues

Biodegradation of organic pollutants in aqueous effluents may be the most effective and least expensive treatment technique. Various applications are possible for this type of bioprocessing system in the fossil and nuclear energy areas, and it may represent the most energy-efficient technique to use for some of the effluent problems in the chemical industry. Certain microbial populations have been shown to degrade phenolics, cyanides, chlorinated hydrocarbons, and other organic compounds. Such bioprocesses operate at relatively mild operating conditions and at high rates, especially if the systems can be designed for good mass transfer. It may even be possible to utilize specially formulated enzymes as the degradation biocatalyst. Some inorganic pollutants such as nitrates and

ammonia have also been shown to be biodegradable, notably when advanced bioreactor concepts (e.g., continuous fluidized beds with particles containing immobilized microorganisms) are used. Preliminary results suggest that bioadsorbents may be used to isolate and remove dissolved heavy metals in aqueous effluent streams. For example, the biomass from several microorganisms and some plant and animal tissue has been shown to accumulate several different types of dissolved metals with distribution coefficients greater than 1000 even when the pollutants are present at less than 1 ppm. In addition, radioactive materials such as uranium, plutonium, strontium, and other fission products have been isolated by bioadsorbents.

Anaerobic digestion has been studied as a potentially useful bioprocess for the volume reduction of carbonaceous solid wastes even when other hazardous materials are also present. Biocatalysts immobilized into a columnar reactor could also be used to remove some organic and inorganic pollutants in off-gas streams.

Although some of these potential bioprocessing concepts have moved through the pilot-plant stage, there is inadequate understanding of the design of large-scale systems except in a restricted, empirical sense. An additional assessment of the most effective form of the bioreagent is needed, and the various types of multiphase bioreactor concepts that will be required in this area must be studied further so that an adequate technological base will be available for design and scale-up.

3.4.2 Remediation of Waste Sites

Bioprocessing systems may play an important part in site remediation programs if contaminated waters are pumped to the surface for treatment or if soils are removed and treated in slurry bioreactors. Both biodegradation and bioadsorption processes should be readily useful in the dilute aqueous solutions recycled in such an area, while biodegradation processes should be particularly effective in treating contaminated soil. Selected biodegradation schemes may also be useful for in situ applications, especially if the biochemistry of such processes are well defined and if injection techniques for the microbial population and the necessary nutrients are optimized.

Bioprocessing approaches appear to be ideally suited for the cleanup of contaminated sites with the potential for high specificity and low energy requirements. However, only preliminary information is available for the effective use of the bioreagent of choice and

the predictive knowledge of the necessary multiphase bioreactor system or the in situ injection techniques.

4. CURRENT SUPPORT FOR BIOTECHNOLOGY RESEARCH

Fundamental biological research in the "new biotechnology" has significant U.S. government support, primarily from the National Institutes of Health (NIH) and the National Science Foundation (NSF). However, since our emphasis is on energy applications, DOE-funded research is of particular interest. Although DOE has several areas dedicated to bioprocessing, the supporting engineering research has only minimal DOE backing. An expansion into long-term, generic engineering research for bioprocessing applications would be most appropriate.

A summary of the existing research support, with an emphasis on DOE, is given below. It is based on several short presentations at the workshop (see Sect. 6.4 for the visual aids that were used) and on input from various other participants during the meeting.

4.1 U.S. DEPARTMENT OF ENERGY

The majority of DOE research support for biotechnology is for the more fundamental biological sciences and for bioprocess development in selected programmatic areas. Some backing for engineering research is also available, although that for long-term, generic, engineering research is relatively limited.

4.1.1 Division of Energy Biosciences

The Division of Energy Biosciences supports research into the investigation of fundamental biological mechanisms in both microbiological and plant sciences (see Sect. 6.4.1). This includes the study of microbial genetics and energetics as well as membrane phenomena. Investigations also include metabolic pathways and microbial ecology. No associated engineering research is supported.

Bioenergetic systems are emphasized in the plant sciences; a significant interest is shown in photosynthesis. The metabolic, genetic, and environmental influences on plant

growth and development are studied, and support is also available for the investigation of the cell wall and of plant-microbial interactions.

It is hoped that this research will provide the fundamental biological basis for future bioprocessing concepts.

4.1.2 Office of Fossil Energy

Over the past several years, the Fossil Energy Program has developed an increasing activity related to utilizing biotechnology to expand the breadth of fossil fuel use (see Sect. 6.4.2). The goals of this program are to explore and to develop new approaches in petroleum resource recovery, coal beneficiation, bioconversion of coal to gases and liquids, and bioprocessing of coal processing wastewater. Most of the support is for relatively applied work that is oriented toward specific process applications. Minimal funding can also be obtained for molecular biology that is directed toward the understanding of some microbial-coal interactions.

4.1.3 Biofuels and Municipal Waste Technology Division

The Biofuels and Municipal Waste Technology Division within DOE is developing a technology base that will allow the increased use of renewable organic materials as fuels or feedstocks to bioconversion processes producing liquid and gaseous fuels (see Sect. 6.4.3). The research, which is relatively applied, is directed toward the production of biomass feedstocks as well as the technology for thermochemical or biochemical conversion of organic feedstocks. Alcohol fuels from biomass and methane from the anaerobic digestion of MSW represent the two major processing thrusts.

4.1.4 ECUT Program

The Energy Conversion and Utilization Technologies (ECUT) Program carries out four main functions (see Sect. 6.4.4): (1) to monitor and evaluate U.S. and international basic scientific research as it may affect energy conversion; (2) to expand the technology base that is generic to energy conservation; (3) to establish concept feasibility for potentially revolutionary conservation technologies; and (4) to effect technology transfer to DOE end-use conservation programs and/or to private industry. Some critical issues that are currently being addressed include the slow rates usually associated with some

bioprocessing concepts, the lack of scale-up and design tools, and the necessity of seriously considering coproduct production. Some of the research supported by the ECUT Program is relatively basic in nature, but the research is both guided by and anticipates the needs of the end user.

A portion of the ECUT program definitely addresses engineering research in support of bioprocessing concepts; however, it is sometimes difficult to maintain long-term backing of broad-based, generic research.

4.2 OTHER RESEARCH SUPPORT

The Engineering Directorate of the NSF has two major programs that support engineering research for biotechnology. Much of this effort is oriented toward biomedical applications, but some of the research is very generic and could impact broad areas. Other governmental agencies such as the U.S. Department of Agriculture and organizations such as the Electric Power Research Institute (EPRI) and the Gas Research Institute (GRI) provide some research and development (R&D) support, particularly in bioprocess development. Both EPRI and GRI have established R&D efforts on the use of bioconversion processes, primarily with fossil fuels. While industry provides extensive R&D support for proprietary biotechnology, it designates very little for engineering research that could have broad applicability.

4.3 EXPANSION OF ENGINEERING RESEARCH SUPPORT

Clearly there is a need for more extensive support of the "bridging" engineering research that will allow the effective coupling of basic biological research with the development of specific bioprocessing systems. An expansion of some of the existing research programs to include additional engineering research would be appropriate, and the establishment of a well-defined bioprocessing research component within the DOE Division of Engineering and Geosciences would appear to be highly desirable (see Reference 4 in Sect. 2.3).

5. RESEARCH NEEDS AND OPPORTUNITIES

In a previous section, the potential impact of bioprocessing on a number of energy-related areas was outlined. However, a significant portion of the meeting was oriented toward those issues and research opportunities that would encourage future development of bioprocessing for energy applications. Although some engineering research is closely tied to a specific application, several important generic research areas cut across many different processing concepts. The consensus of the workshop attendees was that there are four primary generic engineering research areas where focused efforts could lead to greater insight into the fundamental principles underlying successful bioprocessing systems. The most effective bioprocessing concepts will include the necessary biological reagents (microorganisms, enzymes, etc.) contained in advanced bioreactors that are well-integrated and controlled for efficient operation. The product will usually be a complex aqueous mixture requiring effective separation and purification. These various processing elements could form the basis for a comprehensive program on bioprocessing research.

5.1 BIOLOGICAL REAGENTS

Within a bioprocessing system, biological reagents (i.e., biocatalysts or bioadsorbents) are required to carry out necessary chemical and/or physical changes on a feed material (substrate). The biological reagents themselves (microorganisms, enzymes, or other biomass fraction) can be considered as a series of small bioreactors, or microbioreactors, that are contained within a bioreactor vessel. We must be able to predict how each microbioreactor operates in order to understand the dynamics of the larger bioreactor system.

5.1.1 Bioreagent Formulation

The choice of a specific biocatalyst or bioadsorbent will probably be based on the actual bioprocessing needs and selected from various classes of these reagents that have been studied by the basic biological scientists. Often, however, the research engineer can

use a wide range of possible options, some of which can be studied generically. Specific examples of important research in this area are listed below:

1. Study the effects of very high concentrations of microorganisms in aqueous suspension since this is the form that many bioprocesses require.
2. Some advanced bioreactor concepts demand that the bioreagent be immobilized into or onto support particles to prevent washout. Various formulations of the immobilization matrix need to be studied and the effects of the matrix on microbial physiology and on mass transport need to be evaluated.
3. The use of mixed cultures of microorganisms, or mixtures of different enzymes, or combinations of microorganisms and enzymes may be required to optimize some bioprocessing steps. The resulting interactions and synergisms will require predictive mathematical models.
4. Apparently a large number of different types of biomass can serve as good adsorbents for various dissolved metals. Some of these are rather specific for a single metal or a few metals while others adsorb over a broad spectrum. Additional work to determine adsorption and desorption isotherms would be very useful.

5.1.2 Bioreagent Kinetics

We must develop the tools and techniques that will allow us to explore quantitatively the internal behavior of the microreactor (microorganisms, immobilized biocatalyst particle, etc.) and its relations to the external environment. Examples of some of these include the following:

1. Use of advanced bioanalytical instrumentation such as NMR, spectroscopy, fluorometry, FTIR, etc., to probe individual biocatalyst or bioadsorbent particulates.
2. Development of predictive cellular kinetics models with experimental verification.
3. Study of the structure and function of enzyme-active centers, with an emphasis on techniques for enhancing activity, reducing inhibition, and developing predictive kinetic models.
4. Study of transport processes between biocatalyst and adjacent fluids and between the immobilization matrix and other phases.
5. Investigation of cell-surface interactions since many energy applications require the reaction with a biocatalyst at a solid surface.

6. Study of the behavior of cells and enzymes in nonaqueous environments since several energy applications require interaction in that environment.

5.2 ADVANCED BIOREACTOR CONCEPTS

The macrobioreactor or the interactions occurring in the bioreactor vessel must also be fully understood and predictable. Although the conventional stirred-tank bioreactor will continue to have an important place in this technology, it will be even more important to consider advanced concepts such as nontraditional, continuous bioreactors that have a columnar configuration and operate at essentially "plug flow." Other bioreactor configurations, such as those using different geometries and operating schemes as well as those using membranes, should also be considered. In some cases, photobioreactors will have to be used since solar photons represent an important energy source (see Sects. 6.3.5 and 6.3.6 for additional details).

5.2.1 Multiphase Operation

For almost all energy-related applications, these bioreactor systems will operate with multiphases that usually contain at least one liquid, one gas, and one particulate phase. They will generally utilize a liquid, solid, and/or gas feed stream, and biocatalyst or bioadsorbent particulates will frequently be included. We currently have very little predictive capability in this field other than a few, very specific empirical correlations. In general, the design of new facilities follows a tenuous path with successive experimental verification of increasingly larger systems.

5.2.2 Columnar Bioreactor Systems

Perhaps the most important areas of future research for bioreactor systems take advantage of continuous columnar reactors that utilize the bioreagent immobilized into or onto particulates to ensure high bioreagent concentrations and prevent washout, even at high flow rates. This type of bioreactor can operate as a packed-bed or fixed-bed system when the particles are relatively large and the biocatalyst activity requires low flow rates. On the other hand, if there is high reactivity and small bioreagent particulates are used, then the particles are suspended by the fluid upflow and the system operates as a

fluidized-bed bioreactor. So-called air-lift reactors have been successfully used where the contents of the columnar bioreactor are mixed by use of a central draft tube with high gas flow, and trickle-bed reactors can be operated where there is a stationary bed of solids through which the liquid phase "trickles" countercurrent to a continuous gas phase.

Columnar bioreactors, except for the air-lift, operate with the liquid phase in essentially plug flow; however, there is considerable uncertainty concerning phase distribution, interphase mixing, transport between phases, development of concentration gradients, and prediction of the overall productivity. In order to understand fully such systems, several important research needs and opportunities exist:

1. The study of multiphase hydrodynamics will be required for the prediction of phase distribution and mixing.
2. Gas generation, coalescence, and removal must be known in order to help predict phase distribution.
3. Mass transfer between phases will be critically important to allow the overall prediction of productivity.
4. Methods for measuring, controlling, and predicting biofilm thickness will be important for biocatalyst stability and for predicting mass transport and overall productivity.
5. Predictive mathematical models, for design purposes, that take into account all of the dynamic and kinetic mechanisms will need to be developed.

5.2.3 Other Types of Bioreactor Concepts

Currently, stirred tanks, with and without immobilized bioreagents, continue to be the most-used type of bioreactor. Such a system should continue to have important utility for energy applications; in addition, several other specialized bioreactor configurations, such as membrane reactors and photobioreactors, may be useful in this context. Additional research will allow a better understanding of such systems and make it possible to more effectively predict scale-up and productivity:

1. Development of methods for the reduction of fowling of the membrane surface.
2. Study of mixing in a stirred tank with the presence of particulates of immobilized biocatalyst.
3. Development of a photobioreactor configuration that optimizes the adsorption of solar photons.

4. Investigation of interphase mass transport in all types of bioreactors.
5. Development of predictive mathematical models for scale-up that take into account all the known dynamic and kinetic mechanisms.

5.3 BIOSEPARATIONS

Separation science and technology is surely the most potent unifying element in chemical engineering, and the knowledge base and our experience in this area are extensive. Even so, bioprocessing presents a range of novel problems and situations for which our current armament is not entirely satisfactory. Bioprocesses, in general, deal with dilute solutions of materials that may be labile and entirely foreign to the process engineer. There is a lack of adequate thermodynamic knowledge of these important mixtures, and many of the most effective approaches to solving separation problems have not yet been effectively used for bioprocessing products.

5.3.1 Thermodynamics

Most separation concepts will require the distribution of the solute of interest into two or more different phases. There are very few data, useful correlations, or thermodynamic models that allow the prediction of the distribution of the solute over a reasonable range of possible operating conditions. Additional research projects such as those listed below are needed:

1. Study of the effects of "water structure" on solution chemistry and phase equilibria for small- and intermediate-sized molecules.
2. Development of a better understanding of the effects of salts on the phase equilibria for various types of solutes.
3. Devising a more rigorous predictive model for solution properties and phase equilibria, especially for multiple solutes.

5.3.2 Important Separation Technologies

Many of the products from the bioconversion of renewable and fossil feedstocks will be oxychemicals such as organic acids, alcohols, glycols, and several types of aromatics. In most cases, the oxygenated products tend to be highly hydrophilic and, therefore, are difficult to remove from water. It is normal practice to concentrate relatively dilute and

nonvolatile solutes from dilute aqueous mixtures by removing the solvent via evaporation, freeze concentration, or reverse osmosis, etc. However, these are usually very energy-intensive process steps. It is more appropriate to use thermal processes (i.e., distillation) when the minor product constituent is simply more volatile than the bulk solvent. However, low-volatility products may be more effectively recovered by solute removal using separations reagents such as extractants, sorbents, or membrane processes. Direct crystallization is another possibility for the recovery of solutes if they are sufficiently insoluble.

Additional engineering research could allow this largely empirical art to become a predictable science and technology:

1. The need for improved separations reagents (adsorbents, membranes, solvents, etc.) is obvious but could allow greater specificity and capacity.
2. Further study of reversible complexation for biochemical separations could significantly enhance specificity.
3. Multiple aqueous-phase extractions show great promise, especially for macromolecules.
4. Micellar extraction systems should be more fully explored for concepts in which one of the process phases is a liquid organic compound.
5. The use of bioadsorbents, especially for metal extractions, should be more thoroughly examined.
6. New separation concepts that allow recovery and recycle of biocatalysts such as microorganisms or enzymes could economically enhance some types of bioprocesses.
7. Optimum contactor configurations that enhance mass transfer and increase operability need to be developed and studied.

5.4 PROCESS CONTROL AND INTEGRATION

In the past, only minimal attempts have been made to integrate the several bioprocessing steps and to adequately monitor and control the whole system. Although the interest in these research areas is much greater today, additional input could result in well-controlled systems operating at near-optimum conditions.

5.4.1 Bioprocess Integration

Since many of the different steps in a total bioprocess are interactive, it is appropriate to consider the entire bioprocess as a system that encompasses the various process interactions. In fact, two or more of the steps usually carried out in separate vessels in sequence could be optimized into a single processing step. Of particular interest is the combination of the product separation/purification step in the bioconversion or fermentation vessel. This step could be carried out relatively simply by introducing into the bioreactor an extractant or adsorbent that would progress continuously countercurrent to the feed and effluent streams. Thus, the product would be stripped out of the reactor effluent and perhaps even concentrated in the separations reagent that could be collected outside the bioreactor. Not only would this integration conserve processing steps but, by reducing product buildup, might also reduce product inhibition. However, there must be "strategic planning" in deciding which components to separate, the optimum time for separation, and which reagent to use. This intriguing idea will require a significant additional engineering research effort before it can be adequately considered for specific processing systems:

1. The dynamics of the system must be carefully considered; that is, it will be necessary to determine whether a liquid extractant or a solid adsorbent that is more or less dense than the fermentation liquid phase should be used and how it will be introduced or removed from the bioreactor.
2. The change in hydrodynamics and mixing that occurs upon the introduction of another phase should be investigated.
3. Concepts that allow the continuous introduction and removal of the separations reagent must be developed.
4. The study of candidate extractants should include not only an assessment of its specificity and capacity for the product of interest, but also a measurement of its toxicity to microorganisms or enzymes.
5. Recycle of any candidate separations reagents would also have to be investigated.

5.4.2 Measuring Bioprocess Parameters

Our ability to measure critical process characteristics, especially at the micro level, is currently insufficient to allow optimum process control. We need process sensors that are

stable, rugged, inexpensive, and capable of on-line measurements which can be made near real time. In general, we can adequately measure many bulk parameters such as temperature, pH, etc., but it is very difficult to determine concentrations of the biocatalyst, substrates, and products. Some additional points are listed below:

1. In some cases the sensors must be compatible with aseptic operation.
2. Photometric and fluorometric concepts utilizing fiber optics look attractive.
3. On-line NMR may allow a more definitive evaluation of the microbial biocatalysts.
4. Biochemically based measuring devices (e.g., immobilized enzyme electrodes) add the specificity needed for individual chemical constituents if they could be designed for better stability.
5. It would be desirable to use control estimation techniques where measurable quantities are used, along with process and organism knowledge (models), to estimate or infer the unmeasurable but critical process rates and parameters.

5.4.3 Bioprocess Modeling and Control

As indicated above, an alternative to direct on-line measurement of some operating parameters is the use of estimation techniques based on proven models of the process biochemical kinetics and various process dynamics. A hierarchy of such models may be desirable, as indicated in the following considerations:

1. Complex models with many parameters are needed to improve understanding and permit simulation.
2. Systematic methods would be required to reduce complex models to simpler versions that would be useful for on-line process control and optimization utilizing currently acceptable computer control concepts.
3. Ultimately, it will be necessary to have optimization methods that can utilize complex models via small, but very fast, on-line computers and the necessary, new control algorithms.
4. Adaptive techniques are needed that can detect and compensate for changing process characterization brought on by changes in complex industrial media, organism instability, or adaptation.
5. Mathematical modeling, or artificial intelligence techniques, should be investigated to extract the real information from complex physical or chemical signals, (e.g., many

mixture may fluorescence, but how is the complicated signal interpreted to identify the quantity of the rate-controlling or rate-related compounds?).

6. Control techniques are desired which can handle the nonlinear behavior exhibited by most biochemical systems.

Many of the issues noted above represent research needs that are important not only for bioprocesses, but also for a variety of chemical processes and other engineering systems. Thus, generic engineering research in this area could have a broad impact.

6. APPENDIXES

6.1 ORGANIZATION AND MANAGEMENT OF THE WORKSHOP

The Workshop on Bioprocessing Research for Energy Applications was supported by the CEER and was organized and managed by the Center for Bioprocess/Product Development of the University of Virginia. The workshop was chaired by Elmer L. Gaden, Jr., University of Virginia, and Charles D. Scott, Oak Ridge National Laboratory; Giorgio Carta, University of Virginia, served as the executive secretary. Other members of the organizing committee were Arthur E. Humphrey, Lehigh University, and Donald J. Kirwan, University of Virginia.

The workshop consisted of five sessions. A plenary session examined areas of application and current support for research and bioprocess development support. Sessions on bioreactors, bioseparations, and bioprocess integration and control addressed critical needs for fundamental engineering research in these respective areas. Each session was comprised of lead-off presentations highlighting key research issues, followed by discussion and elaboration of these issues by the participants. Conclusions from each of the discussion groups were presented, and other research areas not previously covered were discussed in a wrap-up session. The technical program listing all of the session moderators and speakers is presented on the following page.

Wednesday, November 2, 1988

7:45 am Registration (Carlyle Foyer)

Session I. Energy Technology Areas of Bioprocessing. (Moderator - Dr. A.E. Humphrey, Lehigh University) - Carlyle I & II

8:30 am Welcome - Dr. Oscar Manly, U.S. Department of Energy
8:40 am Introduction - Dr. A.E. Humphrey
8:50 am Fossil Energy - Dr. C.D. Scott, Oak Ridge National Laboratory
9:25 am Gaseous Products from Renewable Feedstocks - Dr. J.L. Gaddy, University of Arkansas
10:00 am Liquid Products from Renewable Feedstocks - Dr. C.E. Wyman, Solar Energy Research Institute
10:35 am Break
10:55 am Nuclear and Hazardous Wastes - Dr. R.K. Genung, Oak Ridge National Laboratory
11:30 am Other DOE Programs Supporting Biotechnology Research:
Biological Energy Research - Dr. R. Rabson, U.S. Department of Energy
Energy Conversion and Utilization Technologies - Dr. J.J. Eberhardt, U.S. Department of Energy
Biofuels and Municipal Waste - Dr. R.F. Moorer, U.S. Department of Energy
Biotechnology for Fossil Energy - Dr. P.C. Scott, U.S. Department of Energy
12:30 am Lunch (Snowden I, II, & III)

Session II. Bioreactor Fundamentals. (Moderator - Dr. D.J. Kirwan, University of Virginia) - Carlyle I & II

2:00 pm Introduction - Dr. D.J. Kirwan
2:10 pm Lead-off Papers
Dr. K. Venkatasubramanian, R.J. Heinz Co.
Dr. H.W. Blanch, University of California, Berkeley
3:00 pm General Discussions
3:45 pm Break
4:00 pm General Discussions
6:30 pm Reception (Brent I & II) and Dinner (Carlyle I & II)

Thursday, November 3, 1988

Session III. Bioseparations Fundamentals. (Moderator - Dr. G.T. Tsao, Purdue University) - Carlyle I & II

8:30 am Introduction - Dr. G.T. Tsao
8:40 am Lead-off Papers
Dr. C.J. King, University of California, Berkeley
Dr. T.A. Hatton, Massachusetts Institute of Technology
9:30 am General Discussions
10:15 am Break
10:30 am General Discussions
12:00 am Lunch (Snowden I , II, & III)

Session IV. Bioprocess Integration and Control Fundamentals. (Moderator - Dr. W.A. Weigand, University of Maryland) - Carlyle I & II

1:30 pm Introduction - Dr. W.A. Weigand
1:40 pm Lead-off Papers
Dr. D.I.C. Wang, Massachusetts Institute of Technology
Dr. M.L. Shuler, Cornell University
2:30 pm General Discussions
3:15 pm Break
3:30 pm General Discussions
5:00 pm Close of Session IV

Friday, November 4, 1988

Session V. Wrap-up Discussions. (Moderator - Dr. E.L. Gaden, Jr., University of Virginia) - Carlyle I & II

8:30 am Introduction - Dr. E.L. Gaden, Jr.
8:40 am Summary Reports from Session Moderators
10:00 am Break
10:15 am General Discussions
12:00 am Close of Workshop

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6.3 SUMMARY OF FORMAL PRESENTATIONS

Four formal presentations were made during the initial workshop session, and two lead-off papers were presented in the three discussion sessions. Summaries of these papers are included in this section.

6.3.1 Bioprocessing Applications for Fossil Energy

Charles D. Scott

Oak Ridge National Laboratory

BIOPROCESSING APPLICATIONS FOR FOSSIL ENERGY

Charles D. Scott
Oak Ridge National Laboratory*

INTRODUCTION

With the advent of modern bioprocessing techniques, an increasing number of concepts that are available for consideration in the processing of fossil fuels. The bioprocessing systems that have been proposed or can be envisioned for the future involve almost all aspects of coal utilization as well as several areas in oil and gas extraction and processing. The advantages of highly specific biocatalytic action, mild operating conditions, and the potential for efficient bioreactor systems make these new processing concepts quite attractive. Unfortunately, very few have been developed to the extent that a firm economic process analysis can be made. Additional research and development (R&D) will be required in order to bring such innovative concepts to the industrial sector. This will require generic bioengineering research on advanced bioreactor systems, innovative separation concepts, and bioprocess control and integration as well as bioprocess development for specific processing systems.

ADVANCED BIOPROCESSING CONCEPTS

The primary bioprocessing concepts for application to fossil energy include enhancing the extraction and recovery of petroleum and other carbonaceous materials; beneficiating coal by removal of sulfur and other components; converting coal to useful liquid or gaseous products; upgrading product quality with removal of detrimental and hazardous components; and utilizing environmental control technology for gaseous and liquid effluents and solid residues. Several of these potential bioprocessing systems will be described in more detail below.

Enhanced Oil Recovery

Secondary recovery of oil is usually accomplished by water or steam flooding to force additional oil out of the geologic formation. Oil recovery can be further enhanced

*Operated by Martin Marietta Energy Systems, Inc. for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400.

by injecting various chemicals such as surfactants, polymers, and solvents that can alter the water-oil interfacial properties or viscosities or the bulk flow patterns. Bioprocessing systems are likely to play important roles in several aspects of enhanced oil recovery. Most current research is directed toward injection of microorganisms into the geologic structure in order to provide for in situ production of the types of materials indicated above.[1,2] Microbial processes can also be considered for the surface production of the same types of chemicals for controlled injection into the geologic structure.

The underground microbial processes are not well defined, and they are almost impossible to control. Further, modeling of the system that would allow quantitative predictions has not been developed. Surface bioprocessing will require efficient and continuous bioreactors integrated with inexpensive separation systems. This type of system must be developed to the extent that the fundamentals are sufficiently well understood so that predictive models are available for design purposes.

Coal Beneficiation

Beneficiation or enhancement of coal quality can serve two purposes: the removal of heteroatom components from the coal and the upgrading of the coal itself. Most research on biological beneficiation has been directed toward microbial removal of pyritic sulfur.[3,4] Recently, the removal of metals, organic sulfur, oxygen, and nitrogen by microbial interactions has also received attention.[4,5]

Removal of these heteroatoms from coals can be affected by (1) choice of bioreactor configuration, (2) particle size or available surface areas, (3) coal slurry density, and (4) the physical and chemical conditions within the reactor. The optimum reactor configuration has not been identified, although a continuous stirred tank or fluidized bed will probably be the best choice. Although this bioprocessing concept has received more attention than any of the others, a predictive capability for process design still does not exist and this is especially true for the bioreactor system.

Conversion of Coal to Liquids and Gases

It has now been shown that some types of microorganisms can solubilize low-ranked coal into an aqueous solution (see Fig. 1).[6,7] In addition, preliminary tests have demonstrated that various enzyme systems in vitro can interact with coal in both aqueous and organic media.[8]

The aqueous product from microbial solubilization of coal can also be used as the feed material for a second microbial processing step in which the gaseous product would be predominantly methane with CO_2.[9] Both processing steps would be carried out at

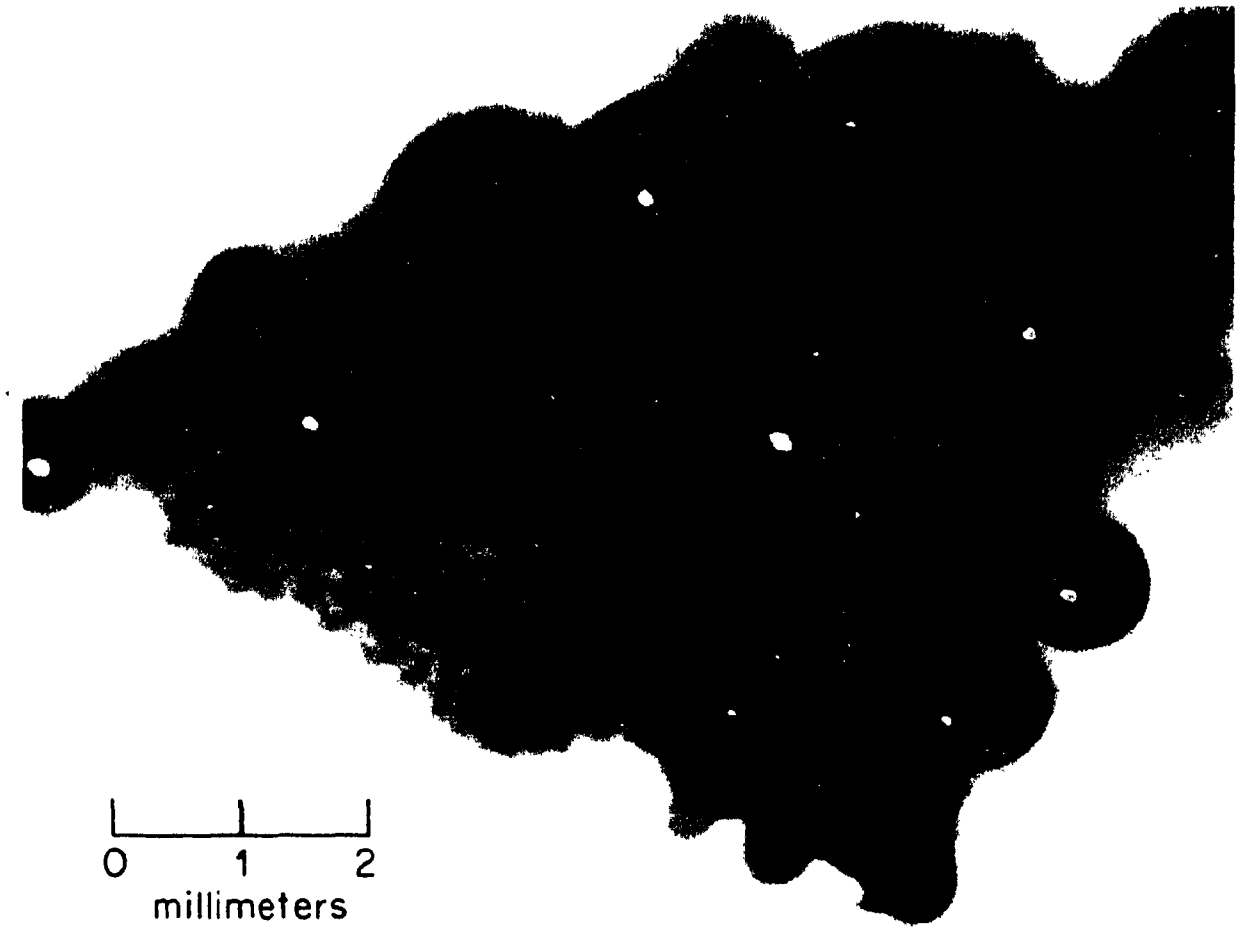

Fig. 1. Liquid droplets produced by fungi in the process of solubilizing lignite coal.

essentially ambient conditions, with the first solubilization step being conducted in air and the second step requiring anaerobic conditions.

The biochemistry of these proposed processing systems needs to be further addressed; however, an equally significant problem area is the choice of the proper coal conversion bioreactor since it will have to deal with coal particulates or other solids and will likely require three- or four-phase operation. The most effective type of bioreactor will probably be a continuous columnar-type system operating either as a packed bed or a fluidized bed (see Fig. 2).[7] Such multiphase systems have been studied on a small laboratory scale for specific applications, but scale-up is still quite difficult.

Product Upgrading

The liquid products from coal conversion processes, petroleum, and oil from shale and tar sands may include undesirable heteroatoms and/or hazardous organic constituents. The heteroatoms (primarily oxygen, nitrogen and sulfur) occur in aromatic compounds, while the hazardous organics are also aromatic compounds, usually multiring, with various substitutions. The materials are removed, in most cases, by severe hydrotreating. We now know that several types of microorganisms will interact with such materials by breaking the ring structure and releasing unwanted constituents.[4]

An emulsion reaction system where the biocatalyst is maintained in the dispersed aqueous phase, which is in intimate contact with the hydrophobic oil phase, will probably be required. Air or hydrogen will probably also have to be added as an interacting substrate. The degraded constituents should be hydrophilic and progress to the aqueous phase, thus cleaning up the liquid product. Emulsion bioreactor systems have had only a cursory investigation. As a result, the fundamental properties of such a system are not known, and there is almost no predictive capability.

Environmental Control Technology

The potential environmental impact of fossil fuels includes the mining and recovery of the primary fuel, the release of hazardous materials in process gaseous and liquid effluents, the ultimate disposal of solid residues, and the effects of large product spills. Advanced bioprocessing concepts have already been shown to be important in the cleanup of aqueous waste streams[10,11] and microbial systems are being considered for stabilizing product spills and residues.[2,4] Microbiological processing has also been proposed for the removal of nitrogen and sulfur oxides from fossil fuel off-gas streams.[4] This approach would require still another class of bioreactor systems -- one that can accommodate a xmoist gaseous stream which must contact the biocatalytic system.

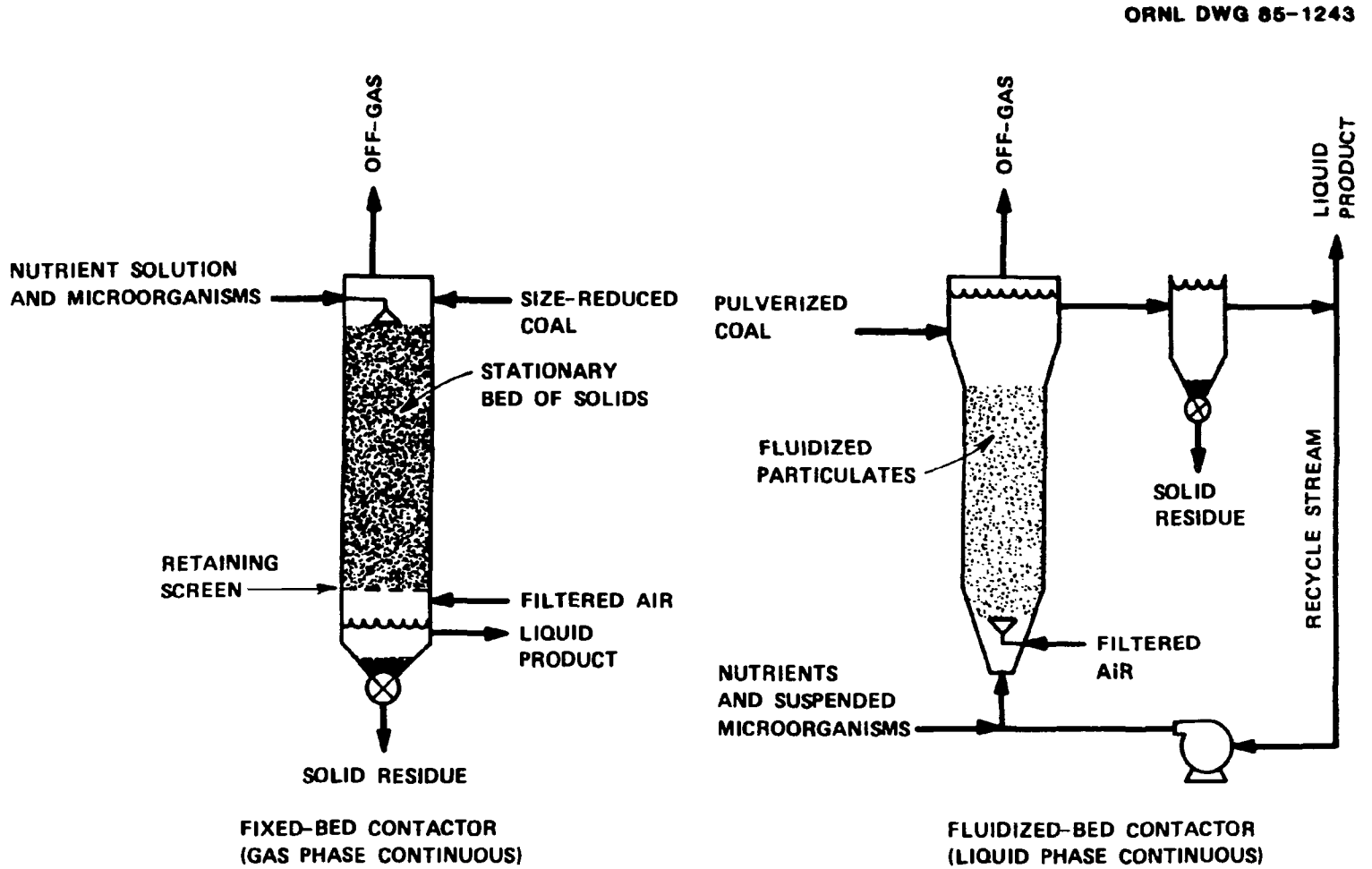

Fig. 2. Two possible bioreactor configurations for the biological solubilization of coal.

Although some preliminary laboratory investigations have been carried out on these applications, the most challenging problems will be the development and understanding of a series of new, innovative bioreactor concepts. This must include a determination of the fundamental mechanisms involved as well as the integration of such reactors into an overall processing scheme.

RESEARCH OPPORTUNITIES

Most of the potential bioprocess applications for fossil energy are concepts that are just beginning to be thoroughly studied. Since no significant R&D background exists for these or comparable systems, advanced approaches are a necessity and readily acceptable. A considerable amount of additional engineering research will be required before bioprocess development can be successful, and much of this effort will appropriately be generic in nature.

Advanced Bioreactor Concepts

One of the most important research areas will be the study of innovative bioreactor systems particularly those that can adequately handle particulates and multiphase operation. Even when conventional stirred-tank bioreactors are used with particulates, it is difficult to predict mixing, hydrodynamics, and mass transfer properties. Even less is known about these properties for continuous columnar systems such as packed beds and fluidized beds, and there is almost no definitive understanding of emulsion-type contactors and gas-phase bioreactor systems.

Research into these advanced bioreactor concepts should concentrate on efficient biocatalytic systems and the fundamental kinetic and dynamic parameters. Coupling hydrodynamics, biokinetics, and mass transfer into a definitive and predictive mathematical model of each of the reactor types could provide the basis for process development and scale-up.

Efficient Bioseparations

Many of the products of bioconversion processes that will be utilized for fossil energy will be a relatively dilute mixture of hydrocarbons either in aqueous solution or in organic solvents. These products and by-products must be recovered, concentrated, and, in some cases, purified before they can be used. Distillation has commonly been used for these types of materials in the past, and it will continue to be an important

processing tool. However, solvent extraction, precipitation or crystallization, adsorption, and other techniques may be the systems of choice for many bioprocessing applications.

Biological agents may also be important for useful separations since certain microorganisms and plant and animal tissues have shown high affinities for dissolved metals. These bioadsorbents could prove to be the most efficient materials for the isolation of trace metals from aqueous streams.

More effective separation systems will require the development of better separations reagents and contacting systems. Research into these areas should provide much useful and needed information.

Integration and Control

Since each of the individual steps can affect the overall efficiency of the bioprocess. it is important to consider the system as a whole. In some cases, it may be more efficient to carry out two or more processing steps, for example, bioconversion and product recovery, in a single vessel. It will also be necessary to monitor important processing parameters and exercise control to achieve optimum operating conditions. Thus, research into better sensing devices, more predictive control algorithms, and computer interfacing will also be important.

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6.3.2 Gaseous Products from Renewable Feedstocks

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GASEOUS PRODUCTS FROM RENEWABLE FEEDSTOCKS

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INTRODUCTION

Renewable feedstocks, or biomass resources, are plentiful in this county, as well as round the world. For example, MSW, agricultural residues and forest residues, available in the U.S.A. annually are 100,400 and 1,000 tons, respectively. If converted into methane, these renewable resources could supply 6, 24, or 60 percent, respectively, or our current natural gas supply. Similarly, energy crops, grown on the 100 million acres of idle, arable forest and rangeland, could supply all the nations' current energy needs (Clausen and Gaddy, 1979).

While biomass resources are sufficient to meet our energy requirements, efficient and economical methods of conversion are essential. Biological conversion techniques are particularly attractive because of potentially high yields and efficiencies. Biological processes are also compatible with the environment and produce specific products, with few by-products for separation and disposal. The principal disadvantages of bioconversion processes is slow reaction rates and the requirement for large reactors. Consequently, reaction kinetics and bioreactor design are especially important.

The components of biomass include hemicellulose (15-35%), cellulose (35-55%), lignin (10-30%), and ash (5-10%). Biomass may be biologically converted into a variety of gaseous products, including hydrogen, methane, and possibly ethylene (Oremland, 1981; Oremland and DesMardis, 1983). Methane is produced by anaerobic digestion of carbohydrate by:

$$(C_6H_{12}O_6)_n \rightarrow 3n\ CH_4 + 3n\ CO_2 \qquad (1)$$

Alternatively, biomass may be gasified to produce synthesis gas, a low BTU mixture of H_2 and CO according to:

$$(C_6H_{12}O_6)_n \rightarrow 6n\ CO + 6nH_2 \qquad (2)$$

The Co and H_2 in synthesis gas may be reacted biologically to yield additional H_2, acetate, or CH_4. The principal gaseous bioproducts from biomass are H_2 and CH_4. These gases have a very low value and economics will not allow large or costly reactors. Commercialization of these processes

will require research to improve reaction rates. There are only two ways to improve reaction rates in biological processes: better microorganisms to improve the biological pathway or better bioreactors to concentrate microorganisms and improve mass transfer. Both methods will be considered in defining the production of gaseous products. The purpose of the following is to examine the microbiology and reaction kinetics of producing gaseous products from biomass, with the objective of analyzing reaction rate limitations and improvements. Various bioreactor designs will be examined to determine parameters that minimize reactor volume. Research needs will be assessed in each of these areas.

MICROBIOLOGY/KINETICS OF GAS PRODUCTION

Production of Hydrogen or Methane from Pyrolysis Gas. Synthesis gas from the gasification of biomass will contain 30-60 percent H_2, 10-30 percent CO, 15-35 percent CO_2, 5-15 percent CH_4, with trace quantities of higher hydrocarbons (Midge, *et al.*, 1983). Unless the water gas shift reaction is employed, the gas will contain about equal amounts of H_2 and CO. These synthesis gases may be biologically converted into H_2 or CH_4. The organisms, Rhodopseudomonas gelatinosa and Rhodospirillum rubrum utilize CO to produce H_2 and CO_2 phototrophically by the reaction:

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (3)$$

If the desired gaseous product is H_2, this reaction can be used with raw synthesis gas to produce a gas containing predominately H_2. Removal of CO_2 would yield nearly pure H_2.

Other bacteria, such as Peptostreptococcus productus, Acetobacterium woodii, and Eubacterium limosum, produce acetic acid as follows:

$$4\ CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad (4)$$

or

$$2\ CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (5)$$

Methane may be produced from acetate by Methanothrix soehngenii and Methanosarcina barkeri as follows:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (6)$$

All methane bacteria utilize CO_2 and H_2 to form CH_4 by the reaction:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2\ H_2O \quad (7)$$

There are two possible routes to CH_4 from synthesis gas: through H_2 by

Equations (3) and (7), or through acetate by Equations (4), (5) and (6). The use of acetate as an intermediate has several disadvantages, including substrate inhibition to methanogens and slow growth and production rates. Therefore, the route utilizing H_2/CO_2 appears to be more promising, even though R. rubrum requires energy in the form of light for growth.

The production of either H_2 or CH_4 from synthesis gas requires the transport of the gaseous substrate from the gas phase, through the liquid phase, to the microorganism (solid phase). The gaseous substrate must be absorbed at the gas-liquid interface and diffuse through the culture medium to the cell surface to be consumed by the microbes. For sparingly soluble gases, such as carbon monoxide, oxygen, etc. and for suspended cells, it has been established that the primary resistance to the transport process lies in the liquid film (Tsao and Lee, 1977; Blanch, 1979; Yoshida, 1982). The rate of transport of gas, N_S^G, is given by:

$$-\frac{1}{V_L}\frac{d\,N_S^G}{dt} = \frac{K_L a}{H}\left(P_S^G - P_S^L\right) \tag{8}$$

where V_L = the volume of the liquid phase,

$K_L a/H$ = the ratio of the mass transfer coefficient and the Henry's Law constant,

P_S^G = the substrate partial pressure in the gas phase, and

P_S^L = the substrate partial pressure in the liquid phase.

Mass transfer is promoted by maximizing the mass transfer coefficient or the driving force, $P_S^G - P_S^L$. The mass transfer coefficient may be maximized by utilizing bioreactors that promote gas-liquid mass transfer.

Equation (8) also shows that the rate of transport of gaseous substrates from the gas phase into the culture medium is faster for higher partial pressures in the gas phase. In the case where the overall reaction rate is transport controlled, that is, the dissolved substrate concentration in the liquid is zero, the rate of transport and thus, the rate of reaction is proportional to the partial pressure in the gas phase. Equation (8) then reduces to:

$$-\frac{1}{V_L}\frac{dN_S^G}{dt} = \frac{K_L a}{H}\left(P_S^G\right) \tag{9}$$

Obviously, operation at higher than atmospheric pressure is advantageous in these situations. On the other hand, high pressure operation causes the concentration of dissolved substrate in the liquid phase to increase. Some gas phase substrates, such as carbon monoxide, may be inhibitors of the metabolism of anaerobic bacteria, so that care must be taken to avoid high dissolved substrate concentrations. One way to avoid the accumulation of high dissolved substrate concentrations is to increase the cell concentration at the same time the pressure is increased.

Anaerobic Digestion. Anaerobic digestion is generally regarded as the interdependent metabolism of complex substrates by a mixture of facultative and anaerobic bacteria to yield CH_4 and CO_2. Three steps are required: hydrolysis of complex organics, such as polysaccharides, by extracellular enzymes to soluble sugars; second, these intermediates are metabolized to acetate, formate or H_2 by acetogenic bacteria; and finally, methanogenic bacteria produce methane and CO_2 from Equations (6) and (7). Careful control of the process is necessary to maintain the proper physiologic balance between the various populations. Methanogenic bacteria are quite sensitive to reduced pH, and an over production of acetate (or other organic acids) will impair or terminate methane production. The parameters that affect digestion include retention time, feed quality and concentration, organic loading rate, temperature, and pH. Significant opportunity exists to develop cultures, perhaps a co-culture, to carry out this series of reactions that will be more stable and exhibit superior kinetics.

The anaerobic digestion process is characterized by slow reaction rates and long retention times (20-40 days). In the digestion of lignocellulosic matter, hydrolysis is considered to be the rate limiting step. It has been found that the kinetics of the reactions may be represented by a first order expression:

$$r_s = \frac{ds}{dt} = kC_o \qquad (10)$$

where r_s = rate of substrate disappearance
s = substrate
t = time
C_o = concentration of substrate in reactor
k = reaction rate constant

Combining this expression with the mass balance in a mixed reactor gives:

$$r_s = kC_o = \frac{C_i - C_o}{t} \qquad (11)$$

where C_i is the inlet substrate concentration.
This relationship may be used to determine the reaction rate coefficient, which characterizes this reaction. For lignocellulosic biomass, the value of the rate constant, k, has been found to be about .07 $days^{-1}$ (Clausen and Gaddy, 1983). From Equation (11), it is noted that with such a low rate constant, retention times (and reactor volumes) will necessarily be large to achieve a high conversion. Therefore, research efforts have been and must continue to be directed toward increasing reaction rates and developing cheap reactors. Methods to increase k by improving the culture, reaction conditions, or the rate limiting step will be considered.

Reaction Conditions. Methanogenic bacteria function within a narrow pH range of 6-8, and optimally at a pH of 7. Slight deviations will severely impair methane production. When the methanogens are inhibited, acids accumulate with a further lowering of pH and methane production soon ceases. Therefore, close pH control is essential. However, the cost of base to adjust pH on a continual basis would be prohibitive. Consequently, pH must be controlled by balancing the microbial population.

Microorganisms in the digester function optimally at either of two temperature ranges: mesophilic, 35°C, or thermophilic, 65°C. Reaction kinetics have been shown to be considerably higher for certain substrates at thermophilic temperatures. However, conversions have also been impaired, for some lignocellulosic residues, probably due to the faster deactivation of cellulase enzymes. Optimal temperatures for anaerobic digestion have yet to be determined.

Culture Enhancement. Efforts have been made to improve reaction rates by both collectively and individually, enhancing the mixed microbial population. Since digestion of biomass is limited by hydrolysis, the addition of celluloytic microorganisms should be beneficial. It has been found that cultures digesting corn stover could be enhanced by addition of Clostridium butyricum (Corder, *et al.*). The ultimate biodegradability was increased slightly to 82 percent over the standard culture. However, the rate

coefficient was increased about 20 percent. Such results indicate the potential for substantial improvement in anaerobic digestion through culture design.

Chelation Enhancement. Trace metals, such as iron, nickel, cobalt and molybdenum, are necessary for the maintenance of a healthy biological population, in anaerobic digesters. These metals are usually present, but may be insoluble or complexed with other elements and unavailable for uptake by microorganisms. Small quantities of chelating agents will allow higher concentrations of trace metals in soluble form for biological growth. A recent study with digestion of sludge found that increases in gas production of up to 50 percent could be achieved with small dosages of EDTA or CA (Ko and Gaddy, 1988). These data show that these improvements are influenced slightly by dosage above 10 μM and affected significantly by retention time, with greater improvements at the longer residence times. Such experiments indicate that there is substantial potential to improve reaction rates through enhancing conditions for microbial growth.

Addition of Methanogens. Laboratory experiments have been conducted to determine the potential improvement in digestion rates of sludge by addition of pure methanogenic bacteria (Ko and Gaddy, 1988). Standard cultures were enhanced with small amounts (1 g/L and 2 g/L) of Methanosarcina barkeri in continuous culture. The gas production from the 1 g/L reactor showed an average improvement over a control reactor of 35 percent for a period of five months. After addition of another 1 g/L, the performance improved to an average of 59 percent for the next four months. Furthermore, the operation of the amended reactor was much more stable.

The significant improvement of the amended reactor is attributed to the ability of M. barkeri to assimilate both acetate and H_2 and CO_2. Through the use of labeled acetic acid, Smith and Mah (1966) found that 73 percent of the methane produced in a standard anaerobic digester came from acetate. Thus, the utilization of acetate by M. barkeri plays an important role in the improvement. Furthermore, the bacteria are also responsible for maintaining the H_2 concentration in the reactor at a very low level in order for the fermentation to proceed efficiently. Hydrogen is involved in all principal biological reactions and is recognized as being the controlling influence on the overall scheme of anaerobic digestion (Bryant, 1979). Hydrogen exerts an

important influence in controlling the proportion of products from the first stage of anaerobic digestion, as well as the degradation of these products in the second stage. Therefore, M. barkeri, which uses both H_2 and acetate, enhances the fermentation by maintaining lower H_2 levels, as well as producing more gas from acetate.

Perhaps the surprising result is not that methanogens improve the rate, but that the improvement is so large and permanent. It has long been recognized that in sludge digestion, the rate limiting step was methanogensis (Ghosh and Pohland, 1974; Kaspar and Wuhrmann, 1978). Therefore, the addition of methanogens would be expected to increase gas production rates. The increased methanogen population functions to speed up the rate limiting step, as well as the other steps. However, the increase was found to be nearly 60 percent at long retention times and lasted for nearly one year. Even greater improvement might result with an increased dosage.

The permanent effect might be explained by considering that a normal culture can only move to a higher methanogen population by an over-production of acetate as substrate for methanogen growth. An excess amount of acetate would lower the pH and adversely affect methanogen performance and growth. Thus, a normal culture might never evolve to a higher methanogen population, given the many other variables that are changing. However, addition of methanogens would allow an increase in the acetate production to sustain the higher cell concentration.

Separate Stages/Pre-Treatment. Since anaerobic digestion is a multi-step process, it is logical to expect that separation of the stages would result in more efficient individual steps and the possible enhancement of the overall process. Consequently, various designs have been studied to carry out the hydrolysis/acetogenesis in an initial reactor, followed by methanogenesis in a separate reactor (Rijkens, 1981; Legrand, *et al.*, 1988), or hydrolysis by pre-treatment followed by acetogenesis/methanogenesis (McCarty, *et al.*, 1983; Clausen and Gaddy, 1978).

In the former system, the requirement for two reactors is offset by smaller individual reactors, as well as higher methane concentrations. Immobilized cell reactors for methanogenesis have been operated at retention times of a few hours, however, retention times for the first stage require

several days. These systems do show potential for improved economics and are worthy of further research.

Methods of pre-treatment have included the use of caustic (Jerger, *et al.*, 1983), acids (Clausen and Gaddy, 1978), and autohydrolysis at elevated temperatures (McCarty, *et al.*, 1983). In all cases, the cost of chemicals or energy have proven prohibitive in the current economy for production of a gaseous product. Also, more severe instability problems in operation of the second stage system are likely.

BIOREACTOR DESIGN FOR GASEOUS PRODUCTS

The bioreactor is the principal capital and operating cost element in the production of gaseous fuels, consequently, bioreactor design is a primary consideration. There are various types of bioreactors, including batch, plug flow, CSTR and immobilized cell reactors, that have been studied for these systems. Each of these will be considered briefly, following a discussion of the important variables controlling reactor volume.

Using Equation (11), it can be shown that the relationship defining the reactor volume, V, is given by (Magruder, *et al.*, 1987):

$$V = \frac{M}{6W_B d\ k}\ \frac{1}{1-X} \qquad (12)$$

where M = volume of gas (methane) produce
W_B = weight fraction of biomass fed
d = density of biomass mixture
X = conversion of biomass

For a given methane production level, factors to reduce reactor volume include: reducing conversion, which is counter-productive; increasing density, which is not possible above fluidity; increasing the rate coefficient, already considered; and increasing the biomass fraction. Consequently, bioreactor designs that allow high cell concentrations and, thereby, high rate coefficients, as well as high biomass concentrations, will minimize reactor volume. Of course, low cost reactors will also be an important consideration.

Batch Reactors. The batch reactor, or semi-batch reactor, has been the predominant design for small scale anaerobic digesters around the world for many years. Large numbers of these systems are used to produce gas from

biomass for cooking and lighting in remote regions. Due to variable loading and lack of agitation or temperature control, these systems do not produce a dependable supply of gas and would not be suitable for large scale application.

More dependable farm systems have been designed and operated to produce both heat and electricity (Clausen and Gaddy, 1983). This simple type of design, with co-generation, can supply all the heating and electrical needs of a typical farm from crop residues from a few acres. Capital and energy costs would be competitive with LPG at about $5 per MM BTU. Batch reactors have also been considered for use in staged operation and in combination with continuous reactors (Legrand, *et al.*, 1987). Such systems have also been shown to have economic potential.

Dry Fermentation/Landfill. In order to minimize reactor cost, inexpensive batch designs, often underground, have been proposed which simulate landfill operation. Although rates are quite slow, anaerobic conditions exist and methane can be collected from shallow wells in landfills (Van Heuit, 1983). Studies of similar batch reactors with low moisture content (dry fermentation) have been shown feasible (Cheng, 1975; Wujcik and Jewell, 1979). Reactor cost per unit volume can be minimized. However, rates are quite slow unless moisture is circulated, which adds to the cost. Also, when free moisture is eliminated (solids concentrations > 15 percent), the density is reduced and reactor volume is increased, as shown by Equation (12).

CONTINUOUS REACTORS

Plug Flow Systems. Plug flow reactors take maximum advantage of concentration profiles in continuous systems to minimize reactor volume. For soluble substrates, this design also allows high cell densities in immobilized cell reactors (ICR). Plug flow reactors have been used successfully for animal waste digestion (Martin and Lichtenberger, 1981). Such systems, however, require an inocula of microorganisms and are not appropriate for lignocellulosic matter, unless a recycle of effluent is provided. Also, ICR systems cannot be used unless soluble substrates are used or frequent shut down for cleaning is provided.

Stirred Reactors. The conventional stirred tank reactor suffers from the major disadvantage that reaction rates are impaired by high conversion in the

reactor. However, the conversion can be used to increase the effective solids concentration and proportionately reduce reactor volume, according to Equation (12) (Clausen and Gaddy, 1987). Fluidity must be maintained in the reactor to promote mass transfer and maintain high reaction rates. The maximum solids concentration for fluidity in the reactor is about 10 percent. However, because of the conversion in the reactor, feed concentrations can be much higher and still maintain 10 percent or less in the reactor. For example, a conversion of 66 percent would enable an increase in feed concentration to 30 percent which would reduce the reactor volume by two-thirds, while maintaining a 10 percent reactor concentration.

This significant reduction in reactor volume is only possible if the reaction rates are not impaired at the higher concentrations. Data for conversion of corn stover and MSW at various solids concentrations, up to 30 percent, are given in Figure 1. The slope gives a rate constant, k, of .068 $days^{-1}$, identical to the result for lower concentrations. The ultimate conversion is still 80 percent, therefore, the full benefit of high solids digestion is possible, up to 30 percent solids.

Cell Recycle. Experiments have also been conducted to determine the effect of separating liquid and cells from the reactor effluent for recycle (Clausen and Gaddy, 1987). Recycle has the effect of increasing microorganism concentration and reaction rate coefficient. These data show that conversions are enhanced by 10-15 percent with recycle of 25-50 percent of the available liquid effluent.

Series Operation. For positive order reactions, such as anaerobic digestion, the reactor volume of stirred reactors can be reduced by arranging several reactors in series. Studies have shown that the optimal number of reactors in series is two (Clausen and Gaddy, 1987). These laboratory experiments have shown that series arrangement gives a 15 percent improvement in conversion and reaction rate over a single reactor with the same total volume.

Immobilized Cell Reactors for Gaseous Substrates. When producing H_2 or CH_4 from synthesis gas, the gas solubilities are very low and mass transfer is generally controlling. Mass transfer may be improved by high agitation rates and with high cell densities. The ICR, or bubble column, allows high cell concentrations and high mass transfer coefficients, without agitation.

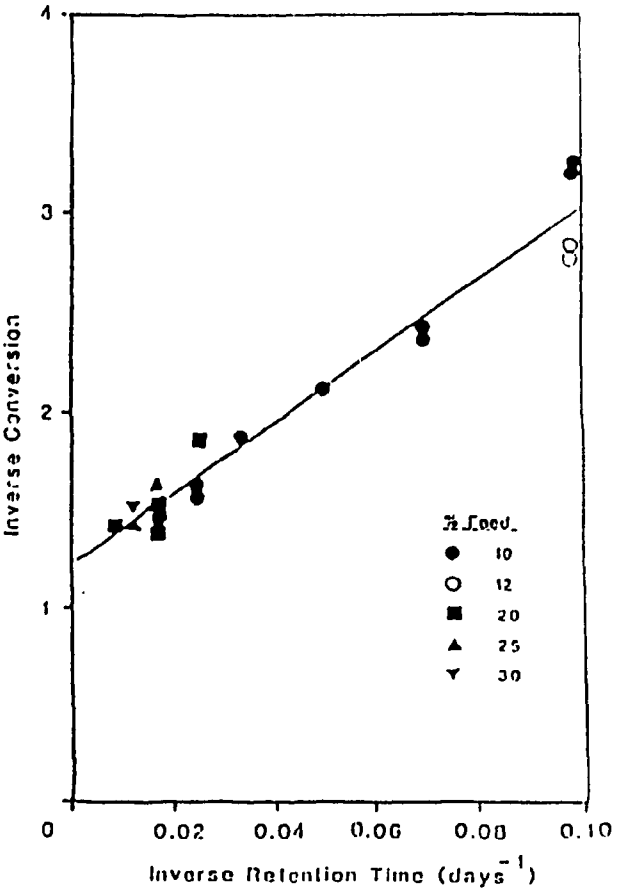

Fig. 1. Conversion-retention time relationship for corn stover/MSW digestion.

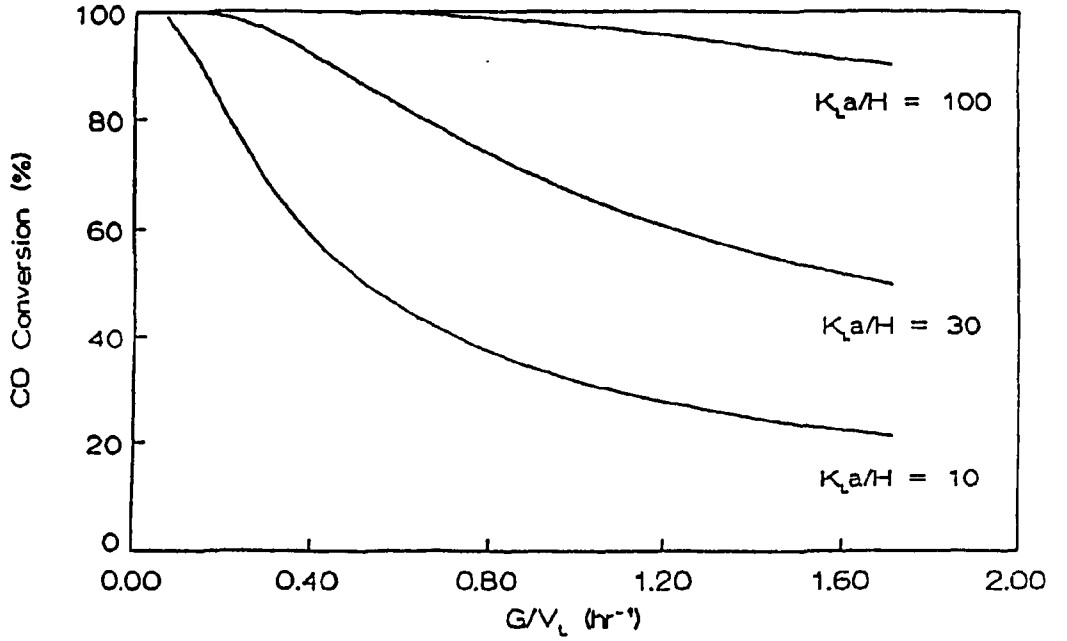

Fig. 2. Carbon monoxide conversion as a function of gas flow rate in a bubble column.

Experiments at different gas flow rates have been conducted with P. productus in a bubble column to develop scale-up parameters (Vega, *et al.*, 1988). A mathematical model has been developed to evaluate conversion of CO under mass transfer controlled conditions. These results are given in Figure 2 for various values of K_La/H, where K_La is the mass transfer coefficient and H is Henry's law constant. As noted, high conversions can be achieved at high dilution rates or low retention times. For example, 90 percent conversion can be obtained in the bubble column with a 30 minute residence time at a K_La/H of 100. It has also been found that reaction rate is proportional to pressure, up to 15 atm. Therefore, the equivalent retention time for a given production rate can be reduced by increasing the pressure. Consequently, 90 percent conversion of CO can be achieved in a column providing only a two minute gas residence time. Clearly, these reactors have great potential for gas fermentations and should receive further study.

SUMMARY

While the value of gaseous products is low and reaction rates are slow, potential for improving technology for biomass conversion is significant and should be pursued. The ability to design improved anaerobic digestion cultures has been demonstrated with cellulolytic organisms and addition of methanogens. Research with various co-cultures may be very fruitful. Enhanced microbial growth and culture performance has been shown feasible by addition of economical quantities of chelators. Pre-treatment of lignocellulosic material has not proven economical. However, separation of the stages has been shown to substantially reduce retention times and increase methane concentrations.

Bioreactor design will have an important influence on the commercial application of this technology. Batch reactors are the appropriate choice for farm systems. Simple and economical underground batch reactors suffer from slow rates and high labor requirements. Plug flow systems are appropriate for animal waste, but have not been applied to crop matter. The CSTR allows operation with high solids, which provides significant reductions in volume. Cell recycle and series operation of these systems enable further volume reduction. For gaseous substrates, the bubble column appears to offer minimal cost and volume. Retention times of only a few minutes are possible at high conversion with these systems.

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6.3.3 Liquid Products from Renewable Feedstocks

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LIQUID PRODUCTS FROM RENEWABLE FEEDSTOCKS

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INTRODUCTION

Biotechnology is a name often used to describe a revolutionary field that has its origin in antiquity. Such products as bread, wine, and cheese have been produced through fermentation processes for thousands of years. Although a fermentation industry that included production of alcohols, enzymes, organic acids, and yeasts developed early in the twentieth century, many of the chemical products based on fermentation technology gave way to those derived from inexpensive crude oil and natural gas in the mid twentieth century. Recently, the staid fermentation sciences changed dramatically with the development of genetic engineering techniques that allow introduction of foreign DNA into microbes, thereby radically altering their ability to produce key products. Through genetic engineering of organisms, products or enhanced capabilities can be achieved for biological-based processes that were never before thought possible. These rapidly moving advances now characteristic of the field of biotechnology have the potential to radically lower the cost of biologically derived liquid fuel and chemical products to the extent that fermentation processes will likely replace many petroleum based processes for fuels and commodity chemicals production, reversing the trends of the mid twentieth century.

In this paper, a brief review will be presented of the potential of biotechnology for production of large volume liquid products such as commodity chemicals and fuels from renewable feedstocks. Emphasis is placed on large volume markets because they could have significant impact on the international competitiveness and security of the United States but the research required to make the technology economically viable is considered too long term to attract the interest of the private sector. Renewable feedstocks are stressed because they provide a domestic, endless supply of raw materials that could be immune to disruption by foreign suppliers and improve international balance of payments. Furthermore, as liquid products derived from these feedstocks are burned, carbon is recycled perpetually between plant matter, liquid products, and carbon dioxide so that carbon dioxide doesn't accumulate in the atmosphere to contribute to the so-called greenhouse effect. Finally, attention is focused on issues in bioprocessing since competitive advantage in areas related to biotechnology may depend as much on advances in bioprocessing as on innovations in genetic

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engineering. In this regard, it is worth noting that many countries such as Japan have made a major commitment to bioprocessing since they recognize its importance to international competitiveness(1).

RENEWABLE FEEDSTOCKS

Several feedstocks could be used as substrates for biological production of large volume liquid products. These include sugar crops, starch crops, lignocellulosic materials, and oil crops such as microalgae. In the United States, sugar prices are generally too high at about $0.20/ pound for sugar crops to be a viable feedstock for production of large volume products that typically have low values. However, corn, a starch crop, sells for only $1.50 to $3.00 per bushel ($0.03 to $0.06/pound), and the total annual U.S. corn production for all uses runs at about 200 million tons. About 800 million gallons/year of fuel ethanol is produced from corn in this country, and since the wet milling process often used in ethanol production leads to a number of valuable co-products, the price of ethanol is moderated from the full price impact of fluctuations in corn prices. Yet, the value of these co-products and their smoothing effect on ethanol prices would probably diminish greatly at moderately higher rates of production. This, along with the limited availability of acceptable land, could hinder substantial growth in ethanol production from corn.

Lignocellulosic materials are comprised of carbohydrate polymers known as cellulose and hemicellulose plus lignin. Agricultural residues, municipal solid waste, and underutilized standing forests are examples of this largely untapped source of renewable feedstocks. Since the major use of lignocellulosic biomass is limited currently to burning of forestry residues for some process heat, significant disagreement exists over the availability and cost of these materials, and the estimates of the resource vary widely. For the United States, the amount of collectible underutilized wood, a term used to describe traditional, naturally standing forest resources that are in excess of that required by the forest products industry, is estimated to range from 90 to 253 million dry metric tons per year for wastes and from 182 to 909 million dry metric tons per year of excess production at a price of $20 to $36 per dry ton. Collectible amounts of agricultural residues in the U.S. are estimated to range from 47 to 364 million dry metric tons per year at a price of $24 to $49 per dry ton. The impact of short rotation forestry, in which hardwood plantations are intensively managed to provide high yields per acre over short harvest times, is more difficult to quantify because it is a unproven technology, but it is estimated that 765 million dry tons could be produced in the United States each year at a cost of $34 to $68 per dry ton. Herbaceous energy crops, terrestrial non-woody plants grown for their energy content, would probably compete with short rotation crops for land use, thus restricting the total U.S. availability of the two to 765 million dry tons per year but at a cost of $26 to $45 per dry ton. The domestic availability of municipal solid waste (MSW), the trash generated by residential, commercial, industrial, and institutional sectors, is estimated to be

about 90 million dry tons per year assuming that only large municipalities with more than 500,000 people are attractive for siting of a capital intensive bioprocessing facility; the estimated cost of MSW ranges from $24 to a credit of $12 in tipping fees after paying for processing of the raw feedstock. In sum, these materials are estimated to provide from 1174 to 2381 million dry tons per year in the United States at prices from $20 to $70 per dry ton, enough feedstock to generate from 130 to 270 billion gallons of liquid products(2). Even though these values are subject to significant uncertainty, they indicate that the resource base of renewable feedstocks is substantial.

LIQUID PRODUCTS FROM RENEWABLE SUBSTRATES

A biorefinery could ferment sugars derived from renewable feedstocks into any of a wide range of liquid products including ethanol, glycerol, acetone, n-butanol, butanediol, isopropanol, acetic acid, butyric acid, citric acid, fumaric acid, itaconic acid, lactic acid, propionic acid, and succinic acid. In addition, lipids, single cell protein, and food grade sweeteners such as high fructose syrup can be produced by bioprocesses. Some of these products, sugars, or other biomass components such as lignin can be chemically converted into such products as furfural, furans, glycols, adipic acid, methyl ethyl ketone, phenols, aromatics, dibasic acids, and olefins.

If we look at existing markets, organic acids, solvents, amino acids, antibiotics, vitamins, industrial enzymes, steroids, alkaloids, and other products of smaller sales volume are or could be produced through bioprocessing. However, of these, the U.S. dollar market for organic solvents and acids is almost an order of magnitude greater than for the world market for the next largest and is larger than the combined total world market of the rest. Closer examination of the U.S. organic acid and solvents segments shows that the potential market for ethanol as an industrial solvent and octane enhancer (~800 million gallons/year) and for production of such products as ethylene represents well over half of the total value of the oxychemicals that could be derived through bioprocessing(3). Beyond the direct conversion of renewable resources into existing product lines, it is possible to substitute new products derived from this resource for many products now based on petroleum. However, substantial speculation is involved in assessing the impact of such substitutions, and regardless, the point can be made that a very large market exists for liquid products from renewable feedstocks. In addition, it is possible, at least in principle, to substitute ethanol for gasoline as a neat liquid fuel, a market which could absorb about 140 billion gallons of ethanol annually.

PROCESSING RENEWABLE FEEDSTOCKS INTO LIQUID PRODUCTS

The details of the process steps for conversion of renewable feedstocks into liquid products can vary significantly with the choice of feedstock and the desired product, but the general process flow scheme generally has common features. Since renewable feedstocks are solid materials that have developed some degree of natural resistance to breakdown by microbial populations, the feedstock must be treated in some fashion to make it susceptible to bioprocessing. Thus, the first major processing element is the feedstock preparation step which usually includes size reduction followed by a chemical or enzymatic pretreatment designed to open up the feedstock structure. For processes based on the enzymatic breakdown of the complex structure of carbohydrates in the feedstock, an enzyme production step is needed; part of the pretreated feedstock is typically used in enzyme production to support microbial growth and induce enzyme production. The enzymes are then added to the remaining pretreated feedstock to catalyze the breakdown of carbohydrate polymers into monomeric sugars in a hydrolysis step. In the subsequent fermentation, the sugars are converted into liquid products by appropriate microorganisms. Following the completion of the biological conversion operations, recovery and purification is required to remove the desired product from the fermentation broth and concentrate it to meet market specifications.

BIOPROCESSING ISSUES

For large scale markets in fuels and commodity chemicals, a specified product must be produced at low price. Today, biotechnology is well suited to production of complex molecules or other unique products with high specificity for specialty applications in which price is a secondary issue. Production costs for bioprocesses are typically high, and yields, product concentrations, and conversion rates can be relatively low. Furthermore, the processes are generally performed in batch operations, which are expensive to operate and require high capital costs compared to their throughput.

Although variations in process design are to be expected with changes in type of feedstock and particularly with changes in target products, significant differences are also found between process designs prepared by different engineering firms for the same application. Much of this variability is caused by the fact that little experience is available on which to base the designs and predictive tools to allow a priori design of bioprocesses are lacking(4). Considerable judgement is involved in selecting a process configuration that is judged ready for application while also being economically viable. Clearly substantial advances are needed in the underlying process technology.

In this section, some suggestions will be offered of advances needed in bioprocessing for economic conversion of renewable feedstocks into large volume liquid products. Although many

of the technology needs discussed have received some attention in the past, progress has not been sufficient for the concepts to be accepted for commercial applications. Thus, further work is required on these technologies or new approaches must be devised.

Feedstock Preparation

The first major process step is feedstock preparation. The key is that the pretreatment must facilitate high yields of product in the downstream biological conversion steps while minimizing the operating and capital costs of the step itself(5); obviously, these goals may be somewhat mutually exclusive. Information is needed on the relationship between pretreatment conditions and substrate structure and their impact on substrate susceptibility to downstream bioconversion. Beyond producing a feedstock that can be converted to product in high yields, it is also desirable that the feedstock preparation step separate major components of the feedstock to generate an economic product slate. Such separations are important in the wet milling of corn since a range of co-products such as oil, protein, and fiber can be recovered for sale in addition to ethanol. If the cellulose, hemicellulose, and lignin fractions of lignocellulosic biomass could be separated in the feedstock preparation step, the process can be configured to take advantage of the unique attributes of each component while avoiding complications arising from carrying along the others. For example, if lignin accompanies the cellulose feed to the enzymatic hydrolysis and fermentation steps, substantial purge streams will probably be withdrawn with attendant loss of cellulose to maintain the lignin concentrations within limits that can be processed effectively. Alternatively, retention of cellulose in a lignin stream intended for processing of lignin to products would likely result in cellulose degradation in that operation.

Biocatalysis and Fermentation

Virtually all commodity chemicals and fuels are produced in continuous processes. Continuous processing lends itself to cost-effective use of equipment since there is little down time and the utilization efficiency approaches 100%. Continuous processes also facilitate application of automatic process control, resulting in improved consistency with lower labor costs. Heat and water balance integration is improved by continuous operation, increasing the efficiency of the operation. Yet, with the exception of aerobic and anaerobic waste treatment facilities and some large plants for ethanol production from corn or sugars, most biological conversion processes are operated in the batch mode. For continuous processes to be economically viable, they must be capable of handling a complex mixture of enzymes, microorganisms, solid substrates, products, and solid and liquid inerts. Continuous processes must also maintain stable operation for extended periods to be economically attractive. The area would benefit from fundamental studies that would allow understanding of the interaction between fluid flow and kinetics in such complex mixtures and how to effectively process these streams.

High yields of product are necessary to maximize revenues while minimizing waste treatment costs(5). In addition, economics are improved with high rates and high product concentrations. Although batch operations realize high rates initially, they are penalized by long downtimes for fermenter turnaround. Continuous stirred tank reactors (CSTR's) are frequently used in continuous operations, but operation at high product concentrations results in low rates while some substrate is swept from the fermenter before it reacts appreciably resulting in loss in yield. Plug flow operation can in principle achieve the high initial rates of batch fermentations without frequent fermenter downtime, but little experience is available on which to base the design of a system handling the complex mixtures of microbes, enzymes, solid substrates, solid and liquid inerts, products, and possible gases typical of conversion of many renewable substrates to liquid products.

Lignocellulosic feedstocks break down into large amounts of glucose and xylose plus significant quantities of galactose, mannose, and arabinose as well as organic acids, extractives, proteins, and lignin, a phenolic polymer. All of these components must be utilized to maximize revenues while minimizing waste treatment costs. Even if a microbial population can convert all of the carbohydrates to product, the tendency of the microbes to sequentially convert sugars due to the diauxic effect must be dealt with if continuous processes are to be viable. A second challenge is to convert the non-carbohydrate components into saleable products.

Aseptic operation is essential to maintain the desired microorganisms and enzymes in the fermenter. A variety of techniques are employed to prevent invasion by undesirable microorganisms such as minimizing the number of vessel penetrations, use of specialized equipment and fittings, sterilization of the reaction media, and establishment of other design criteria. However, this protection can be costly and may be insufficient for the long periods required in continuous processing. Alternate approaches such as immobilization of desired microbes and/or enzymes, operation at high product concentrations that are toxic to most organisms other than those desired, flocculating microbial populations, or selective separation schemes are possibilities, but little information is available with which to assess the effectiveness of these approaches.

Control of microbial populations becomes even more acute when dealing with recombinant microorganisms that have been tailored to process a particular feedstock component, produce a desired product, or achieve enhanced capabilities. Such microorganisms are often at a competitive disadvantage to both invading microbes and to those of the same species which have lost their genetic modification. Although selective pressures such as incorporation of antibiotic resistance in the plasmid are frequently employed to maintain the genetically modified microbe, the quantity of antibiotics required for large volume products is usually too great to be cost effective. It is desirable to develop and understand engineering approaches such as immobilization of the recombinant microorganisms to improve the economic use of genetically modified microbes for large volume markets.

Production of enzymes and, to some degree, growth of microorganisms are expensive, and engineering strategies would be valuable to extend the life of biocatalysts. An improved understanding of the effects of environmental parameters on microbial and enzymatic activity would facilitate design of effective catalyst regeneration systems. It would also be useful to explore methods to overcome inhibition or deactivation of biocatalyst activity.

Mixing is an important problem in large scale fermentations. It has frequently been assumed that agitation must be adequate to suspend solid substrates for use of renewable feedstocks. However, recent evidence suggests that only enough fluid circulation is required to prevent stagnation of product and the resulting inhibition of kinetic rates(6). More information is needed to understand the relationship between kinetic rates and fluid and solid movement for particulate substrates. High solid loadings are frequently desirable to achieve adequate product concentrations to minimize downstream processing costs, but the viscosity of the fermentation broth becomes so high above about 10% solids concentrations that conventional agitators aren't effective. Thus, novel agitator designs are needed. In addition, an improved understanding of the shear rate distribution in agitated vessels and the impact of shear rates on performance for high viscosity, complex broths would be valuable. Devices are needed as well to impose known shear rate profiles on non-Newtonian fluids for such investigations.

For aerobic conversions, an improved understanding is needed of oxygen transfer in complex viscous mixtures. The low solubility of oxygen in water presents a significant problem to efficient bioprocessing. Since oxygen is continuously depleted as the fermentation proceeds, the medium must be constantly aerated, and the more viscous the medium, the more difficult it becomes to supply adequate oxygen to maintain high rates. Some approaches which could be studied to improve oxygen supply are operation under pressure to increase solubility, use of oxygen rich gas, and changes in fermenter design or operation.

A number of advanced designs could achieve some or all of the objectives mentioned. These include co-immobilized biocatalysts, fluidized beds of immobilized biocatalyst, inclined settlers, and packed beds of immobilized biocatalysts. However, such systems have generally not received attention for processing the complex mixtures frequently encountered in bioprocessing lignocellulosic biomass to liquid products, and an improved understanding is needed for operation with such mixtures.

Recovery and Purification

The accumulation of product in the fermenter generally inhibits conversion rates. Yet, although rates and yields will increase if the product can be maintained at low concentrations, downstream processing costs rise sharply as more water must be processed for each unit of product(5). Thus, alternate approaches such as extraction or vacuum distillation of product from the fermenter appear desirable to maintain low product concentrations in the fermenter while still providing high product

concentrations for downstream processing. Since limited success has been achieved to date with integrated product recovery/fermentation systems, more information is required to improve existing options(7). New approaches merit attention if they show promise for minimizing end-product inhibition of fermentations while overcoming shortcomings experienced to date. Better data is also needed on the impact of product concentration and recovery approaches on kinetic rates.

For continuous fermentations, recovery of unused substrate from spent material and inerts will probably be important to achieve high yields. Furthermore, it is also desirable to recover active enzyme and microorganisms from the fermentation milieu for recycle back to the process. Fundamental knowledge is needed of the important property differences that will facilitate these separations. New separation schemes such as reversed micelles might be applied to this problem. In an alternate approach, it may be possible to design the process so that active biocatalysts and/or unused substrate are retained while spent materials and inerts are swept from the fermenter; information is required on the fluid dynamics of complex mixtures to intelligently design and assess such concepts. Immobilized biocatalysts could serve the function of retaining microbes or enzymes, but immobilization isn't typically applied in conjunction with solid substrates characteristic of many renewable feedstocks.

Although distillation is frequently employed for product recovery and purification, the unit operation chosen for this step must suit the product volatility, concentration, contaminants, and purity requirements. For products with high volatility such as ethanol, modern distillation processes are reasonably cost effective, and radical improvements in alternate separation schemes are needed to supplant it as the preferred choice. However, since water is usually the primary media for fermentations, product recovery by distillation requires that water be evaporated for products such as glycerol that are non volatile or less volatile than water. In such cases, distillation is a costly option given the high heat of vaporization of water and its large amounts for the dilute product streams(3). Recovery operations such as ion exchange, extraction, ultrafiltration, pervaporation, chromatography, affinity separations, and other approaches are possible alternatives to distillation, but improvements are generally needed in these technologies before they will be cost effective for large volume products. A better fundamental understanding of these approaches can lead to improvements in the technologies or new developments that overcome their shortcomings. The use of organic solvent based fermentations presents another approach to reduce the energy requirements for product recovery since low boiling solvents typically have a lower heat of vaporization than water.

Scale-Up

In the petrochemical industry, plants are generally constructed as large as possible to take advantage of economies of scale since equipment costs typically increase with the 0.6 power of size. For conversion of renewable feedstocks to liquid products, the economies of scale must be traded

off against increased costs for feedstock transportation. Nonetheless, Archer Daniels Midland operates a 255 million gallon per year grain to ethanol plant in Decatur, Illinois, and over 75% of the fuel ethanol produced in the United States is by plants with capacities in excess of 40 million gallons per year(8).

If we look at the design for a seemingly small by comparison 25 million gallon per year plant for ethanol production from lignocellulosic biomass, the fermenters for enzyme production and cellulose hydrolysis would be on the order of 2.5 million gallons capacity each while the ethanol fermenter would hold approximately another 1.3 million gallons based on today's technology. Obviously, this is a significant scale-up from laboratory scale fermenters of a few liters or even from pilot units of 1500 liters. Yet, to assess the potential of conversion of renewable feedstocks into liquid products requires that engineers estimate the vessel capacities, associated process equipment sizes, and utility requirements at such scales, and the designs should be reliable to allow accurate assessment of the technology's potential. If the decision is made to build a plant, the accuracy must be even better or substantial losses of capital, delays in construction, and other severe financial problems can result.

Reliable tools are needed to allow engineers to predict the performance of large scale bioprocesses for conversion of renewable resources into liquid products. Empirically derived models are often not satisfactory, particularly for scaling over large changes in capacity, since production scale processes behave differently than those at the pilot scale due to changes in factors that control performance. Thus, a solid understanding must be developed based on fundamental principles controlling process performance. Such accurate models are needed for the entire bioprocessing sequence from feedstock preparation through fermentation and on to recovery and purification.

Bioprocess Monitoring and Control

Accurate monitoring and control are valuable for bioprocesses to minimize labor costs while maximizing productivity. Both batch and continuous processing can benefit from advances in these areas since steady state operation generally improves the performance of continuous processes while better approaches to monitoring and control could improve the productivity of batch processes. Sensors and instruments for on-line measurement of solid substrate and product concentrations, inerts, enzyme activity, and microbial populations are very limited. In addition, the monitoring of bioprocesses for conversion of renewable resources into liquid products is complicated by the presence of the numerous components in the fermentation broth. Bioprocessing for production of large volume liquid products would greatly benefit from automatic control. Development of artificial intelligence systems could allow consistent operation of facilities to insure reliable process performance, reproducible product quality, and reduced operating costs.

CONCLUSIONS

Renewable materials provide inexpensive feedstocks that are available in large quantity compared to the current markets for liquid organic chemicals and can make a significant impact on even the huge liquid fuels market. These substrates free us from reliance on politically unstable sources of fuels and chemicals, reduce the international balance of payments deficits for the United States, and don't contribute to the accumulation of carbon dioxide in the atmosphere and the associated greenhouse effect. Bioprocessing technology can convert this resource into a diverse range of liquid products with high selectivity and therefore, efficiency. However, traditional bioprocessing approaches are costly, and improvements are required to keep pace with the advances now possible through modern biotechnology. Otherwise, the applications of the rapidly moving field of biotechnology could be restricted to high value products in such areas as health care, and we would not see the benefits of biotechnology for production of a diverse slate of high volume products. On the other hand, since considerable foreign emphasis is now being placed on advancing bioprocessing, the international competitiveness of the United States in the critical area of biotechnology could be lost without development of improved bioprocesses for its application.

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6.3.4 Bioprocessing Applications in the Management of Nuclear and Chemical Wastes

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BIOPROCESSING APPLICATIONS IN THE MANAGEMENT OF NUCLEAR AND CHEMICAL WASTES

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INTRODUCTION

The projected requirements for waste management and environmental restoration activities within the United States will probably cost tens of billions of dollars annually during the next two decades.[1-2] Expenditures of this magnitude clearly have the potential to affect the international competitiveness of many U.S. industries and the continued operation of many federal facilities. In fact, the U.S. Department of Energy (DOE), the U.S. Department of Defense (DOD), and other federal agencies already face profound challenges in finding strategies that manage budgets and priorities while bringing their sites and facilities into compliance with current statutes and regulations and with agency policies and orders. While it is often agreed that current technology can be used to address most waste management and environmental restoration needs, it is also argued by many that the costs of implementing current technology will be too high unless the standards and schedules for compliance are relaxed. Since this is socially unacceptable, efforts to improve the efficiency of existing technologies and to develop new technologies should be pursued. A sizable research, development, and demonstration effort can be easily justified if the potential for reducing costs can be shown. Bioprocessing systems for the treatment of nuclear and chemically hazardous wastes offer such promise.

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BACKGROUND

Hazardous Waste Laws

Many federal and state statutes establish the basic compliance requirements for waste management activities. The Toxic Substances Control Act (TSCA), the Resource Conservation and Recovery Act (RCRA), and the Comprehensive Environmental Response, Compensation and Liability ACT (CERCLA), otherwise known as Superfund, and their various amendments have the most far-reaching impacts. The management of mixed wastes (i.e., waste co-contaminated with hazardous chemicals and radioactive materials) is especially complicated for facilities such as those managed by the DOE because the Environmental Protection Agency (EPA) and state governments have jurisdiction over hazardous materials while the Nuclear Regulatory Commission (NRC) and the DOE are responsible for radioactive materials. Final strategies for the management of mixed wastes will depend on the results of ongoing negotiations among DOE, EPA, and state governments. However, it is reasonable to assume that the management of both radioactive and chemically hazardous wastes will eventually be integrated under a RCRA-like philosophy that requires cradle-to-grave responsibility for hazardous materials. Under this philosophy, radioactivity would be added to the criteria currently used to identify hazardous materials.

TSCA will have an impact on bioprocessing applications through regulatory authority over the development and use of intergeneric microorganisms, especially their release to the environment. RCRA and the Hazardous and Solid Waste Amendments of 1984 require EPA to prohibit the land disposal of many untreated wastes and to set standards for the treatment, storage, and disposal of hazardous wastes. CERCLA requires EPA to establish a program for environmental restoration of contaminated sites. While CERCLA does not regulate treatment,

it requires EPA to develop and promote innovative technologies for application at Superfund sites. The 1987 Superfund Amendment and Reauthorization Act (SARA) required EPA to establish various research, development, and demonstration programs. The best known of these is the Superfund Innovative Technology Evaluation (SITE) Program. The goal of the SITE Program is to have ten annual field demonstrations of innovative technologies through 1991.

A potential incompatibility seems to exist between the goals of the RCRA and CERCLA programs. RCRA regulates rather than promotes technologies and does not pursue innovation. In fact, RCRA sets standards based on Best Demonstrated Available Treatment Technology (BDAT). In many cases, standards are based on optimal incinerator performance. RCRA currently has not recognized biodegradation as BDAT. The need for Superfund demonstrations to comply with RCRA inevitably limits the application of biodegradation systems.[3]

Magnitude of Compliance Costs

The generation of hazardous waste within the United States exceeds 300 million tons annually under current RCRA criteria. More than 90% of this waste is generated by the manufacturing industry, and 70% of the manufacturing total is derived from the chemical sector.[4] The Congressional Budget Office has estimated that the 1984 amendments to RCRA could result in annual expenditures of $11 billion by private industry by 1990.[5] Environmental restoration activities on current or abandoned industrial sites contaminated with hazardous chemicals may cost $100 billion and require 50 years to complete with current technology. By 1990, Superfund (CERCLA) and other waste-related regulations could cost U.S. industry $20 billion annually.[5] Costs for bringing federal facilities into compliance have not been established, but they will undoubtedly be significant (i.e., billions of dollars) and will represent a significant drain on federal agency budgets.

The Potential of Bioprocessing Applications

Several prestigious studies have identified significant opportunities for reductions in waste management costs through the development of bioprocessing options. The area of hazardous waste management has recently been reviewed by a committee of the National Research Council as part of a study of research needs and opportunities in chemical engineering.[6] In this review and others,[7] the development of biodegradation processes for the detoxification of currently generated chemically hazardous waste was cited as a promising area for research and development based on engineering considerations. The National Science Foundation has also sponsored a recent workshop on biotechnology for the management of hazardous waste.[7] This workshop documented the need for and potential of interdisciplinary bioprocessing R&D intended to produce new technologies for waste management. Needed interfaces among the fields of microbial biochemistry, genetics, ecology, environmental microbiology, and chemical and environmental bioprocess engineering were reviewed, and a comprehensive R&D agenda encompassing bioreactor systems development was proposed.

Our understanding of biodegradation processes at the molecular level has been rapidly expanding over the past decade. For example, the use of genetically engineered microorganisms as well as the use of indigenous organisms isolated from various environments can offer dramatic increases in the specificity and efficiency of mineralization of many organic compounds. Coupled with the possibility of pursuing low-temperature, low-pressure processes, these advances suggest the potential for cost-effective technologies. The potential for exploitation of such microorganisms within engineered bioreactor systems has been widely recognized,[7-8] but the development of bioprocesses for waste management is still limited by several factors. These include the limited characterization of microorganisms for

specific applications and a paucity of data and experience supporting process design, scale-up, and operation. Since waste management practitioners have high legal and financial liabilities, the acceptability and eventual deployment of newly developed technologies may depend on successful field demonstrations that define costs and eliminate performance uncertainties.

The problems of hazardous waste management that may be addressed by biotechnology are generally of two types - either the treatment of currently generated wastes or the remediation of toxic waste sites. In situ bioremediation technologies are not considered bioprocessing applications for the purposes of this discussion. The use of bioprocessing applications in the treatment of currently generated wastes may be accomplished through destruction or detoxification processes (e.g., by biodegradation) or through segregation of hazardous materials from the bulk of the waste (i.e., by biosorption processes). In the special case of mixed waste, which is prevalent on DOE sites, detoxification may refer to the removal of chemically hazardous characteristics from a given waste to yield a low-level radioactive product suitable for subsequent disposal under agency orders.

Bioprocessing applications can also be a part of site remediation programs if contaminated waters are pumped to the surface for treatment (e.g., by either biodegradation or biosorption processes), or if soils are excavated and treated (e.g., in slurry bioreactors where biodegradation processes are involved).

Biodegradation and biosorption processes are readily functional in dilute aqueous solutions and, therefore, are of great interest since a large fraction of the hazardous waste generated in industry and from site remediation programs is in that form. The development of bioreactors exploiting biodegradation and biosorption processes must obviously accommodate some or all of the following fundamental phenomena:

* removal of wastes from the aqu[illegible]tem by biomass;
* action of extracellular enzymes [illegible]astes outside of microorganisms;
* transfer of degraded or transformed chemical species through the cell wall;
* action of intracellular enzymes on wastes (products) inside of microorganisms;
* conversion of products into cellular components, use of products for energy, or storage of products for potential future use; and
* elimination of metabolic wastes or unnecessary products from cells.

If these phenomena are indeed to be accommodated during the treatment of dilute wastes, highly efficient pollutant/biomass contacting systems must be devised. Methods for developing and maintaining high concentrations of active microorganisms and for monitoring their viability and activity must be demonstrated.

The ability to maintain a small ecosystem within the bioreactor may be required where mixed substrates, biotransformations involving intermediates, and changing influent conditions are present. Mass transfer rates and biotransformation kinetics, as well as the variables that control them, must be carefully studied for particular applications if efficient design and operation of bioreactors for waste management are to be achieved. Understanding and controlling complex bioprocessing applications will require advances in methodologies for monitoring biotransformations.

Other obvious considerations for bioreactor development include the need for separation of biomass from treated waste streams and the management of excess biomass or any gaseous wastes released from the system as potential wastes. This will be especially relevant in cases where removal without destruction has occurred, as in the cases of inorganic, nonbiodegradable, and radioactive pollutants.

If bioprocessing applications are developed for the detoxification of soils, the biomass/pollutant contacting problem is considerably different from that in the general case discussed above. Contact between microorganisms and a pollutant initially sequestered within a porous, surface-active medium (i.e., soil) is required. Expertise in hydrology, geochemistry, and interfacial/surface sciences should be part of all integrated approaches to this problem.

It is worth noting that the development of bioprocessing applications may address certain TSCA and RCRA concerns. With respect to TSCA, environmental releases of engineered microorganisms can be avoided if treatments are conducted in closed systems rather than in the environment. With respect to RCRA, the placement of hazardous wastes in or on the ground can be avoided.[11] Bioprocessing in closed systems may provide efficient and acceptable near-term treatment while generating the data and experience needed to use selected biodegradation processes in eventual in situ applications.

RESEARCH, DEVELOPMENT AND DEMONSTRATION OPPORTUNITIES

There are several general characteristics that should apply to RD&D programs in this area. First, a view toward timely transfer of new biotechnology to the private sector should be maintained. Under the schedule pressures driven by hazardous waste laws, solutions to waste management problems are being selected as early as possible. If savings from innovative approaches are to be realized, the development and deployment of new technologies must begin as soon as possible. Collaborative research among universities, national laboratories, and the private sector should be encouraged. Second, technologies should be demonstrated on a scale adequate to answer questions about design and scale-up issues and about economic viability. Economic questions should be answered within a total systems context that addresses cradle-to-grave responsibilities. Comparisons to conventional physical and chemical systems, with an emphasis on incineration, should be

developed to facilitate the technology selection and approval processes. Finally, the development of mobile pilot units for field work should be sought. Field studies on real wastes often identify different problems and produce different results than do laboratory studies. Lacking relevant data and operational histories, both the waste management industry and government agencies have been reluctant to recommend innovative treatment technologies. Concern for liabilities for damages resulting from failures of technologies is widely expressed.[8]

The opportunities for RD&D activities dealing with bioprocessing applications for waste management are innumerable. However, any work in the following areas which has the above characteristics and is successfully completed would have important national significance.

Groundwater Treatment

Most aqueous waste treatment operations (approximately 70%) on Superfund sites deal with contaminated groundwaters and leachates. Organic chemicals are the major problem. Solvents are the predominant contaminant, with trichloroethylene being of major concern.[8-9] The presence of heavy metals has also been widely noted. The development of bioprocessing approaches should include consideration of both biodegradation and biosorption operations and their integration into total treatment systems.

Contaminated Soil Treatment

Most solid waste treatment operations on Superfund sites deal with contaminated soils (approximately 54%) and sludges from pits and lagoons (approximately 18%). Contaminants include organics, solvents, heavy metals, other inorganics, and pesticides.[10] The development of slurry bioreactors should be pursued with an emphasis on treatment of contaminant/soil/water systems that are not amenable to in situ bioremediation. Both PCBs and PAHs are organics of major concern.

Treatment of Aqueous Wastes Containing High Concentrations of Heavy Metals and Nitrate Compounds

Processes in the nuclear fuel cycle produce liquid low-level radioactive waste (LLLW) streams containing high concentrations of nitrates.[11] Management of these streams is complicated by the presence of nitrates which may be leached from the solidified LLLW that is being prepared for disposal. Biodenitrification of LLLW streams that are characteristic effluents of the nuclear fuel cycle of national defense activities should be pursued.

Volume Reduction of Solid Low-Level Radioactive Wastes

Various DOE facilities, and probably numerous other concerns, produce large volumes of solid low-level radioactive wastes.[11] The disposal of this material is problematic and expensive, and any means of efficiently reducing its volume would be important. Even if waste segregation methods are employed to remove wastes suitable for disposal in sanitary landfills, a significant fraction of the residual waste will be cellulosic material amenable to anaerobic digestion. The feasibility of using this process within integrated waste treatment systems should be analyzed, and needed RD&D activities should be pursued. Obviously, the gas, liquid, and solid discharges would require special attention to determine the ultimate fate of radioactive materials.

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6.3.5 Bioreactor Fundamentals

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BIOREACTOR FUNDAMENTALS

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INTRODUCTION

Over the last ten years a number of both novel bioreactors and biologically-catalyzed reactions have appeared, some of which have the potential to open new avenues in the use of enzyme and microbial processing for energy and chemicals production. Thus we shall consider the bioreactor to encompass both the reactor itself and the enzymatic or cellular process occurring in it. Bioreactor fundamentals can be examined from the perspective of the types of products formed or reactions catalyzed, noting that in the case of environmental control, the "reaction" considered may be adsorption. This product focus is important, as the economic and technical feasibility of many processes depends on a variety of factors, only one of which is the suitability of a particular bioreactor. Prior to the advent of genetic engineering, novel bioreactors were often developed to overcome limitations imposed by the organism (eg. extractive fermentation to reduce product inhibition), whereas today biological approaches may be available. Appropriate alteration of the microorganism or enzyme may result in conventional bioreactors being entirely suitable for the product under consideration.

There is a considerable body of literature concerning conventional bioreactors, including stirred tank and pneumatically agitated reactors. We shall focus attention on *novel* bioreactors, as summarized in Table 1 (adapted from [1]).

There continue to be a number of fundamental problems that need to be addressed in the area of multiphase bioreactors and these will be examined in terms of the application of conventional bioreactors for some of the products of interest in the following sections.

BIOREACTORS IN THE PRODUCTION OF GASEOUS FUELS

Methane formation by anaerobic digestion of organic wastes is a well established practice in wastewater treatment. It is typically used to reduce the volume of activated sludge solids and to handle waste particulates arising from primary treatment processes. A mixed population of organisms act on solid materials to produce organics (eg. cellulases, lipases other hydrolases). Short chain fatty and volatile acids are then produced by *acid formers*. About 70% of the methane produced arises from acetic acid produced in this step. Obligate anaerobes (the methanogens) produce methane from these volatile acids; this is thought to be the rate limiting step in anaerobic digestion. These organisms have an optimum pH of around 7.0 to 7.8, and thus good liquid phase

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Table 1. Examples of Novel Bioreactor Systems.

Bioreactor Design	Cell System Used	Typical Products
Air-lift	Bacteria, yeast & fungi	S[illegible]rimary & secondary metabolites, biosurfactants
Fluidized bed	Immobilized bacteria, yeast, fungi & activated sludge	Ethanol, secondary metabolites, wastewater treatment, bioadsorption
Membrane bioreactors (hollow fiber, flat sheet)	Bacteria, yeast, plant cells	Primary metabolites eg. ethanol, lactic acid, enzymes and proteins
Modified stirred tank	Immobilized bacteria, yeast & fungi	Primary metabolites
Modified packed bed	Immobilized bacteria, yeast & fungi	Ethanol, acetic acid, other organic acids
Tower and loop bioreactors	Bacteria & yeast	SCP, ethanol
Vacuum bioreactors	Bacteria & yeast	Volatile primary metabolites
Photochemical bioreactors	Photosynthetic bacteria & algae	Glycerol, β-carotene

mixing is required to reduce local concentrations of acids. Limitations in the design and operation of anaerobic digesters are primarily a result of the complexities of the biological processes occurring in the digester. Methanogens are not well understood and the dynamics of their interactions with other bacteria in the mixed culture needs to be defined. The concurrent production of H_2S by sulfate-reducing bacteria presents a problem in utilization of the resulting digester off-gas.

The situation is simplified if we consider methane production from a well-defined substrate (eg. agricultural residue) using defined cultures. Opportunities exist for improvement of the anaerobic bacteria involved, although cloning in such organisms presents difficulties. Some engineering efforts along these lines with obligate anaerobes are underway [2].

BIOREACTORS IN THE PRODUCTION OF LIQUID FUELS

Ethanol production from renewable feedstocks has received considerable attention over the past fifteen years. One of the limitations of the alcohol fermentation has been product inhibition by ethanol. Various reactor configurations which alleviate inhibition by *in-situ* removal of ethanol have been examined. Coupled with bioreactors which provide high cell concentrations by use of cell retention or recycle, high volumetric productivities may be obtained. Vacuum and flash fermenters show high productivities (> 30 gm/l.hr), but at the expense of increased capital costs. Other approaches to product removal, eg. pervaporation, have not been sufficiently examined from economic and operational standpoints to assess their applicability. Although various ethanol recovery schemes (eg. liquid-liquid extraction, adsorption) have been proposed, distillation appears to be the method of choice. In general, the economics of ethanol production is dominated by feedstock costs and

improvements in bioreactor design and ethanol recovery show only marginal improvements [3]. As distillation costs are relatively insensitive to inlet ethanol concentration in the fermentation range (1 to 8% w/v), improvements in the ethanol tolerance of the producing microorganism beyond 10% are not likely to provide significant economic advantage.

The production of methanol from natural gas or other methane sources may provide an alternative to high temperature, high pressure catalytic processes. The high energy associated with disruption of the C-H bond (104 Kcal/mole) has made design of heterogeneous metal catalysts functioning at ambient conditions difficult, although progress has been reported [4]. Catalytic conversion of methanol to higher alkanes provides a means of providing a transportation fuel.

The methanotrophs are able to use methane as a sole carbon source, the first step being oxidation of methane to methanol by methane monooxygenase. The three component enzyme has been isolated from *Methanococcus capsulatus* and characterized [5]. The use of an immobilized methane monooxygenase presents several bioreactor challenges. The enzyme is comprised of 3 components; component C is an NADH acceptor reductase, which transfers electrons to component A, the oxygenase which interacts with the hydrocarbon substrate. Component B is involved in the coupling of substrate oxidation to electron transfer. Thus all three components must be present and active for reaction. Methane is sparingly soluble in aqueous solutions, and thus a conventional immobilized enzyme agitated or packed bed bioreactor employing an aqueous phase would require exceedingly large volumes. Solubility can be improved by use of non-aqueous solvents. Several bioreactor configurations can be envisioned to enable the enzyme components to retain full activity in a continuous non-aqueous phase. These are described below.

Reverse Micelle, Microcapsule and Liquid Membrane Containing Bioreactors

There are several approaches for use of enzymes with non-aqueous solvents. The enzyme may be sequestered in a reverse micelle, formed by the addition of a surfactant to a small volume of water and a bulk organic phase. The enzyme thus functions in an aqueous mini-phase, with substrate present at high concentration in the organic phase. This has advantages in that the structure of the water surrounding the enzyme may exhibit different properties. Rate enhancements as a function of the micelle size have been observed. Separation of the micelle from the continuous organic phase may be difficult, due to the small size of the micelles (of order hundreds of Angstroms).

An alternative approach is to encapsulate the enzyme within a microcapsule, formed by interfacial polymerization or similar techniques. Reactants can be transported through the capsule membrane, while enzyme(s) and hydrophilic cofactors are be retained within the capsule. This approach offers promise for cofactor recycle and overcomes the limitations of substrate and product inhibition. The relative fragility of microcapsules need to be addressed; approaches using varying polymeric encapsulants are being examined.

Liquid membranes also provide a means of maintaining an enzyme in an aqueous phase while providing substrate and product transport to that phase from a surrounding organic layer. Transport of charged species may be facilitated by use of liquid ion-exchange materials (eg. quaternary alkyl amines).

BIOREACTORS IN THE PRODUCTION OF CHEMICALS

Aerobic Fermentation Systems

The number of chemicals produced by biological catalysis is rather limited. A number are produced by aerobic fermentation (eg. organic acids, amino acids and various polysaccharides), where the transport of oxygen from the gas to the liquid phase often limits reactor productivity. Although considerable attention has been focussed on reactor configurations which provide improved oxygen transfer capabilities [6], the major difficulty in design and analysis of large scale bioreactors is the lack of understanding of the basic physicochemical processes which govern the operation of the two-phase process. A large number of empirical correlations for the liquid film mass transfer coefficient (k_La) exist.

Bioreactors may be grouped according to the method of energy input for dispersion of the gas phase and for agitation of the liquid phase. Mechanical agitation in the form of impellers is perhaps the most common; stirred tanks, with and without baffles to direct internal flows, provide high shear and good gas dispersion characteristics. External pumps can also be employed to provide energy input, usually by recirculation of broth. The third class of aerobic bioreactors are those where energy is provided by gas expansion, as is the case with bubble columns, air-lifts and tower fermenters. In all cases, the generation of a large interfacial area is critical. Bubble breakup and coalescence govern this process, but to date little is understood of the factors which govern these individual processes in fermentation broths. Studies are required to delineate the effects of liquid viscosity and interfacial tension on bubble breakup and coalescence in laminar and turbulent flows. The results of such studies can then be coupled with a knowledge of flow profiles in a bioreactor of a particular geometry to determine the rate of oxygen transfer. In the case of immobilized cells or enzymes, the three phase nature of the reactor introduces further complexities and mass transfer must be considered in such systems.

Anaerobic Fermentation Systems

A number of chemicals can or have been produced by anaerobes. These include acetic acid, lactic acid, acetone, acetoin, butanol, butanediol and isopropanol. These economic feasibility of these processes is governed by (a) conversion of sugar substrates, (b) product yield and selectivity, (c) reactor productivity and (d) product separation and purification [7]. In general, the first two factors are the most important.

Various bioreactor configurations have been examined to overcome product inhibition, generally observed in these fermentations. The most common approach is *extractive fermentation*, where a second liquid phase is contacted with the broth, either *in situ* or external to the bioreactor. Transfer of the product to the second organic phase relieves inhibition and permits a greater amount of substrate to be converted. This can also improve selectivity, as is the case in the acetone-butanol fermentation. In the case of lactic or acetic acid production, a complexing extractant can be employed, containing a phase transfer catalyst (eg. phosphene oxides, tertiary or quaternary alkyl amines). The solvents employed are generally non-toxic but have relatively poor distribution coefficients for the products of interest. Solvents with better K_D values tend to be toxic at aqueous saturation levels. Thus a large inventory of non-toxic solvent or a high recycle rate of solvent must be employed. Other approaches include the use of membrane bioreactors, where cell retention permits dilute feeds to be fermented

at high rates and conversions. However, these processes are governed by the cost of the feedstock and only relatively modest economic improvements can be expected with improved fermentation productivity or product recovery schemes.

Aerobic Bioconversions

Regio- and stereospecific biological oxygen insertion reactions offer significant opportunities for production of chiral alcohols and epoxides, in addition to methanol formation described earlier. Monooxygenases have relatively low specificity as a result of the conformation of the binding site, but some interesting specific transformations have been reported [8]. Alkane monooxygenases are generally cytochrome P_{450} or non-heme iron type and produce non-discriminatory active oxygen. Some of the substrate of interest are water soluble, but others, eg. alkyl cyclohexanes, are only sparingly soluble. Thus bioreactors containing enzyme in micelles or microcapsules with substrate present in a water-immiscible organic phase (as described earlier) may have advantages. In the case of reactions conducted by whole cells, eg. the epoxidation of propene by immobilized *Mycobacterium* [9], the oxidation of isoprenoid hydrocarbons (pristane and related hydrocarbons contained in shale oil) by *Rhodococcus*[10] , various non-aqueous solvents can be employed in a packed bed bioreactor using immobilized cells. In the first example, oxygen and propene solubilities are higher in organic media, and thus high volumetric productivities can be achieved. These aspects of bioreactor design need to be further explored.

Anaerobic Bioconversions

Immobilized cells and enzymes can conduct a variety of important single and multiple reactions involved in the production of chemicals. Packed-bed and fluidized bed bioreactors are commonly employed for these purposes. In general, the identification of the microbial route or enzyme yielding the desired product is the limiting step in the development of such processes. Once this has been accomplished, several factors relative to bioreactor design are involved. These relate to the often inhibitory nature of the reactants (eg. degradation of refractory materials), the limited solubility of reactants and products, and the need to regenerate cofactors. Bioreactor designs which permit facile cofactor recycle are needed. Several approaches have been examined at the laboratory scale, including cofactor retention by attachment of the cofactor to a water miscible polymer and retention of the cofactor by a low molecular weight cut-off membrane. However, when organic solvents are to be employed as the continuous phase, other approaches must be examined. Microencapsulation may be a promising alternative.

BIOREACTORS IN ENVIRONMENTAL CONTROL

The removal of hazardous waste materials arising from energy related processes represents a significant biotechnological challenge. Many of these materials are present in small concentrations and are often toxic to microorganisms. There are several examples of successful employment of microorganisms to remove radionuclides from waste streams, remove phenolics, chlorinated biphenyls etc. In general, packed or fluidized bed reactors have been employed, with the microorganism attached to support particles. The uptake mechanism may be active or passive. Typically, the main problem involves selection of a microorganism with the desired properties. Conventional immobilization strategies appear to suffice.

In the case of recovery of toxic metal ions from waste streams, conventional ion exchange resins fail to exhibit selectivity when used as secondary steps, following chemical treatment. The design of highly selective adsorbents is thus desirable and much can be learned from biological peptides and carbohydrates [11] that can be translated into the design of biomimetic adsorbents.

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6.3.6 Bioreactor Fundamentals

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6.3.6 BIOREACTOR FUNDAMENTALS

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(A summary of the paper prepared by C. D. Scott)

INTRODUCTION

The industrial application of bioreactor systems is slowly evolving from the classical batch stirred tanks to continuous operation utilizing several different approaches. Many of these advanced concepts are just beginning to be introduced. For example, we are now seeing improved aeration and agitation techniques and process monitoring and control systems are becoming meaningful. New and improved bioreactor configurations are also being considered. These include continuous columnar systems such as airlift and tower bioreactors as well as membrane systems. It has been demonstrated that for some of these new concepts, the use of immobilized biocatalysts allow high productivities, at high flowrates without catalyst washout.

Some bioreactor concepts must accommodate much different biocatalysts than the microorganisms or enzymes that have been usually considered. For example, mammalian and hybridoma cells are now extensively used and plant cells and even insect cells are being investigated. These different types of cells are, in general, more fragile and require a reactor design that has reduced shear force while maintaining good process control and mass transport.

Research needs in this area certainly include advanced bioreactor designs that operate on a continuous basis with improved process control. The latter will require stable and sensitive on-line monitoring of various process parameters with advanced conputer interfacing. Scale-up criteria must be predictable and the system should be amenable to aseptic operation with good operational characteristics such as low pressure drop and imporved CO_2 removal.

6.3.7 Separations for Recovery of Biologically Produced Chemicals

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INTRODUCTION

Since biomass is highly oxygenaated, chemicals and chemical intermediates made from biomass by fermentation, pyrolysis or other means are also oxygenated. Thus the most readily achievable products are carboxylic acids, alcohols, glycols and related substances[1]. Recovery of these chemicals poses a challenging separation problem, for several reasons:

1. These oxygenated products tend to be highly hydrophilic, have low activity coefficients in aqueous solution, and are therefore difficult to remove from water.
2. The products appear in dilute aqueous solution, posing the problems of recovering from a low-activity medium and handling and processing a large amount of water per unit quantity of product.
3. The solution containing the product is usually complex, containing substantial quantities of similar products and substrates, as well as cellullar matter.
4. Except for the simplest carboxylic acids and alcohols, the products are less volatile than water.
5. Residual solvents or other separating agents may have toxic effects upon organisms, if the separation is carried out in the presence of organisms or if the aqueous medium is to be recycled to the reactor.
6. Most organisms of interest are sensitive to elevated temperatures.

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7. Fermentations to form carboxylic acids typically perform best at a pH somewhat above the pK_a of the acid, with the result that the product is largely ionized.

As has often been noted[2], for lower-value chemicals such as ethanol the cost of recovery can be a large fraction of the market value of the product.

CANDIDATE SEPARATION PROCESSES

Solvent Removal vs. Solute Removal

The most common approach for concentrating solutes present in dilute solutions is removal of solvent (the bulk component, i.e., water for a dilute aqueous solution) by processes such as evaporation, freeze concentration, reverse osmosis or ultrafiltration. These methods do not fractionate among the various other substances in solution. Also, evaporation and freezing are energy-intensive. Energetically, it is preferable to remove the desired solute(s), e.g., the product, from solution[2].

Avoiding Consumption of Chemicals and Production of Waste Streams

The classical approach for recovering citric acid and similar carboxylic acids from fermentation solutions involves adding calcium hydroxide to precipitate the calcium carboxylate, and then reverting to the free carboxylic acid through addition of sulfuric acid[3]. This technique requires consumption of lime and sulfuric acid and creation of a waste calcium sulfate solution. Solute-removal methods which avoid consumption of chemicals and production of such a waste stream are desirable.

Vaporization Processes

Distillation or stripping is attractive as a means of recovering the desired product when it is more volatile than water. Vaporization processes are the approach of choice for recovering products from the butanol-acetone-ethanol fermentation[4]. Relative volatilities can be enhanced by use of azeotropic and extractive distillation, as has been done for years for recovering ethanol made by fermentation[5] and for recovering acetic acid[6]. Unfortunately, only the simpler aliphatic, monofunctional carboxylic acids and alcohols have sufficient volatility be recovered by preferential vaporization of the product. For substances of lower

volatility it would be necessary to distill the water overhead, which is energy-intensive and does not provide the wherewithal of fractionating among the remaining solutes.

Extraction, Sorption, and Membrane Processes

For low-volatility products, recovery by solute removal implies extraction, adsorption, preferential transport through membranes, or various elaborations upon these processes. Problems with these processes include limited capacities and selectivities, as well as the need for regeneration. One approach toward overcoming these limitations is the use of reversible chemical chemical complexation, described below.

Solvents used for extraction can also be contaminating, if dissolved solvent comes into contact with organisms. Toxicity effects from dissolved solvents in the aqueous phase can be alleviated if extraction is carried out in the absence of the organism and if the residual solvent is somehow removed before the aqueous medium is recycled to the bioreactor. Processes for removing residual dissolved solvent before recycle include atmospheric or vacuum steam stripping, inert-gas stripping, and extraction with a non-polar solvent[7].

Crystallization

Direct crystallization is another possibility for recovery of sufficiently insoluble substances, e.g., fumaric acid.

Solute Ionization

For adsorbents and extractants which take up a carboxylic acid solute in the un-ionized form, the capacity is reduced to the extent that the solute is ionized in solution, i.e., if the pH is close to or above the pK_a for the solute. This increases the incentive for high-capacity separating agents. Similarly, driving forces for vaporization or crystallization of the free acid are reduced by solute ionization.

Charge-mosaic ion-exchange processes can remove salts from aqueous solution preferentially, but consumption of chemicals and discharge of a salt-bearing waste stream are required if the salt is to be reconverted to the free acid.

Recently, "water-splitting" bilayer membranes have been developed, having the potential for creating the corresponding acid and base by electrodialysis of a salt in aqueous solution[8].

ADSORPTION PROCESSES

Adsorbents have the virtue of being relatively non-contaminating and free of toxic effects. Although adsorbents are often thought of as being best limited to the removal of trace materials, regenerated adsorption processes can also be economically attractive for bulk-recovery processes.

Activated carbon provides high capacities for recovery of moderately polar organics. Thus, for example, a typical activated carbon will take up about 25 % w/w acetic acid (dry basis) from a 3% w/w solution of acetic acid in water[9]. Bulk solution is taken up to fill the pore volume, resulting in a total sorbate containing about 25% w/w acetic acid in water[10].

For most organic solutes, activated carbon provides a relatively inert surface, so that solute uptakes are determined primarily by activity coefficients in the aqueous solution, i.e., "hydrophobicity"[11]. Solutes effectively adsorbed are therefore those which serve to suppress the surface tensions of aqueous solutions. By this criterion carbon adsorption is effective for solutes such as acetic and lactic acids, but not for glycerol or sucrose. Selectivity among solutes is limited, also reflecting relative activity coefficients in aqueous solution. Phenols are exceptions, being taken up at higher capacities by virtue of a chemical interaction with the carbon surface.

Polymeric sorbents are another possibility and those with low degrees of chemical functionality are usually more readily regenerable than carbons. However, the non-functional polymeric adsorbents (e.g., macroreticular styrene-divinylbenzene copolymers) have substantially lower capacities and greater water uptakes than activated carbons[9,10]. Although such adsorbents have usually been pre-wetted before use, interesting and potentially useful properties can be gained if they are used in the non-wet state[12]. Much less water is taken up since the pores remain air-filled, and it is possible to fractionate among solutes on the basis of volatility since diffusion within the pores is rate-limiting. Solutes of surprising low volatility (e.g., butanediols) will adsorb at substantial rates, since the transport path is short.

REVERSIBLE CHEMICAL COMPLEXATION

Ordinary solvents provide low equilibrium distribution ratios (capacities) for the more polar organic solutes. Similarly, ordinary polymeric membranes with sufficiently high capacity provide poor selectivities between low-molecular-weight polar organics and water in reverse osmosis processes. Extraction, membrane and sorption processes can be enhanced through the use of reversible chemical complexation with appropriate agents[13]. In the case of extraction, reactive liquid extractants are used. For membrane processes a reactive agent can be impregnated into the membrane ("facilitated transport", or solid-supported liquid membranes). Complexing sorbents can be made by incorporating functional groups onto the surface or into the bulk structure. Complexation can also be implemented through adductive crystallization, azeotropic or extractive distillation, or foam or bubble fractionation.

Chemical complexation can provide higher capacities for dilute solutes, as well as greater selectivity among solutes, due to differing reactivities. The type of interaction (typically hydrogen bond, or acid-base) is selected so as to be strong enough to give the desired effect, but not so strong as to preclude economical regeneration. It is important to identify complexation interactions which are reversible and involve no side products.

Tertiary amines, phosphine oxides and phosphates serve as effective complexing agents for carboxylic acids[13,14]. Amine extractants give substantially higher equilibrium distribution ratios than phosphates and are less costly than phosphine oxides. The optimum molecular weight for a tertiary amine extractant is determined by offsetting factors of high capacity and low water solubility of the amine and the complex. Tertiary amines with alkyl side groups in the range trioctyl to tridodecyl appear to be suitable for most carboxylic acids. Amine and pyridyl sorbents also give substantial capacities for uptake of carboxylic acids[15].

For alcohols and glycols, it has been found that phenols give substantially higher equilibrium distribution ratios than other solvents, and it has been shown that this extra capacity results from a stoichiometric complex, at least at high dilution[16]. Simple phenols have significant aqueous solubilities and are toxic to many organisms. For

situations where a solvent is to be in direct contact with an organism, e.g., extractive fermentation, aliphatic alcohols and carboxylic acids of low enough solubility to avoid toxicity appear to give the highest equilibrium distribution ratios for ethanol, although these are still low -- e.g., 0.25 for 1-tridecanol[17].

For extraction of carboxylic acids with amine extractants, it has been shown that the chemical nature of the diluent accompanying the extractant in the organic phase has a major effect upon the extraction equilibrium. For extraction of carboxylic acids with tertiary amines, diluent effects have been interpreted quantitatively in terms of complex stabilities and tendencies for overloading -- i.e., complexes with multiple acids per amine[18,19]. There is also a similar, but weaker, diluent effect on equilibria for extraction of ethanol with m-cresol as extractant[16].

Use of chemically active diluents implies some residual solubility of the diluent in the aqueous phase, with possible resulting toxicity. There may also be residual solubility of the extractant or complex, although this is more readily avoided. This problem can be overcome by precluding direct contact of the extractant with the organism and recovering dissolved extractant in one of the ways noted above, by using a sorbent or membrane containing phenolic functionalities, or even by encasing the organism within a protective coating[20].

REGENERATION

With adsorbents, extractants and related agents, it is necessary to have a means of regeneration, so as to recover the desired product and produce fresh separating agent for recycle. Regeneration sets the energy consumption and is often the most challenging aspect of a process.

For sufficiently volatile products (e.g., acetic acid), regeneration can be accomplished by distillation of an extract[21] or heating and vaporization from a sorbent having sufficient thermal stability[15,22].. It is possible to accomplish additional separation between the product and co-adsorbed water through azeotropic distillation directly upon an adsorbent bed[23].

Solvent leaching provides another avenue for regeneration of activated carbons and non-functionalized adsorbents. The adsorbed solute can be removed into relatively few bed volumes of an appropriate solvent (e.g., methanol or acetone)[24]. Leaching with such solvents is much less effective for amine-based polymeric sorbents, which are otherwise effective for

recovery of carboixylic acids[15]. Pyridyl sorbents are much more regenerable and have satisfactory capacities, but swell excessively[15].

Relatively non-leachable or non-volatile carboxylic acids can be made more leachable or volatile through esterification, which can be carried out by passing an alcohol vapor through a bed of an appropriately treated carbon[23].

One general approach for regeneration of extractants or sorbents containing low-volatility solutes is to alter an environmental variable so as to change the distribution ratio or partition coefficient substantially. Regeneration through thermal swing has become commercial for extraction of citric acid with tertiary amine extractants[25]. Another possibility is to swing the distribution ratio by changing the composition of the diluent, e.g., through distillation[18,19,26]. This approach can be used in combination with temperature swing. pH can also be a swing variable; however, a pH swing implies consumption of chemicals and creation of a waste salt stream.

We are currently exploring two additional approaches for regeneration of amine extractants laden with low-volatility carboxylic acids:

1. For acids with low solubilities, it is possible to evaporate a volatile diluent from an amine extract, thereby causing the acid to precipitate. Preliminary experiments with fumaric acid show that it is possible to recover overloaded acid in this way.

2. Consumption of chemicals with a pH swing can be avoided if the pH is changed through addition of a volatile base, such that the resulting carboxylate salt can be decomposed thermally, yielding the product and the base for recycle. Carboxylic acids can be back-extracted from tertiary amines into aqueous ammonia effectively, but the resulting ammonium carboxylate forms the amide upon heating.

Following a related concept put forward by Urbas[27], a carboxylic acid can be back-extracted into an aqueous solution of a volatile trialkylamine (e.g., trimethyl), yielding the trialkylammonium carboxylate, which cannot form the amide. The aqueous solution of the trialkylammonium carboxylate is then heated and concentrated. The carboxylate decomposes, giving the product acid and the trialkylamine for recycle.

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6.3.8 Bioseparations: Some Research Directions

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BIOSEPARATIONS: SOME RESEARCH DIRECTIONS

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INTRODUCTION

The goal of this paper is to outline areas that appear fruitful for future research into bioseparations, with an emphasis on generic processes that can have an impact on energy-related applications. These applications include the production of oxychemicals for fuels and feedstocks, and polymers such as xanthan gum for use in tertiary oil recovery operations. In many cases, as in the bioconversion of starch to ethanol, crude preparations of enzymes are required, and efficient methods are needed for their isolation and purification from fermentation media.

The traditional separation sequence of solids removal followed by product isolation, purification and polishing is universal. The degree to which each step is undertaken, however, depends on the product to be isolated and on the final product specifications. The bulk of the volume reduction is obtained in the product isolation step using filtration, membrane separations and precipitation, although solvent extraction is also beginning to play a significant role in this area. Adsorption or chromatographic processes and, possibly, electrophoresis are found in the purification and polishing steps of many processes, but it is only adsorption that will be used in the recovery of bulk commodity chemicals.

Jud King has covered in some depth adsorption and reversible chemical complexation processes for the recovery of carboxylic acids and alcohols, with an eye to identifying the constraints

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and difficulties that dominate the economics of these separations. In this discussion, we point out that for the most part equipment used in these processes is generic, and that the question is one of optimization, although there are still opportunities for the innovative development of new processes. Primary areas for research are in materials science, where solute/solvent/surface interactions are of paramount importance, and in solution thermodynamics for the prediction of solute structure/property relationships. It is unlikely that the stringent product purity requirements placed on high-valued pharmaceutical and diagnostic protein preparations will be needed in the energy-related fields, and thus affinity separation processes should not be a point of focus in our discussions.

MEMBRANE SEPARATIONS

Membranes have played an important role in recent years in bioproduct recovery operations. Microfiltration can be used for cell harvesting and pyrogen removal, while ultrafiltration is geared towards macromolecular solutes such as proteins and other biopolymers in the 10 to 500 kilodalton range. The recovery of oxychemicals using membranes has been demonstrated using pervaporation, electrodialysis and supported liquid membranes. In principle, these processes are well-understood, although in some cases, such as in retention of the integrity of supported liquid membranes, there are still technical problems that need to be resolved. The primary inroads to be made in improving membrane operations lie in the materials science arena, with the development of new membrane materials able to withstand high temperatures (to facilitate sterilization), strong acids and bases, and organic solvents. A better understanding of solute-surface interactions is needed if the near-irreversible adsorption of proteins on the membrane surfaces, and the plugging of membrane pores by cells are to be minimized. Further improvements in membrane design and applications will be forthcoming once their performance characteristics can be

predicted a priori, and solute and solvent fluxes can be estimated solely on the basis of the membrane structural features. The design of membranes from first principles for specific applications is a goal worth striving for.

The traditional use of membranes in ultra- and microfiltration processes has, from an engineering perspective, been well-optimized and is not in need of fundamental research. Promising, non-traditional uses of membranes have been identified, however, and are described below as examples of novel engineering approaches for the recovery of bioproducts.

Hollow Fibre Membrane Extractors

Conventional solvent extraction techniques call for the dispersion of one phase as droplets in the second, continuous phase to ensure high interfacial surface areas for mass transfer. The mechanical energy required to achieve this dispersion is imparted by agitation of the two phases. In many instances, subsequent disengagement and separation of the phases can be hampered because of the formation of emulsions, or because of poor drop settling characteristics owing to high continuous phase viscosities, or to low density differentials between the two phases. These problems can be avoided to a large extent through the use of hollow fibre membrane extractors, where one phase is introduced to the lumens of the membrane bundle, while the second phase is fed to the shell-side of the module. The interface between the two phases is immobilized within the pores of the membranes, and since very high membrane areas can be accommodated in hollow fibre modules, it follows that high mass transfer rates should be attainable. In these devices the membranes do not serve their traditional functions as semi-permeable barriers to solute transfer, but rather act as "momentum barriers" preventing the transport of momentum from one phase to the other, thereby minimizing the risk of emulsion formation. Also, phase disengagement is no longer a problem, and countercurrent solvent extraction processes can be carried out readily if the two phases have close, or even identical, densities.

This concept has been shown to be feasible in the extraction of both low molecular weight oxychemicals and proteins. The general engineering principles are well-established, and there is not much incentive to conduct further, basic research in this area. As with liquid membrane systems, however, there is much that can be done in the materials science area. This ranges from the development of more solvent-compatible membranes and membrane-potting compounds, to the synthesis of membranes having the required physical properties such as wetting characteristics and uniform pore sizes to ensure retention of the interface within the membrane pores.

Rotating Membranes as an Alternative to Cross-Flow Filtration

In the recovery of bioproducts from fermentation media, micro- and ultrafiltration processes are frequently used. Owing to concentration polarization effects, a build-up of the macromolecular solutes and colloidal particulate matter occurs on the membrane surface. The presence of this "gel layer" (a misnomer!) can have severe detrimental effects on the performance of the filtration device, leading both to loss of capacity and to deterioration in selectivity. The need for reducing the extent of gel layer formation has led to the development of cross-flow filtration modules, where the flow tangential to the membrane surface reduces the concentration boundary layer and redistributes the solute retained at or near the membrane surface. However, to ensure sufficiently high shear rates, significant pressure drops must be tolerated over the unit. These cause higher transmembrane fluxes, with higher fluxes at the feed end leading to increased concentration polarization effects. The net result is that at a certain, critical pressure drop the gel layer is established to such an extent that there are no further increases in transmembrane flux with increasing pressure gradients along the device. The recently developed rotating membrane systems offer a means for circumventing these problems, and indicates the important role that engineering can play when based on a strong grounding in fluid mechanics and

transport phenomena. The unit consists of an outer cylindrical shell and an inner, concentric cylindrical membrane. Either one or both of these cylinders can be rotated at speeds of 2000 to 3000 rpm such that flow instabilities develop in which roll cells, called Taylor vortices, are formed. These vortices can provide the surface shear rates and cross-channel mixing required to minimize concentration polarization effects without the need for high tangential flowrates, and low pressure gradients can be tolerated along the device. Moreover, the centrifugal forces acting on the macromolecular solutes at the membrane surface may also assist in the inhibition of the concentration polarization.

Much fundamental work is needed in the full characterization of these devices. The elegant analysis of Taylor in 1926 did not allow for the distortion of the vortices as a result of either the axial fluid flow or the lateral convection through the cylinder surfaces. This is an area where traditional chemical engineering and transport fundamentals can be put to good use, in contrast to the hollow fibre membrane extractors where the pertinent transport processes are well-understood.

The rotating membrane concept has illustrated the potential for engineering solutions to some of the downstream processing problems associated with concentration polarization effects. What it does not do is address the importance of surface chemistry in establishing an adsorbed protein layer on the membrane surfaces, or in the plugging of the membrane pores by cells and cellular matter. Again the importance of understanding solute/surface interactions is emphasized, and exploitation of this understanding in the design and development of new materials for such applications should be a major research thrust.

Micellar-Enhanced Ultrafiltration

The use of ultrafiltration in the recovery of low molecular weight solutes is possible only if some means is devised to ensure rejection of the solute by the membrane. One possible route is to take up the solute in a larger molecular assembly of colloidal dimensions that is itself rejected by the membrane. It

has been demonstrated that if surfactants are added to an aqueous stream at concentrations above their critical micelle concentration so that they form supramolecular aggregates, then hydrocarbon solutes can be solubilized within their apolar cores, and removed from the free solution. When the solution is subjected to ultrafiltration, the micelles are rejected by the membrane and therefore so are the solubilized solutes. The solute, now enriched in the retentate, can be recovered from the surfactants by inducing a phase change, or precipitation of the surfactants.

A number of variants of this approach can be conceived, including the use of suitably derivatized star polymers, or even large adsorbent particles, for protein recovery. While the general principles have been established, and no new engineering considerations have been introduced, this process still offers the challenges of concentration polarization observed in conventional ultrafiltration operations. With the surfactant systems in particular, the high surfactant concentrations at the membrane surface may fall in a region of the phase diagram where the spherical micelles are not favoured, but rather some other aggregative structure, possibly even liquid crystalline, is formed.

Research challenges in this area are again related to solution thermodynamics. What are the driving forces for the solubilization of the solutes, and particularly for the selectivity in the solubilization process? What chemical modifications can be introduced in the detergents or polymers to enhance the selectivity and performance of these systems?

ADSORPTION AND CHROMATOGRAPHIC OPERATIONS

These processes are widely used in the recovery of both high value-added products and bulk commodity chemicals. Little distinction in terminology is made between the adsorptive processes, in which the solute/adsorbent interactions are

sufficiently strong that the entire bed is loaded with the product before being regenerated, and chromatographic operations, where the solute/particle interactions are considerably weaker, and separation is attained because of the large number of "plates" in conventional columns. In the former case, chemistry dominates the effectiveness of the separation, while hydrodynamic effects dominate the efficiency of chromatographic operations. In both cases, conventional packed columns are usually employed, but the high pressure drops across the beds tend to lead to crushing of the particles if they have insufficient mechanical strength. The production of crush-resistant packings is an important generic research area falling under the material science heading.

Many of the problems associated with packed beds can be reduced significantly if radial flow beds are used instead of the traditional axial flow beds. Here, the feed is introduced radially into an annular packed bed region of lower flow path length, thus leading to reduced pressure drops for the same feed loading per packed volume. Alternatively, the use of cylindrical channels rather than spherical particles can reduce form drag significantly without adversely affecting the performance of the device. This is an area where engineering science has contributed to solving certain problems, although the engineering principles are well-established and there is no need for detailed research on these aspects.

One other area where efficient adsorption processes can be improved on is the use of affinity membranes, where the fluid is passed through the membrane pores, which are suitably derivatized to interact selectively with the solute of interest. Very high volumetric capacities can be obtained using hollow fibre modules.

SOLVENT EXTRACTION

The importance of chemical complexation in the recovery of carboxylic acids and alcohols using solvent extraction has been covered well in Jud King's presentation. Our emphasis here will

be on the use of other solvent extraction processes primarily for the recovery of proteins and whole cells.

Two-Phase Aqueous Polymer Systems

Most hydrophilic polymers are incompatible with each other in aqueous solutions, and tend to phase-separate when mixed together, even in fairly low quantities. Each of the resulting immiscible phases is predominantly water (80 to 90% by weight), the remainder consisting primarily of only one of the two phase-forming polymers. The benign environment offered by these aqueous polymer phases to labile proteins and sensitive cells, which can distribute unevenly between the phases, makes these polymer solutions ideal solvent pairs for the recovery of proteins from fermentation media. However, two-phase aqueous polymer systems generally do not have favourable distribution characteristics for uncharged, low molecular weight products, and are not useful for direct concentration and purification of these products. Even charged species are expected to partition fairly evenly between the phases unless the polymers are suitably modified to encourage strong, specific interactions between the products and the polymers. Nonetheless, these systems can offer significant advantages when it comes to integration of fermentation processes with product recovery, particularly if the products are inhibitory (as they often are!). Since the biocatalysts will usually be confined to one phase (the lower, dextran phase in PEG/dextran systems) and the solvent product is distributed evenly between the two phases, it is clear that product can be removed continually by removing the top phase, and replenishing it with fresh polymer solution. The product can then be recovered using, e.g., distillation or extraction with an organic solvent, and the top phase regenerated for recycle to the bioreactor. In some cases, as in the bioconversion of macromolecular substrates such as starch and cellulose, the substrates themselves can serve as one of the two phase-forming polymers.

Two-phase polymer systems show excellent potential for use

in the in-situ recovery of biocatalytically produced oxychemicals such as alcohols, ketones and carboxylic acids. However, there are many facets of the process that are still poorly understood, particularly relating to the solution thermodynamics responsible for the partitioning of solutes and biocatalysts between the two phases, and the actual phase separation process itself. While significant progress has been made in developing correlational approaches for predicting protein distribution coefficients in these systems, and in the fundamentals of bioparticle partitioning, there is still much to be gained from a concerted research effort in this area, which is one that offers significant promise for meaningful research progress. In addition, the development of new, low-cost polymer systems with favourable rheological and physiochemical properties will continue to be an important practical goal.

Other areas for research would entail the development of new polymer systems with top phases having an enhanced capacity and affinity for the bioproduct. It has been shown with proteins that adsorbent beads which distribute preferentially to the top phase, and for which the proteins have a high affinity, can lead to enhanced separation capabilities. For low molecular weight products similar advantages may be forthcoming using traditional adsorbents added to the two-phase polymer system. It might also be feasible to rely on the solubilizing power of surfactant aggregates such as micelles, which can be induced to stay in the top phase, to modify the partitioning behaviour of the oxychemicals sufficiently to warrent their use in large-scale operations. Again, a major research objective would be to understand the solution thermodynamics of the process, and to elaborate on the nature of the micelle/polymer interactions.

Reversed Micellar Solutions

Reversed micelles are nanometer-scale surfactant-stabilized water pools that, under the right conditions, can form spontaneously in organic solvents. Our interest in reversed micelles as effective solvents for many bioproducts is based on

the observation that they are able to host a large array of hydrophilic species, including proteins, amino acids and other ionized solutes, within their polar cores, frequently with a high degree of selectivity. They show particular promise as extractants for the recovery, concentration and purification of biocatalysts such as alpha-amylase, used widely in the bioconversion of starches. They also show promise for the selective extraction of a range of amino acids and other charged solutes, which, to a greater or lesser extent, depending on the nature of the side chain residue, associate with the surfactant layer stabilizing the micellar water pools.

There is at present strong incentive to explore these processes further, both in the development of new solvent/surfactant pairs, and in the characterization of the fundmental interactions responsible for the selectivity of the solubilization processes.

Coacervates

In a number of polymer and detergent systems it is possible to induce phase change by moderate swings in temperature, and to use this in the selective recovery of bioproducts. For instance, surfactants can be added to a single-phase system at a given temperature, and then the system temperature can be raised to induce the phase transition, with a simultaneous distribution of the solutes between the two phases

SUMMARY OF PROMISING RESEARCH AREAS

Much of the equipment that is used in traditional chemical engineering settings and in biotechnology is fairly well characterized, and while there is certainly room for improved mathematical descriptions of these units, as well as more reliable scale-up information, it is doubtful that significant new advances will be forthcoming from this avenue of research. Occasionally new concepts such as the rotating membrane appear, and these are certainly worthy of the traditional engineering and

transport analysis type of study. However, it would seem that more attention must be paid to the materials science aspects of these systems, as it is primarily the nature of the solute/surface interactions that is important in these processes. A case in point is the minimization of protein or cell adherence to membrane surfaces, which can reduce both selectivity and capacity significantly.

Solution thermodynamics is an area that deserves strong attention. For bulk chemicals production, solvent extraction still offers significant potential for efficient product recoveries, particularly when integrated with the fermentation processes. The distribution of the solutes between the two phases, or between a liquid and solid adsorbent, will depend on a range of molecular interactions, including electrostatic and hydrophobic interactions, hydrogen-bonding and steric influences.

The specific design of more selective complexing agents, solvents and sorbents can now be approached from a more fundamental point of view by drawing on the significant advances made in the areas of molecular modelling. The pharmaceutical industries rely routinely on computer modelling in the design of new drugs, and it is now possible to explore the effects on tertiary structures of proteins introduced by genetically modifying the primary peptide sequences. These approaches can certainly be adopted to the design of affinity agents, and can also be extended to investigate environmental effects on protein interactions with the solvents and surfaces. This will be an important area for future research.

Transport properties of solutes in separation processes will still remain an important research objective, particularly in relation to solvent, membrane or adsorbent structure.

6.3.9 Bioprocess Integration and Control Fundamentals

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BIOPROCESS INTEGRATION AND CONTROL FUNDAMENTALS

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INTRODUCTION

The control of bioprocesses is important to ensure achieving the necessary product concentration, productivity and conversion efficiency. However, one of the major problems facing bioprocess control is the lack of sensors to monitor the pertinent variables. This critical need has been addressed throughout the years but unfortunately advances have been rather minimal. In view of the importance of biosensors and monitoring techniques, this topic will again be repeated in this workshop.

Biosensors and monitoring in energy related research are quite generic since fundamental discoveries and applications are translatable to other biotechnology systems. Therefore, advancements can be considered to be useful to all sectors of the bioprocess industry. It is the intent of this paper to address some of the present and future needs in bioprocess sensor and monitoring which will ultimately lead to optimal process control. This latter subject will be addressed in the companion paper in this workshop to be presented by Professor Michael L. Shuler.

The second area to be addressed ~~in this~~ paper deals with the integration of bioprocesses. In recent years, reasonable efforts have been made by the Biochemical Engineering community to examine this concept. The concept to integrate bioprocesses is quite natural since solutions could alleviate some of the bottlenecks in the use of biological systems for the production of fuels and chemicals. It is imperative, however, to realize that energy related research is often addressing large-volume and low cost processes. Therefore the economical constraints to integrate bioprocesses must be at the forefront in addressing this approach. It is the intent of this paper to briefly review the past and present status of bioprocess integration as well as to identify some of the research opportunities.

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BIOPROCESS SENSORS AND MONITORING

Needs and Opportunities in Mutation and Selection

In any bioprocess for energy or chemical production, the heart of the technology will be the microorganism which is able to achieve the necessary product concentration at a high rate of production and accompanied by the highest conversion efficiency. The initial research task to find the desirable organism will be achieved through mutation and selection. Specifically, the fundamental needs in this area are:

- Selecting hyper-producers capable of achieving high product concentration, high rate of product formation and efficient conversion efficiency.
- Mutation and selection to ensure that the strain is able to tolerate high concentrations of the product. This is important since in most bioprocesses the cost of recovery is often the major economical barrier.
- Making certain that the genetic traits of the selected strain is stable over long periods of continuous or semi-continuous operation.

At a first glance, one realizes that the needs stated above are within the realm of geneticists and microbiologists. However, the systematic solutions to achieve these objectives will require efforts in cross-disciplinary collaborations. Some of the opportunities to achieve these objectives in sensor research include:

- The ability to assess in a single cell the intracellular enzyme levels that are critical for product formation.
- A systematic method to differentiate cell morphology as a possible selection method to isolate hyper-producing strains. This approach has been used successfully in the past in the antibiotic industry as well as the production of biopolymers.
- An automatic method to screen a large population of microbial strains based on differences in physical and chemical properties. For example, one might hypothesize a positive correlation between size of a cell to its product tolerance. Thus, the ability to screen a very large population and select those physically different strains for its product tolerant characteristics might be quite useful.

- Development of fundamental strategy where one is able to differentiate physiological differences between low-producers and hyper-tolerant strains based on genetic basis.

It can be seen that the opportunities stated above will all require the ability to systematically examine large microbial populations. Therefore, the fundamental underlying principle to achieve this objective will be automation. Thus, research devoted towards the use of existing instruments such as the cell sorter and cytofluorometer are implicit in the future research programs.

Intracellular Activity Monitoring

The intracellular biological activities of the cell ultimately dictate its ability to produce the desired product at the appropriate rate and concentration. The fundamental needs in this area of research are summarized below.

- Activity-Functional Relationships: There is a fundamental need to address the intracellular enzymatic or other activities as to their functional relationship to product formation. For example, one might hypothesize that the decrease in the specific enzyme activity might be causatively related to product tolerance. The activity could in turn be corrected through other methodologies.
- Quantitative Biochemical Pathway Analysis and Understanding: The ability to analyze the biochemical pathways within the cell and the possible influence of the extracellular environment on the pathway could aid in the optimal operation of a bioprocess. Although this type of analysis is being performed by many laboratories, the actual experimental monitoring of the hypothesized pathways and activities has yet been performed. This quantitative understanding could lead to the better design through genetic manipulations or through on-line process control which ultimately leads to optimal product formation.
- Rapid, On-Line and Simple Sensors: All of these needs require that a rapid preferably on-line and last but least a simple to operate sensing or monitoring devise be developed. It can be stated that we are a long way from achieving this need. However, even realizing the complexity of this problem, it is imperative that research in this area be initiated.

There are a number of possible approaches that represent research opportunities to achieve the above objective. Brief summaries on this possible approaches are outlined below.

- In Situ NMR: In recent years, the use of on-line nuclear magnetic resonance has begun to show its usefulness in detecting in vivo biological activities. There is no doubt this approach will be effective through further research and development on the quantification of intracellular events. This type of a research program will, however, involve multi-disciplinary talents.
- Selective Fluorescence in Combination with Cytofluorometry: The use of fluorescence specific for intracellular proteins and other macromolecules in combination with cell sorting and cytofluorometry has similarly been shown to be effective in assessing intracellular biological activities. This approach should be encouraged with vigor in future research programs.
- "Intelligent Sensors": The concept of an intelligent sensor is one where the biochemical pathway is first analyzed through computer simulations. These algorithms are in turn related to other environmental variables as to their influences on the intracellular events. Thus, the mathematical simulations and computations act as "intelligent sensors" to effect alteration of the pathway either through genetic manipulations or through environmental process control.

Sensors for Reactants and Products Monitoring

There has always been the need to increase our bioprocess monitoring addressing the microscopic and extracellular environment. In recent years, a number of research groups have been addressing these needs. Some of the specific needs which are generic to many bioprocesses are identified below.

- Quantitative Monitor of Suspended Solids: Suspended reactant solids in energy research include materials such as cellulosic biomass, coal, hydrocarbons, lignin etc. In this same category, suspended cell mass should also be included. To quantitatively assess the degradation of solid reactants and the monitor of cell mass on-line are two needs that have been constantly stated in many previous workshops. Some advances in recent years have been made but a more vigorous research effort is also needed.

- Liquid Phase Solutes: Versatile liquid phase solute monitoring will be needed for all bioprocesses related to energy research. Ideally, these monitors should be able to perform rapid on-line analysis in a complex milieu such as a fermentation medium.

Recent advancements in analytic chemistry offers some excellent research opportunities in the area of new concepts for sensors and monitoring. Some of these are still at the exploratory level while others are beginning to be developed as prototypes. Some possible examples for research opportunities in this field are briefly presented below.

- Light Scatter Spectra Analysis for Solids and Cell Mass: Research in my laboratory has recently been focused on the spectra analysis range from 300 nm to 700 nm to quantitatively measure both cell mass and other suspended solids. We have previously demonstrated that light scatter spectra in the absence of other suspended solids can be measured using an on-line fiber optic probe during fermentation. Our most recent efforts have been using the technique to measure cell mass concentration in the presence of suspended solids. Through a unique spectra analysis algorithm, we have shown both cell mass solids and suspended solids can be measured on-line during fermentation. This method appears to be quite generic but requires further research in refinements.
- Solute Detection and Quantitation: Two analytical methods both show extreme promise to affect on-line measurement of dissolved solutes have recently been reported. The Fourier Transform Infrared (FTIR) spectra analysis being developed at Lehigh University and the National Bureau of Standards represent an exciting analytical method for on-line solute determination. The second approach is the research using <u>in situ</u> nuclear magnetic resonance (NMR) being pursued at the University of California, Berkeley and Colorado State University (now Texas A&M). Here again, the use of NMR as a monitor offers an excellent opportunity to advance our needs in process sensors.
- On-Line High Speed HPLC: This method is being developed by many instrument manufacturers and is already being used for on-line process monitor. However, the present systems still require sample preparation but nevertheless have shown its versatility and rapidity.

- Culture Fluorescence: The use of fluorescence to detect solutes and cellular activities has been studied for many years. The use of added fluorophores to provide additional detection capabilities represents an intriguing extension of fluorometric methodologies.

Monitoring in Immobilized Biological Systems

There is no doubt that the use of immobilized cells and other biological entities will be important in energy related bioprocess research. However, the advantages provided through cell immobilization are accompanied by other complications. These complications are well known by researchers in this field and therefore will be summarized quite briefly. Specifically for immobilized systems such as encapsulation, gel entrapment, hollow-fiber and membrane devices, adsorption in porous matrices etc., all encounter the following problems.

- Inability to quantify immobilized cell concentration and its biological activity.
- On-line determination of reactants, product and biological activity profiles in the immobilized state.

The present status to assess immobilized cell systems include off-line methods such as sectioning of the matrix followed by specific staining to quantify the necessary concentration gradients. This approach, although quite eloquent, is tedious as well as difficult. It does not lend the ability to perform rapid in situ analysis and thus would be difficult to implement on-line process control. Other strategies include inference of the biological activities and concentration profiles through the use of other measurable parameters in combination with computer modeling and simulations. This approach is only partially useful but is limited in its generalities.

There are a number of research opportunities to assess the actual states of immobilized cell systems. A brief presentation on the present and future opportunities are presented below.

- On-Line NMR: Similar to the use of on-line NMR for solute and intracellular activities, the potential of on-line NMR measurements appears to be quite vast for immobilized cell systems. This concept is being pursued by a number of researchers including the University of California, Berkeley, RPI and Colorado State (now at Texas A&M). Efforts in the application of on-line NMR should be accelerated.

- Micro-Sensors: The development of micro-electrodes for dissolved oxygen monitoring is well known. With the developments from the electronic industry, the ability to fabricate precision micro-electronic devices appears to be an ideal extension for biosensor research. One can envision the actual placement of micro-electronic devices to monitor pH, oxygen and possibly other solutes within immobilized matrices. Printed circuits associated with these micro-electronic devices are also easy to envision. This area of research, however, will require cross-disciplinary collaborations to effectively use the talents of all parties.
- Recombinant DNA Technology in Monitoring: The developments in recombinant DNA and other molecular biological sciences offer some interesting possibilities to advance the fundamentals in bioprocess monitoring. For example, DNA probes are presently used to finger-print DNA sequences with high accuracy and specificity. Efforts in this direction to examine its feasibility as a biosensor should be implemented.

  The second example to use recombinant DNA technology is presently being pursued in my laboratory as well as other universities (e.g. Technion University, Israel). This is the use of R-DNA to clone and express the luciferase genes into other organisms. The luminescence from replicating cells offers a direct measure of <u>viable</u> as well as the ability to quantify the total number of cells. One can envision this method in combination with micro-sensors could make a major breakthrough to monitor both free cells as well a those in the immobilized state.

INTEGRATION OF BIOPROCESSES

<u>Present Status of Bioprocess Integration</u>

The majority of the past and present research to integrate bioprocesses has been in the production of fuels, solvents and chemicals. Generally, the objectives have been to increase the rate of production and the reduction of product toxicity. In most instances, <u>in situ</u> product removal has been the main focus. These studies have included physical removal for volatile products using vacuum during fermentation or gas stripping of the volatile materials. Alternatively, <u>in-situ</u> liquid-liquid extraction has also been demonstrated when

the product does not possess sufficient vapor pressure to flask off the solute. Various types of membrane-coupled fermentations have been reported including the use of reverse osmosis, ultrafiltration and electrodialysis membranes. In addition, membrane-coupled solvent extraction integrated with the fermentation reactor has also been reported to eliminate the physical contact of the biological system with the toxic solvent. Lastly, various adsorbents such as charcoal and ion exchange resins have been integrated with the bioreactor to remove products formed during fermentation.

It is this writer's opinion that the principle of in situ product removal during fermentation has been amply demonstrated. Furthermore, a slight modification such as a new fermentation system using the past demonstrated methodology is not what is needed for the future. What will be needed and could represent research opportunities will be presented later in this section.

Integrated biological processes, especially for ethanol production has also been reported. These include the simultaneous saccharification of feed stocks such as cellulose and starch in combination with fermentation to produce ethanol. The incorporation of recombinant DNA technology to enhance product formation is also being examined. The latter includes the expression of the cellulase genes in yeast to affect the direct conversion of cellulosic biomass to ethanol. Other attempts to express xylose isomerase genes into yeast so that the simultaneous catabolism of xylose can be affected together with the six carbon sugars. All of these systems are natural extensions of what one is unable to achieve without these modifications. However, it should be stated that none of these biologically integrated concepts have been developed in actual industrial systems.

Opportunities in Integration of Bioprocesses

One might ask why none of the demonstrated feasible integrated processes has attracted commercial practice. This question leads to the possible research and development opportunities for the future. Some of the pressing questions which should be addressed are summarized below.

- What is the critical bottleneck? In many instances, the use of integrated bioprocesses has not addressed the critical bottleneck. For example, to increase the ethanol volumetric productivity through in situ product removal does not contribute significantly to the reduction of the overall economics. Therefore, in future research

for bioprocess integration, the critical bottleneck should first be defined before embarking on seeking "a new mouse trap".

- Simplicity, Scaleability and Cost: In addressing means to integrate bioprocesses, one must consider the question of technical feasibility. This is further reduced to the simplicity or complexity of such a process. Many integrated processes are quite eloquent on paper but often are unscaleable or are too costly. Thus at the onset of any new research in this area, a preliminary engineering feasibility analysis should be performed to address the commercial realities.
- Engineering Versus Biological Solutions: One should also realize that bioprocess bottlenecks allow two methods for possible solution: engineering versus biological. This is certainly the case for simultaneous saccharification and fermentation. Although the molecular basis of product toxicity has not been studied in detail. The biological approach could very well be more feasible than brute force engineering methods. Therefore a real research opportunity exists to address generically the mechanism of product toxicity at the molecular and physiological level. The outcome from these studies in combination with engineering integration methodologies could very well be the long range solution.
- Research, Development and Prototype Integration: One of our real weaknesses in bioprocess research is the lack of following-up from the laboratory setting to the prototype development. A new concept is often reported and published but process refinement is seldom continued. This lack must be corrected especially in integrated processes where much developmental and engineering analyses must be performed in a collaborative setting. Thus the university researcher must involve industrial counterparts throughout the research and development program. This relationship is excellently practiced by our counterparts in Germany and Japan. Perhaps at the onset of any research on bioprocess integration at a university, an industrial partner must be established prior to any research funding.

INTEGRATION OF DOWNSTREAM PROCESSING

The last area which I would like to address is on the integration of downstream processing. Unfortunately research and development to integrate recovery processes in biological systems are not that active. There are several factors contributing to this lack of activity. One obvious answer is that the integration of large volume product recovery does not in reality involve biochemical engineers. These often are within the realm of classical chemical engineering principles. The second reason for the lack of activity is the nature of energy related products: low value commodities. The incentive to integrate downstream processes is thus more relevant to high-value products: e.g. recombinant DNA therapeutics.

In preparing this paper, I spent considerable time in analyzing whether integrated downstream processing could indeed enhance bioprocesses in fuel and chemical production. The only examples of possible opportunities for research are not terribly exciting but will be presented for completeness. It is my hope that I am incorrect in my assessment, and other more novel and clever concepts might be presented at this Workshop as future research opportunities.

Research Opportunities in Downstream Integration

It is my opinion that integrative downstream processing will involve intermediate-value products related to the energy and chemical industries. For example, the use of biopolymers such as Xanthan gum for enhanced oil recovery could be such a product candidate. In this example, innovative research for biopolymer recovery where integration might contribute towards cost reduction could be quite significant. More specifically, whole-broth processing integrated to affect increased biopolymer concentration and to reduce purification costs might be considered. Since one of the major costs in biopolymer recovery is the recovery of the solvent, one might consider increased solvent recovery efficiency through simultaneous extraction and distillation. This latter approach does not fit within the focus of bioprocess research *per se*. From my own limited knowledge and perspective, it is not easy to identify where real research opportunities lie in the integration of downstream processing in the energy and chemical arena.

6.3.10 Issues on Bioprocess Integration, Modeling, and Control

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ISSUES ON BIOPROCESS INTEGRATION, MODELING, AND CONTROL

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INTRODUCTION

All fields accumulate "truisms". These concepts, approaches, rules-of-thumb, can become unquestionably accepted with time. In a field, such as bioprocessing, where art and tradition play as an important role as science such "truisms" can become very firmly established. Often these "truisms" are effective in constructing workable bioprocesses.

However, these "truisms" can be dangerous - dangerous to devising truly more effective and more nearly optimal bioprocesses. Often these "truisms" lead to automatic constraints as new processes are examined. These constraints can form the walls of a box; the real solution may lie outside the box. Significant improvements in bioprocesses can probably be achieved only if we continuously reexamine the constraints we place on ourselves. The most important constraints are those that remain unconscious; we as a profession must actively seek to question ourselves and each other to uncover such constraints on our thinking.

The rapid evolution in our understanding of molecular biology and chemistry of macromolecules as well as tremendous increases in computational and analytical capabilities means that many of the "truisms" of 15 or 20 years ago are no longer applicable.

The purpose of this paper is to discuss how bioprocess integration of recovery and fermentation can lead to novel reactor configurations with potentially enhanced productivity and how computer-based models of cellular systems may lead to

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improvements in bioreactor operating and control strategies. Further models may aid the process integration task by providing a complete overview of a process and integration of components.

Bioprocess Integration

Recognition is growing of a simple fact: separate optimization of fermentation and recovery does not necessarily yield the optimal process. Traditionally the strain development, fermentation, and recovery experts worked essentially in isolation. A lack of formal training of engineers in basic biological concepts and of biologists in engineering and process concepts has made an integrated view of the bioprocesses difficult to obtain. Systems have been optimized sequentially with only modest feedback from downstream to upstream. With improved training of both engineers and biologists it has become possible to begin to build better processes.

One form of this integration is to try to couple some aspects of recovery and purification with the bioreactor. Motivation for such approaches has come initially from a desire to relieve product inhibition and thereby increase reaction rates and/or allow the use of a more concentrated feed. However, other advantages may accrue such as improved selectivity for the product of interest, conversion of a primarily intracellular to extracellular product, and protection of a product from degradation.

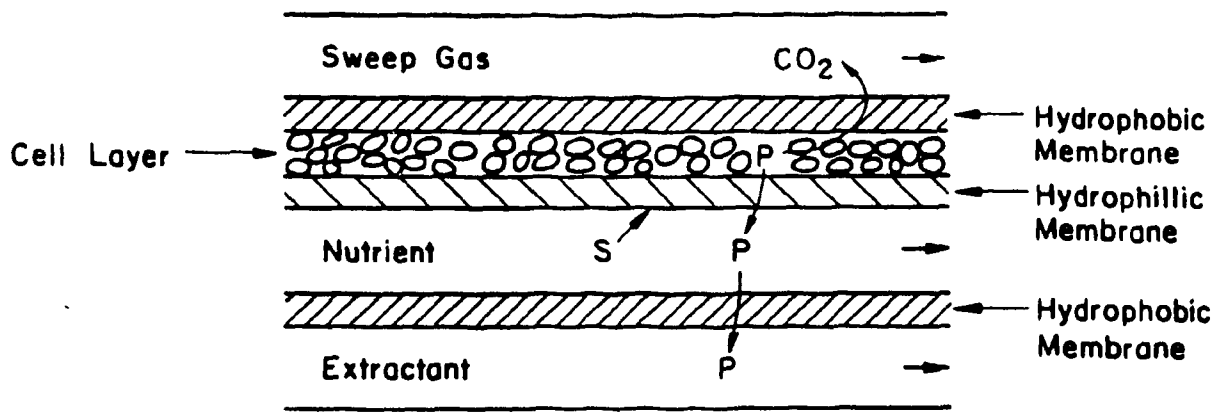
Isolated examples of attempts at bioprocess integration stretch back towards the beginnings of modern biochemical engineering. The energy crisis and interest in ethanol production spawned in the 1970's many suggested approaches to bioprocess integration. Some early approaches to such process integration include vacuum fermentation (1,2), membrane processes (3), addition of solid adsorbents (4), and liquid extractants (5). Recent reviews are available from Wang (6) and Diaz (7) and a section of Biochemical Engineering V (8) is devoted to integration of reaction and recovery.

Process integration has become an important component of the efforts of my laboratory. I will use these to illustrate

some aspects of bioprocess integration.

We have developed (9,10) a reactor system that allows the use of tributylphosphate (TBP) as an extractant of ethanol from fermentation broths. Although TBP had been recognized as an attractive solvent for this purpose (11), its direct use for *in situ* extraction was thought to be impossible due to toxicity (e.g. 12). The question we asked was "why was TBP toxic?". The answer involved recognizing that a solvent could be toxic due to two features: (1) a chemical or molecular level toxicity due to solvent dissolved in aqueous broth, and (2) a physical or phase toxicity due to direct interaction of yeast with droplets of emulsified solvent. It turned out that TBP's toxicity was solely due to phase toxicity (9). Thus constructing a membrane-based reactor (Fig. 1) allowed us to circumvent this phase toxicity.

FIGURE 1. Schematic diagram of the multimembrane unit. Glucose and ethanol are represented by S and P respectively.



For example, with a hydrophobic membrane the solvent will readily fill the pores of the membrane. If the aqueous solution is at a pressure higher than the solvent, but less than the critical entry pressure of the aqueous solution into the membrane pores, then the solvent is effectively immobilized in the membrane. Such a system allows *in situ* solvent extraction of product while preventing emulsification of solvent and phase

toxicity. The primary advantages for the ethanol fermentation are the use of more concentrated feeds, relief of feedback inhibition, and reduced distillation costs for ethanol recovery.

The use of *in situ* adsorption onto neutral resins can be used with other products to alter cellular biochemistry. For example, we have worked with the production of ajmalicine from suspension and immobilized cultures of the plant, *Catharanthus roseus* (13,14,15). *C. roseus* makes about 30 different alkaloids, one of which is ajmalicine. Ajmalicine is used to treat circulatory problems. Suspension cultures of *C. roseus* can make relatively large amounts of ajmalicine, but the product is stored intracellularly. Common extraction procedures involve solvents that are selective for the indole structure, but not so selective among the various indole alkaloids.

If products are released to the extracellular environment, the cost of recovery and purification is often dramatically reduced while providing increased flexibility in reactor design and "reuse" of biomass (an important consideration with slow growing biomass). The neutral resin Amberlite XAD-7 (Rohm and Haas) selectively removes ajmalicine from a mixture of indole alkaloids based primarily on differences in pKa (13). Ajmalicine adsorbs very easily at pH 5.6 which is the pH of the medium used to culture *C. roseus*. The addition of resin to the culture medium leads to significant preferential increases in ajmalicine production without any deleterious effects on the culture (14). Further the resin by itself, or more effectively in combination with calcium-alginate immobilization, and/or use of eliciters to stimulate secondary metabolism, will give rise to much increased levels of extracellular ajmalicine (15). Some results are shown in Table 1.

TABLE 1. Effects of Resin Addition and other Parameters on Extracellular Ajmalicine Production.

Treatment	Extracellular Ajmalicine mg/L
1. Growth Medium	1 to 2
2. Production Medium	4 to 5
3. Production Medium + Eliciter + Immobilization but no resin	15
4. Production Medium + Resin	33
5. Production Medium + Resin + Eliciter + Immobilization	91

Not only is the amount of ajmalicine increased, the process leads to greater purity. With 91 mg/L extracellular ajmalicine about 89.5 mg/L will be on the resin. The closely related alkaloid, serpentine, is present at an extracellular level of 1.6 mg/L with essentially none of that on the resin. Under some growth conditions the intracellular concentration of ajmalicine and serpentine are nearly equal. Thus, the use of *in situ* adsorption increases significantly and preferentially ajmalicine production, allows extracellular production, and leads to significant concentration and purification of the desired product.

Not only can this integrated view be expressed through the addition of extracting agents, it can be expressed at the molecular level. For example, we have found that we can achieve protein excretion into the extracellular compartment from *E. coli* using appropriately constructed plasmids. Excretion depends on a high level of overproduction from a plasmid-encoded gene. We have achieved good excretion of β-lactamase and human epidermal growth factor (16,17). The motivation for this approach has been to greatly simplify recovery and purification rather than any potential increases in reactor productivity.

In all of these three examples it was necessary to challenge the common wisdom (i.e. TBP is toxic and cannot be used *in situ*, ajmalicine is intracellular and cannot be excreted without damage to the cell, and *E. coli* cannot preferentially excrete proteins extracellularly). In all these

cases we have shown that the common wisdom or "truism" was a false constraint. There may be many opportunities for bioprocess integration that we as a field have ignored because of self-imposed, but unnecessary constraints.

Models and Integration

Process integration involves not only the physical integration of recovery and purification, but ultimately the conceptual integration of the process. The earlier such integration takes place, the more efficiently a nearly optimal process can be generated. Process mathematical models may play a useful role in this regard.

The process begins with selection of a strain and strain improvement. Programs utilizing the techniques of artificial intelligence have been constructed at MIT ("BioPath") and Cal Tech ("MPS") for examining the effects of molecular level manipulations on the production of metabolites. These models have been used as techniques to identify potentially rate-limiting steps in a pathway or to identify alternate metabolic routes to a given product. These models suffer from all of the difficulties normally associated with the techniques of artificial intelligence (e.g. the difficulty in abstracting the true rules by which a knowledge base is manipulated). Further, such models are incomplete with respect to cellular regulatory mechanisms and mechanisms for transducing extracellular information into intracellular responses. Also, the prediction of intrinsic rate constants is very difficult from first principles. I believe that this latter factor is an important constraint and completely new approaches to such modeling will be necessary before such models will be generally useful. However, the prediction of yield and optimal choices of substrates is a worthy goal and greatly impacts rest of the process.

Once the characteristics of the organism (or organisms) are known models of reactor performance become important. Bioreactor models will be discussed in more detail in the next

section. Models capable of predicting dynamic behavior are essential to process control and also for developing models where the mode of operation is essentially transient. Such dynamic models really require a high level of complexity. For the purposes of process integration less complex models may be satisfactory; such models predict primarily material balance information and reactor scheduling. Commercial packages (e.g. Aspen Technology) are being developed to fulfill such needs.

Further, reactor models can be coupled to models of recovery and separation. An example of such a program is "Bio Sep Designer" developed at MIT (G. Stephanopoulos). Such a model is not concerned so much with dynamic responses but in exploring possible alternatives in process configuration and choice of techniques. When such models are coupled to reactor models, the implications of changes of upstream parameters on the whole process can be examined. Further economic models can be overlaid on the combined process models to determine economically attractive combinations.

Thus models can greatly assist the task of process integration. However with current models the emphasis is on configuration and not dynamic performance. Further, these models do not readily admit the inclusion of novel reactor designs or unusual reactor operating strategies. More robust and intrinsically realistic bioreactor models are needed.

Bioreactor Models and Control

Models of cultures have been classified as structured or unstructured and segregated or non-segregated (18). Structured models break the culture into sub-components. Segregated models recognize that individual cells will vary from one and another while non-segregated models assume that only the population-averaged value of a parameter is important and not its distribution among individuals in a population.

Unstructured and non-segregated models have totally dominated the field because they are computationally fairly simple and do not demand extensive experimental data for verification.

However, their range of usefulness is greatly restricted. Unstructured non-segregated models cannot, based on first principles, make accurate predictions of dynamic behavior in bioreactors (19). Thus structure is critical. The most realistic of models will include both structure and segregation. When approached from the population point of view such models lead to integro-differential equations that are essentially intractable.

We have circumvented that problem by writing highly structured models of single cells and then using those models in a finite-representation scheme to model a population (20). These models are capable of making accurate *a priori* predictions of dynamic performance of bioreactors. When our group began developing such models we faced the prevailing view that: (1) cells were too complex to justify construction of such a model, and (2) even such single-cell models would be too computationally involved to be tractable on available computers. This view had a fair degree of validity twenty years ago. However, advances in computer technology have outpaced our ability to correctly incorporate increasing detail into our model. More importantly advances in molecular biology have provided both the necessary insights into cellular mechanisms and the data required for parameter estimation to make the construction of complex models relatively easy. The prime limitation on the use, development and extension of such models is a human one - our ability to convert existing insights and information in the biological literature into appropriate mathematical statements. For models to be realistic and useful the engineer or modeler must understand subcellular biology.

Let's consider the primary dilemma modeling faces. Simple models are usually highly empirical, unrealistic and unreliable for predicting dynamic behavior. Complex models, as we have constructed from *E. coli* (20), may be viewed as too specific (restricted to single strain) and too computationally demanding for on-line control.

These apparent deficiencies of complex models are addressable and present real research opportunities. The first concern is generality. Clearly strain differences are important. However, the basic structure of cellular metabolism remains similar between strains. The actual number of parameters that may be different from one strain to another may be small. Techniques to identify parameters causing such differences need to be refined, but in most cases presents a straightforward problem. The model itself provides a basis for assisting in isolating which features of the predicted metabolism differ from preliminary observations.

Associated with model complexity is the need to evaluate a large number of parameters. First the reader should recall that it is not the number of parameters that determines a model's degree of artificiality, but rather the number of non-intrinsic adjustable parameters that differentiates models from curve fits. A model with 100 independently determined parameters is more meaningful than one with two freely adjustable parameters. For many organisms sufficient literature data is already available to allow independent evaluation of most parameters.

Although we have model E. coli in the greatest detail we have written reasonably detailed models of a lower eukaroyte, Saccharomyces cerevisiae (21) and a higher eukaroyte, Chinese Hamester Ovary (CHO) cells (22). Further a highly detailed model of a gram positive bacterium, Bacillus subtilis, has been written (23) at the University of Pittsburgh. Thus complex models have been formulated representing a wide spectrum of cell types. The construction and testing of such models by experienced personnel can be done in a few months. However, it is critical to develop techniques for the general construction of structured realistic models.

The computational requirements for such models can vary considerably. Our models of CHO cells and S. cerevisiae can run on personal computers. Our base E. coli single-cell model for glucose-limited growth will go through one cell cycle (45

min) on an IBM AT (or clone) with a math coprocessor in less than three minutes with a suitable streamlining of computer code (Gary Stanlake, personal communication). The single-cell model can be used as a structured but non-segregated model of a population. At the other extreme a population model using 250 single-cell models of plasmid containing E. coli is routinely run at the Cornell Supercomputing facility. Thus the highest level of realism is not now approachable for on-line control. However, quite highly structured but non-segregated models can be utilized with very modest demands on computer time.

The most highly complex models can make good a priori predictions of dynamic behavior. Thus, they can serve three important functions: (1) testing a variety of operating strategies (e.g. periodic cycling, optimal timing for inducing plasmid-encoded protein synthesis, effects of changes in nutrient composition, etc.), (2) providing a dynamically correct simulator to test different process control strategies, and (3) to serve as "parent" models for the rational formation of less computationally intensive models.

One example is the use of the modal technique (24) which converts our E. coli model with 18 components into a three pool model by considering the response time characteristic of changes of each component and focusing only on those whose time scale corresponds to the time scale of observation (e.g. for growth). Such reduced models are potentially superior to ad hoc models with the same number of components in that such reduced models are derived from a realistic base and involve inherently fewer assumptions.

My own belief is that models even more realistic (and hence complex) than current models are required. Such realistic models provide a high degree of potential accuracy in making a priori predictions over a wide range of environmental conditions because few assumptions must be made. Such complex models can serve as a rational base for reduction to less complex, computationally faster models.

Although the above discussion has focused on pure populations in homogeneous environments, analogous needs exist for mixed culture systems or systems with a heterogeneous environment. However, both of these cases would increase complexity.

Computationally fast models can play an important role in on-line control. However, even the most realistic model will be imperfect. Control strategies to augment and correct model-based predictions are needed for on-line control of processes. High level process control systems are in a state of rapid evolution. This writer does not claim any great expertise in these systems, but believes that the potential of process control has been barely exploited. Currently computer control (25) of fermenters involves primarily data logging and manipulation to yield such process parameters as O_2 uptake, CO_2 release, respiratory quotients, and mass balance information (e.g. nutrients, antifoam, acid and base). Closed loop control, primarily on temperature, pH, aeration/agitation, and liquid level is common. More sophisticated control based on predictions of dynamic response is almost totally lacking. Attempts to directly influence subcellular metabolism are virtually absent. A lack of metabolically accurate models of the culture is the primary obstacle to implementation of more sophisticated control approaches.

A related obstacle is the development of appropriate sensors and measurement techniques, but I believe that even with sensors (and with on-line HPLC we are approaching the threshold of achieving many insights into metabolism) the main limitation will be on how to use the information intelligently. The development of models and control strategies is lagging developments in measurement techniques.

Finally, we should briefly discuss the use of "expert systems" for control of fermenters. The pharmaceutical industry has regulatory constraints that make the exact repetition of the fermentation process important. Expert systems provide an effective tool to duplicate the best of past

performance. Such an approach relies on the very inexact extraction of knowledge from expert operators. Expert systems alone are difficult to implement on new processes or processes still undergoing optimization. Model-based control systems augmented by some features of expert control would be much more suitable for new processes than a strategy relying solely on rules derived from experts.

Summary

Perhaps the primary constraint on bioprocess integration and development of models and control strategies are self-imposed constraints on what is possible. An important role for academic bioprocess engineering is to continuously re-examine these constraints for their validity.

More specific suggestions are:

1) A need for a better fundamental and predictive understanding of the interactions of solvents and solvent mixtures on cellular membranes and extractive characteristics for a variety of potential fermentation products.
2) Development of integrated bioprocess models that can relate changes in cellular metabolism to reactor performance and the efficiency and configuration of the separation system.
3) Development of more realistic models of cellular metabolism coupled with techniques for rational reduction to less complex more computationally efficient models.
4) Development of model structures allowing rapid generalization from one strain to another and from one species to another.
5) Development of model-based control strategies to effectively exploit advances in measurement techniques and sensors.

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6.4 VISUAL AIDS USED BY STAFF OF THE U.S. DEPARTMENT OF ENERGY

Four staff members of the U.S. Department of Energy presented summaries of biotechnology research and development currently being supported by that agency. Copies of the visual aids used during those presentations are included in this section.

6.4.1 Division of Biological Energy Research

Robert Rabson

U.S. Department of Energy

Division of Biological Energy Research
(Division of Energy Biosciences)

Scope: To discover and define fundamental biological mechanisms that might be employed as the basis of future energy-related biotechnologies.

Areas Covered: Microbial Sciences, Plant Sciences

Division of Biological Energy Research

Areas supported in the plant sciences:

- Bioenergetic systems - Photosynthesis
- Control of plant growth and development (metabolic, genetic, and environmental)
- Stress response mechanisms
- Genetic transmission and expression
- Plant-microbial interactions
- Plant cell wall structure and function

Division of Biological Energy Research

Areas supported in the microbiological sciences:

- Lignocellulose degradative mechanisms
- Mechanisms of fermentations
- Genetics of neglected microorganisms
- Energetics and membrane phenomena
- Thermophily and anaerobiosis
- Microbial ecology and associations
- One- and two-carbon metabolism
- Microbial interactions with plants

ENERGY BIOSCIENCES
(BIOLOGICAL ENERGY RESEARCH)
RESEARCH TRENDS

- COMPLEX CARBOHYDRATES
  - A. STRUCTURAL STUDIES INCLUDING NEW ANALYTICAL PROCEDURES
  - B. BIOCHEMICAL AND PHYSIOLOGICAL PROPERTIES: SYNTHESIS, ROLE IN CELL RECOGNITION AND METABOLIC REGULATION

- OTHER POTENTIAL BIOTECHNOLOGY TARGETS
  - A. MOLECULAR BIOLOGY AND MECHANICS OF ENERGY TRANSFORMING PROCESSES, E.G., PHOTOSYNTHESIS, ION TRANSPORT
  - B. MECHANISMS OF CONVERSIONS OF BIOPOLYMERS (LIGNIN, CELLULOSICS) TO SIMPLER USEFUL PRODUCTS
  - C. GENETICS, PHYSIOLOGY AND BIOCHEMISTRY OF NEGLECTED BUT POTENTIALLY USEFUL MICROORGANISMS (ANAEROBES, THERMOPHILES)
  - D. PLANT CHROMOSOME ISOLATION AND ORGANIZATION

- INTEGRATION OF BIOLOGICAL INVESTIGATIONS WITH POWERFUL NEW ANALYTICAL PROCEDURES OF CHEMISTRY AND PHYSICS

6.4.2 Fossil Energy Activities in Biotechnology

Paul C. Scott

U.S. Department of Energy

U.S. DEPARTMENT OF ENERGY FOSSIL ENERGY ACTIVITIES IN BIOTECHNOLOGY NOVEMBER 1988

INTRODUCTION

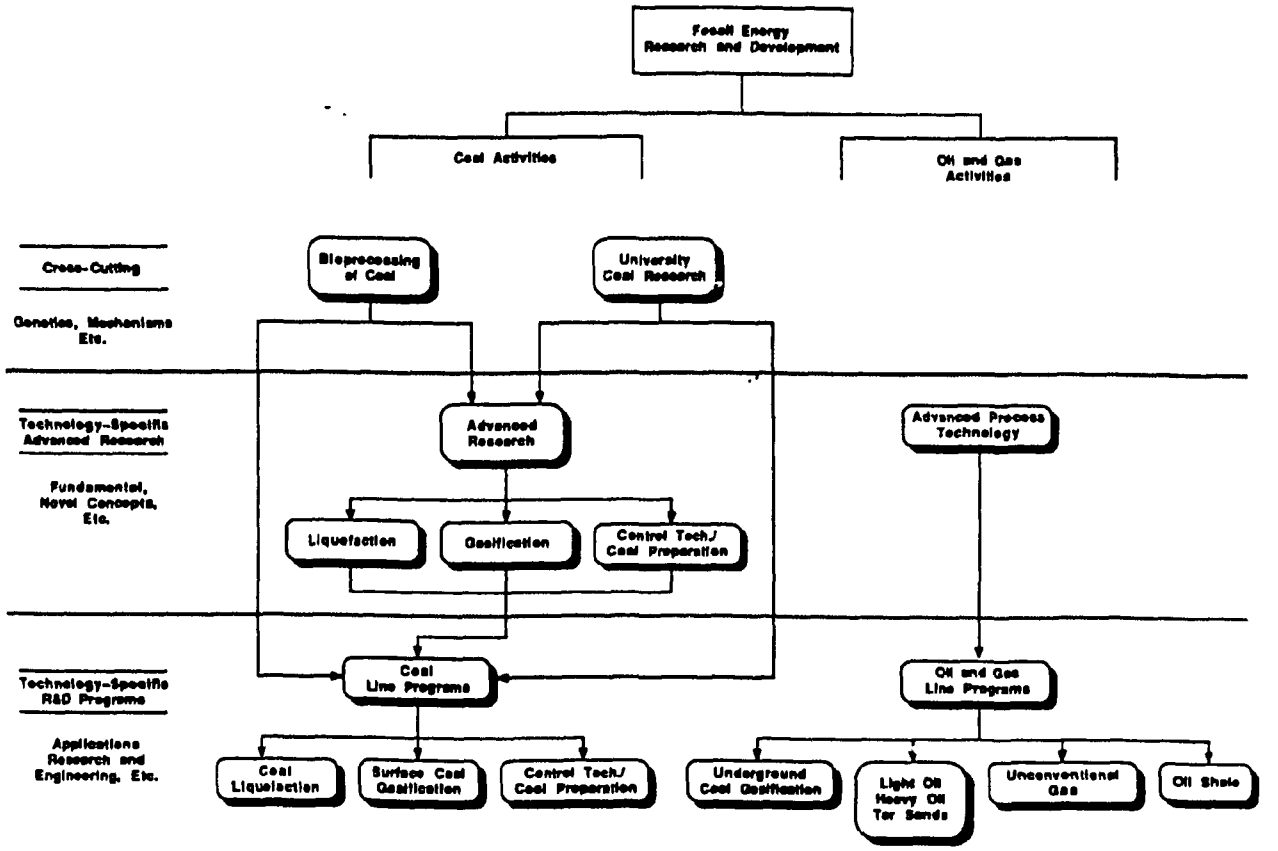
Activities in biotechnology are integral to the Fossil Energy mission and thus share the objectives of that mission. These objectives are to:

- o Increase the contribution of coal by:
  - improving the environmental, technical, and economic performance of coal-based systems; and
  - increasing the number of areas of application and flexibility of use of coal-based systems.
- o Increase the effective resource base for premium gas and liquid fuels through:
  - enhanced resource recovery; and/or
  - production of liquid and gaseous fuel analogs from coal, shale, and tar sands.
- o Couple process technology research with appropriate environmental research to economically manage environmental impacts of processing and using fossil fuels.
- o Couple process technology research to advanced research activities needed to understand fundamental scientific and engineering phenomena.

To support these goals, several Fossil Energy programs have been seeking, over the past several years, to explore the use of new approaches in biotechnology to obtain and use fossil energy resources.

During the period 1984-1988, the Fossil Energy Program has committed approximately $16 million for biotechnology-related research. The activities in biotechnology are funded through several different Fossil Energy subprograms. Relationships between the subprogram activities are shown in Exhibit 1.

EXHIBIT 1. RELATIONSHIPS BETWEEN FOSSIL ENERGY R&D



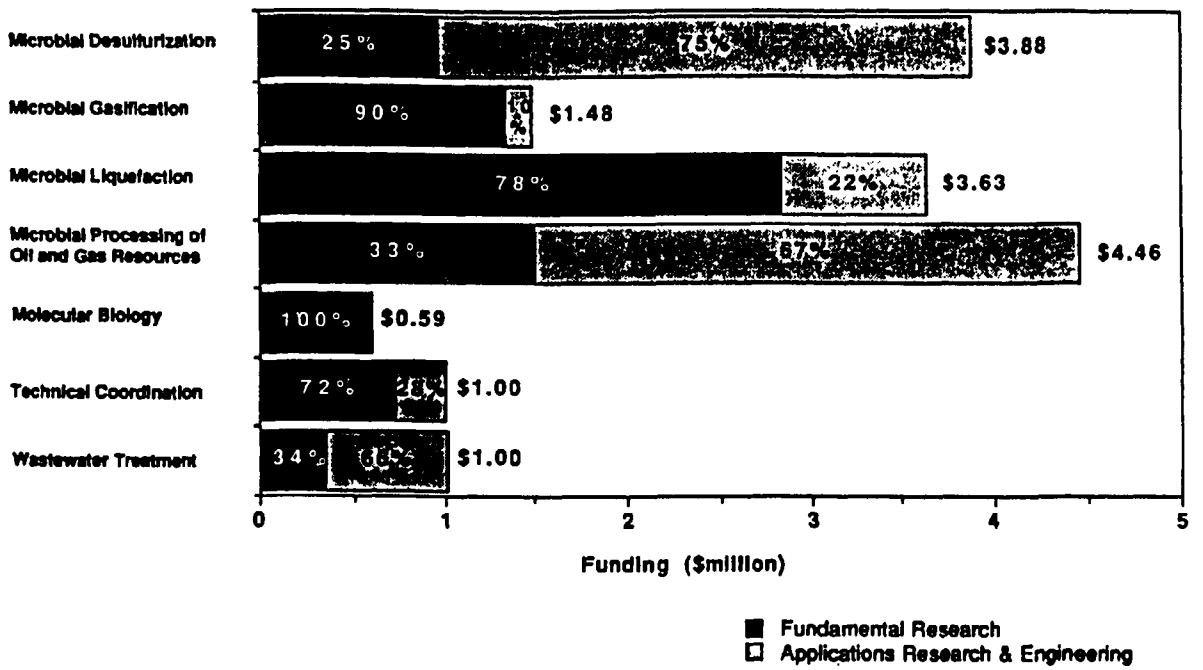
In the coal activities, the most fundamental and crosscutting programs are Bioprocessing of Coal, dealing with genetics, mechanisms, etc., and University Coal Research, providing research information and results for all of the other programs. The Advanced Research activities in Coal Liquefaction, Coal Gasification, and Control Technology/Coal Preparation seek to obtain fundamental information and explore novel concepts for a specified end-product (e.g., a coal-derived liquid or gas, or a desulfurized coal). The Coal Technology R&D programs (also called Line Programs) are more engineering oriented, and conduct applied R&D through the proof-of-concept stage.

In the oil and gas activities, the Advanced Process Technology Program supports fundamental research and explores novel concepts related to oil and gas resources, while the Oil and Gas Technology R&D programs are more engineering oriented, similar to the Coal Technology R&D programs.

Fields of research in biotechnology have included molecular biology, coal desulfurization, coal gasification, coal liquefaction, treatment of coal conversion wastewater, and processing of oil and gas resources. The funding of these activities is summarized in Exhibit 2.

Exhibit 2.
Fossil Energy Activities in Biotechnology, FY 1984-1988

Total Funding $ 16,042,000



The following provides a brief summary of the activities in each generic area in terms of (1) fundamental research and (2) applications research and engineering.

Fundamental Research Activities

The principal aim of the fundamental research activities is to elucidate the underlying principles and improve the understanding of microbial processing of fossil fuel resources. An additional objective is to explore novel approaches and seek breakthroughs in the use of biotechnology for recovery or utilization of such resources. The following is a synopsis of current fundamental research activities in each of the bioprocessing technical areas:

Molecular Biology

- Investigation of genetic control mechanisms of organisms that have specific interactions with coal.

- Determination of the feasibility of introducing catalytic activity into biological molecules such as antibodies.

Microbial Desulfurization

- Study of the mechanism of thiophene metabolism by mutants of *E. coli*.

- Characterization of metabolic features of microorganisms that degrade materials containing organic sulfur.

Microbial Liquefaction

- Screening of organisms and investigation of the biochemistry of bond breaking to produce liquid products.

- Development of analytical techniques for product analysis, investigation of fungal morphology, and isolation and characterization of enzymes.

Microbial Gasification

- Biological conversion of synthesis gas to methane, including optimal culture, mass transfer, and reaction kinetics.

- Sequential conversion of coal to liquid then gaseous products.

Wastewater Treatment

- Investigation of reactor operation and mathematical modeling for anaerobic biological treatment of wastewaters derived from coal conversion processes.

Microbial Processing of Oil and Gas Resources

- Determination of the feasibility of using microorganisms to improve oil mobility within a reservoir.

- Modification of refractory materials, including heavy oils, tars, and kerogens.

- o Isolation and screening of microorganisms to provide laboratory support for field experiments.

Technical Coordination

- o Planning and management of conferences and workshops related to bioprocessing of fossil fuels.

- o State-of-the-art reviews and assessment of research needs in the field.

Applications Research and Engineering

Applications Research and Engineering covers the development of a technical knowledge base to a proof-of-concept stage for improved fossil energy technologies. In the biotechnology field the current range of activities include:

Microbial Desulfurization

- o Removal of organic sulfur by mutagenesis of bacterial organisms (microbial process optimization).

- o Removal of inorganic sulfur by thermophilic organisms.

Microbial Liquefaction

- o Development of optimal conditions for the use of microorganisms to produce liquid fuels from coal-derived synthesis gas.

Microbial Gasification

- o Investigation of the potential of anaerobic bioconversion of low rank coals to methane.

Wastewater Treatment

- o Process studies on biooxidation reactors for wastewater treatment.

Microbial Processing of Oil and Gas Resources

- o Field experiments to employ microorganisms to increase oil mobility.

Technical Coordination

- o Assessment of biotechnology applications in:
  - Coal preparation,
  - Coal utilization,
  - Environmental control technology, and
  - Petroleum, heavy oil, and oil shale technologies.

Summary

Over the past several years, the Fossil Energy Program has developed an increasing activity related to utilizing biotechnology to expand the breadth of fossil fuel use. This program is now exploring and developing new approaches in petroleum resource recovery and in coal benefication and processing, in order to achieve the goal of producing clean fuels.

6.4.3 Bioprocessing for Biofuels

Richard F. Moorer

U.S. Department of Energy

BIOPROCESSING FOR BIOFUELS

WORKSHOP ON BIOPROCESSING RESEARCH FOR ENERGY APPLICATIONS

RICHARD MOORER
BIOFUELS AND MUNICIPAL WASTE TECHNOLOGY DIVISION
DEPARTMENT OF ENERGY

MISSION OF THE BIOFUELS AND MUNICIPAL WASTE TECHNOLOGY DIVISION

- **PROVIDE THE TECHNOLOGY BASE FOR THE PRIVATE SECTOR TO INCREASE SUPPLIES OF ORGANIC FEEDSTOCKS SUITABLE FOR USE DIRECTLY AS FUELS FOR ECONOMIC CONVERSION PRODUCTS.**

- **PROVIDE THE TECHNOLOGY BASE FOR THE THERMOCHEMICAL AND BIOCHEMICAL CONVERSION OF ORGANIC FEEDSTOCKS TO ENERGY PRODUCTS, FOCUSSING ON LIQUID AND GASEOUS FUELS.**

- **PROVIDE EARLY TRANSFER OF TECHNOLOGY TO PRIVATE INDUSTRY BY VERIFYING TECHNICAL FEASIBILITY/ READINESS OF INTEGRATED FEEDSTOCK PRODUCTION AND CONVERSION TECHNOLOGIES THROUGH COST-SHARED PROJECTS.**

PROGRAM ELEMENTS

FEEDSTOCK PRODUCTION

- **TERRESTRIAL CROPS**
- **AQUATIC SPECIES**
- **MUNICIPAL SOLID WASTE**

CONVERSION TECHNOLOGIES

- **BIOCHEMICAL PROCESSES**
- **THERMOCHEMICAL PROCESSES**

BIOCHEMICAL CONVERSION

- **ALCOHOL FUELS (LIQUIDS)**
- **ANAEROBIC DIGESTION (GASES)**

ALCOHOL FUELS RESEARCH

- **PRETREATMENT**
- **ENZYME PRODUCTION**
- **ENZYMATIC HYDROLYSIS**
- **FERMENTATION**
- **LIGNIN CONVERSION**

BIOPROCESSING RESEARCH NEEDS

ALCOHOL FUELS

- BIOPROCESS MODELLING
- CONTINUOUS PROCESSING
- CONSERVATION OF BIOCATALYSTS
- PRODUCT SEPARATION
- MICROBIAL MAINTENANCE
- TRANSPORT PHENOMENA
- ENVIRONMENTAL CONTROL
- PROCESS INTEGRATION
- SCALE-UP

AQUATIC SPECIES RESEARCH

- **PRODUCTION**
- **EXTRACTION AND CONVERSION**
- **ALGAL PROCESS ENGINEERING**

BIOPROCESSING RESEARCH NEEDS

AQUATIC SPECIES

- **CONTINUOUS VS. BATCH PROCESSING**
- **MAXIMAL CELL/LIPID PRODUCTION**
- **CO2 UTILIZATION**
- **CULTURE MAINTENANCE**

ANAEROBIC DIGESTION RESEARCH

- ENHANCED BIOCONVERSION
- IMPROVED REACTOR CONCEPTS
- PROCESS ENGINEERING EXPERIMENTS
- LANDFILL GAS RECOVERY AND WASTEWATER TREATMENT

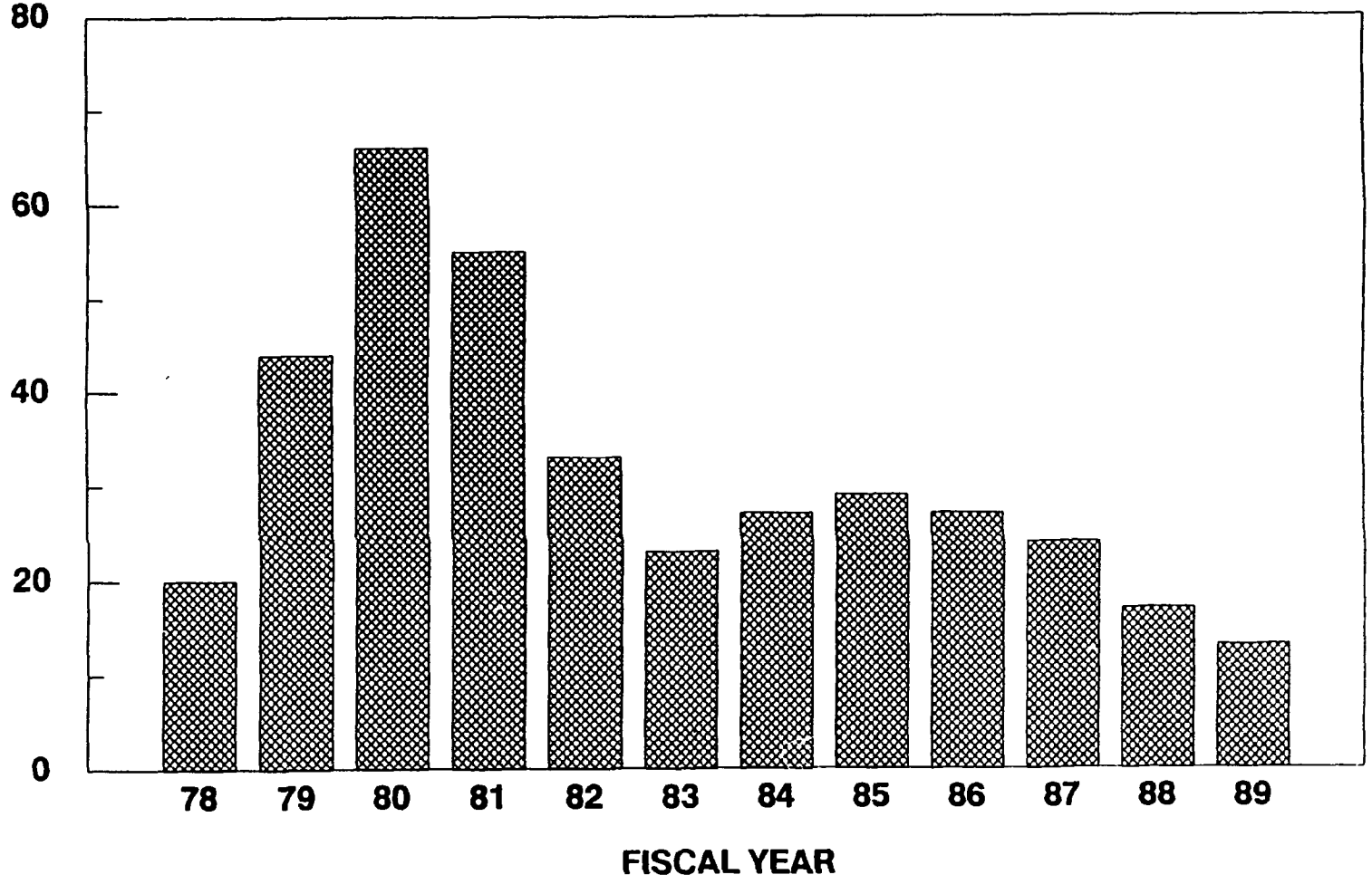
BIOPROCESSING RESEARCH NEEDS

ANAEROBIC DIGESTION

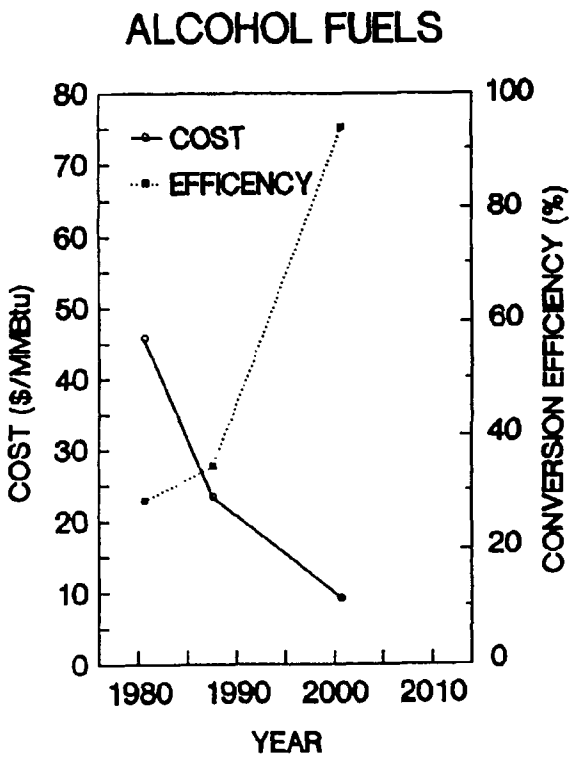
- **NOVEL BIOREACTOR CONCEPTS**
- **IMPROVED CELL DENSITY/ENZYME LEVELS**
- **PROCESS MONITORING AND CONTROL**
- **PRODUCT RECOVERY AND ENRICHMENT**
- **SCALE-UP**

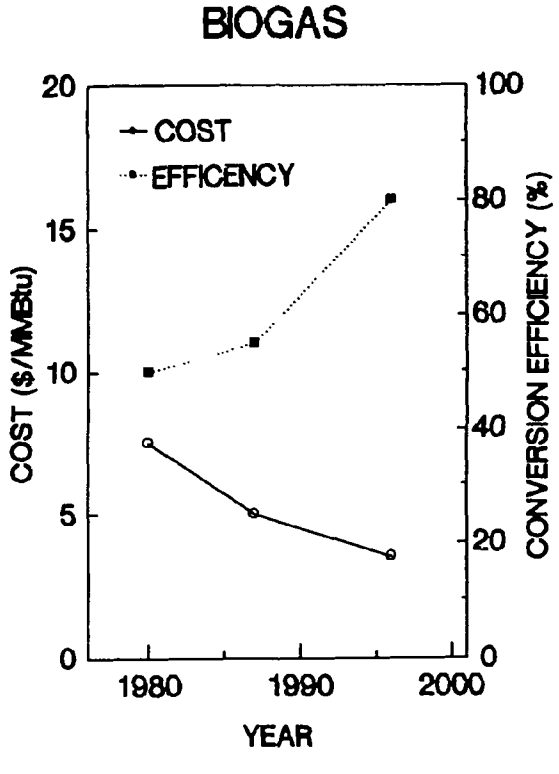
BIOFUELS BUDGET HISTORY

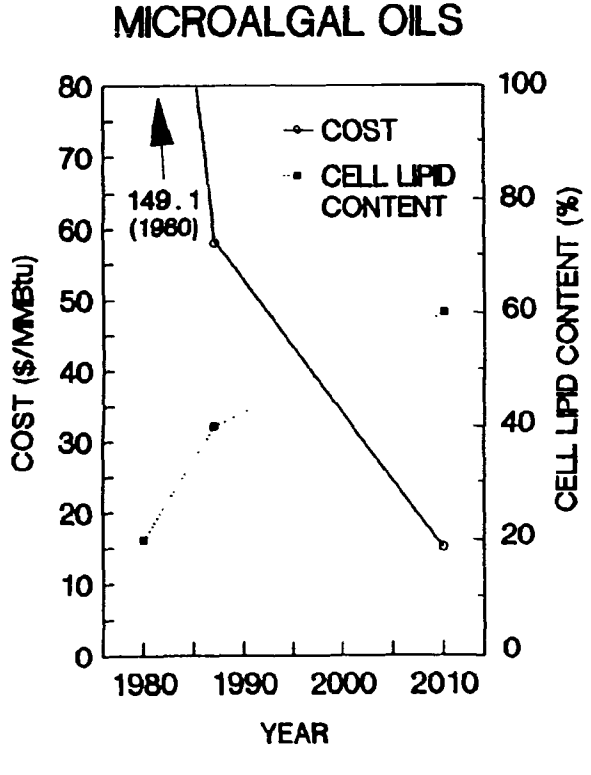
($ IN MILLIONS)



BIOFUELS PROGRAM PROGRESS







6.4.4 The Energy Conversion and Utilization Technologies (ECUT) Biocatalysis Program

Leonard Keay

U.S. Department of Energy

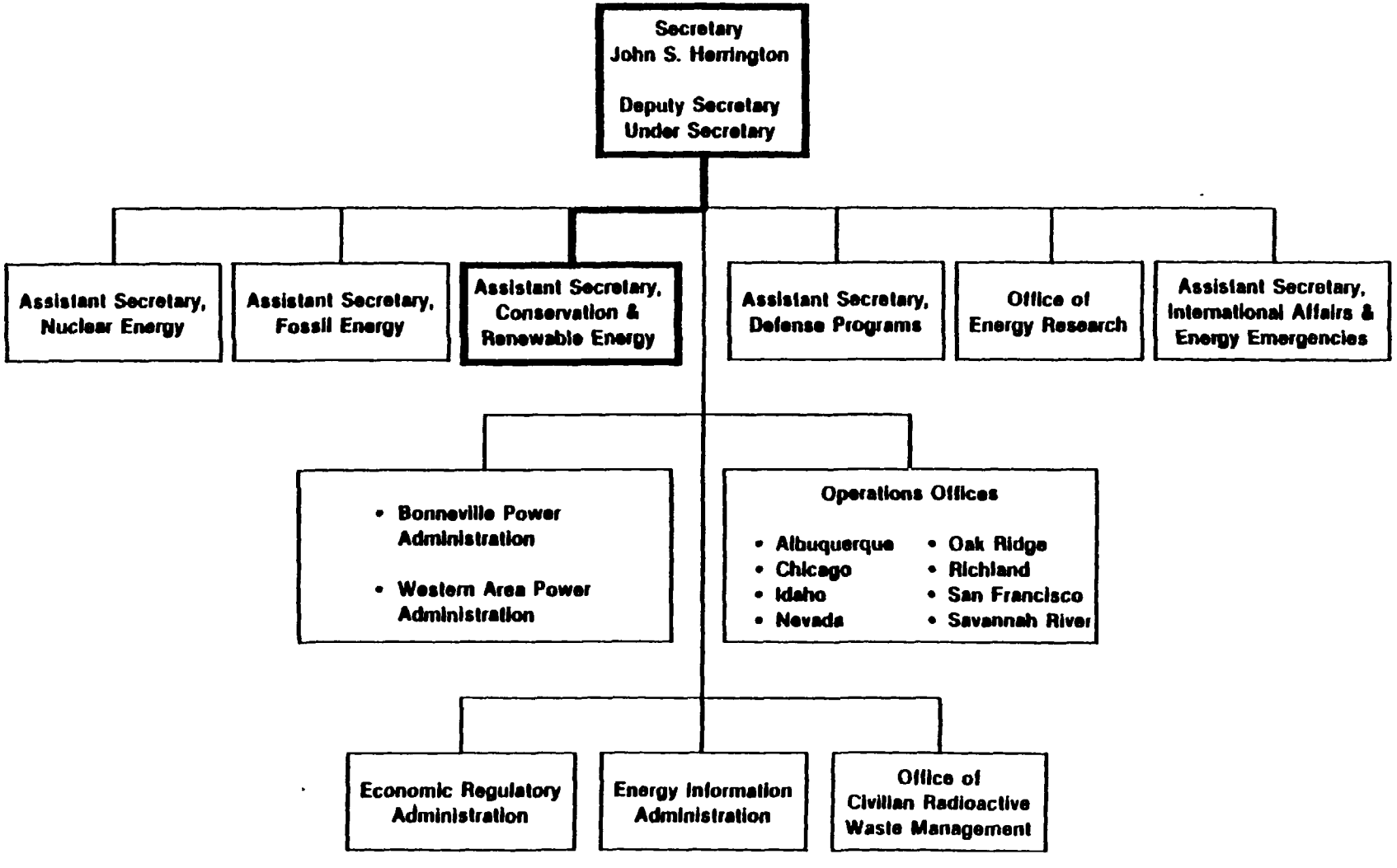
THE ENERGY CONVERSION AND UTILIZATION TECHNOLOGIES (ECUT) BIOCATALYSIS PROGRAM

DR. JAMES J. EBERHARDT
DIRECTOR, ECUT PROGRAM

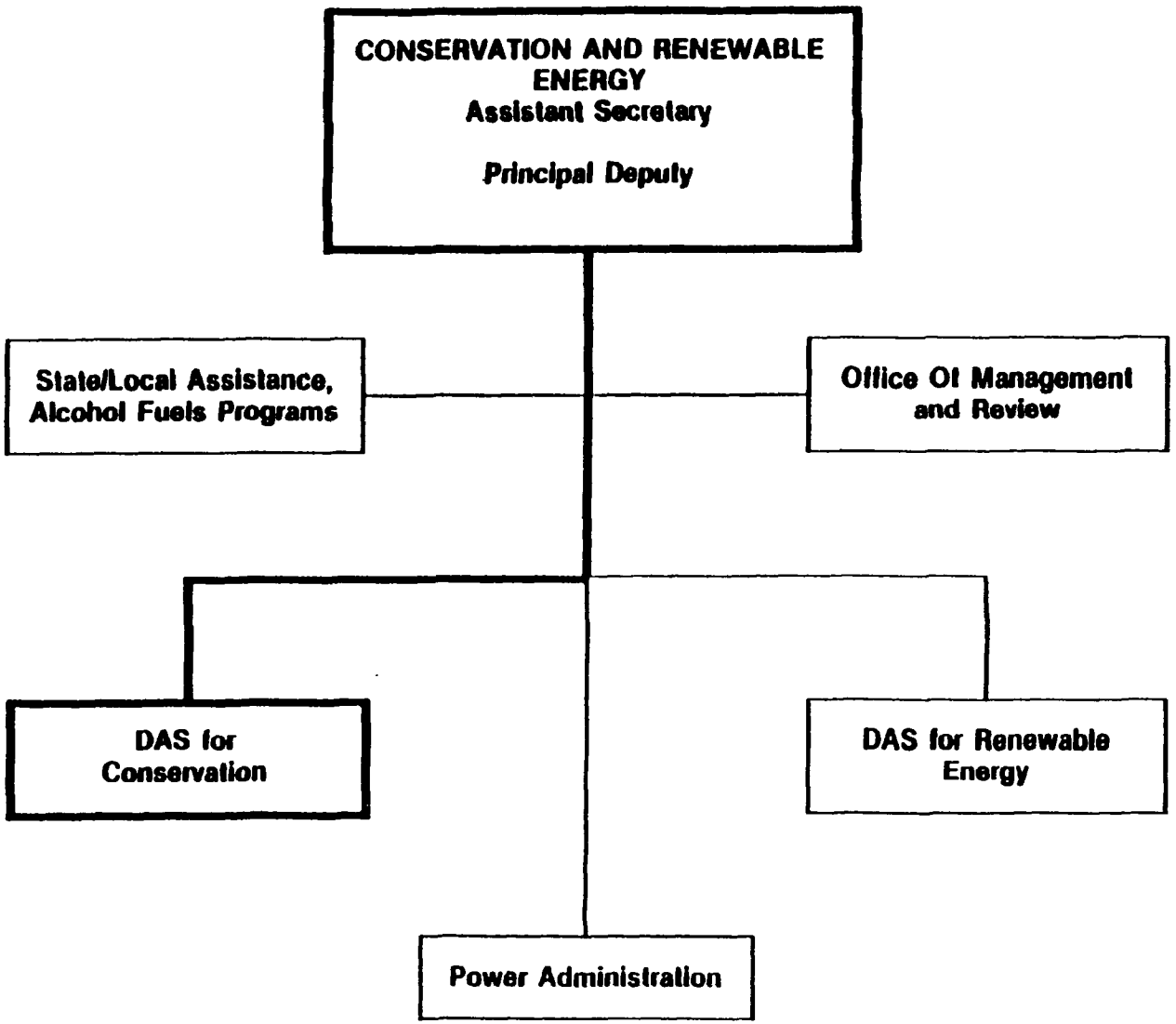
OFFICE OF ENERGY UTILIZATION RESEARCH
OFFICE OF CONSERVATION AND RENEWABLE ENERGY
U.S. DEPARTMENT OF ENERGY

Presented at the Workshop on Bioprocessing Research
for Energy Applications Sponsored by The Council
for Energy Engineering Research
November 2-4, 1988
Alexandria, VA

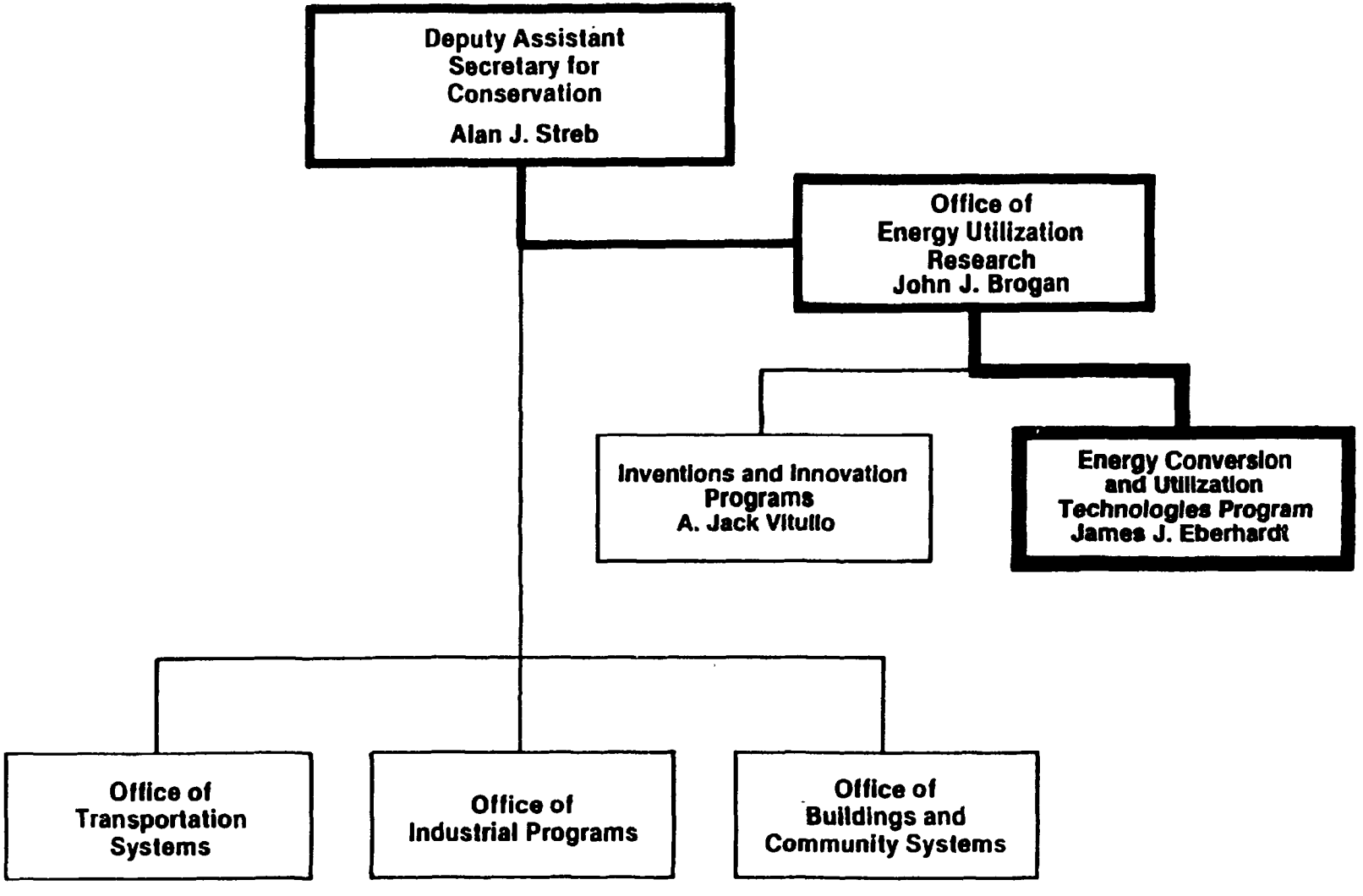
Elements of the Department Organization



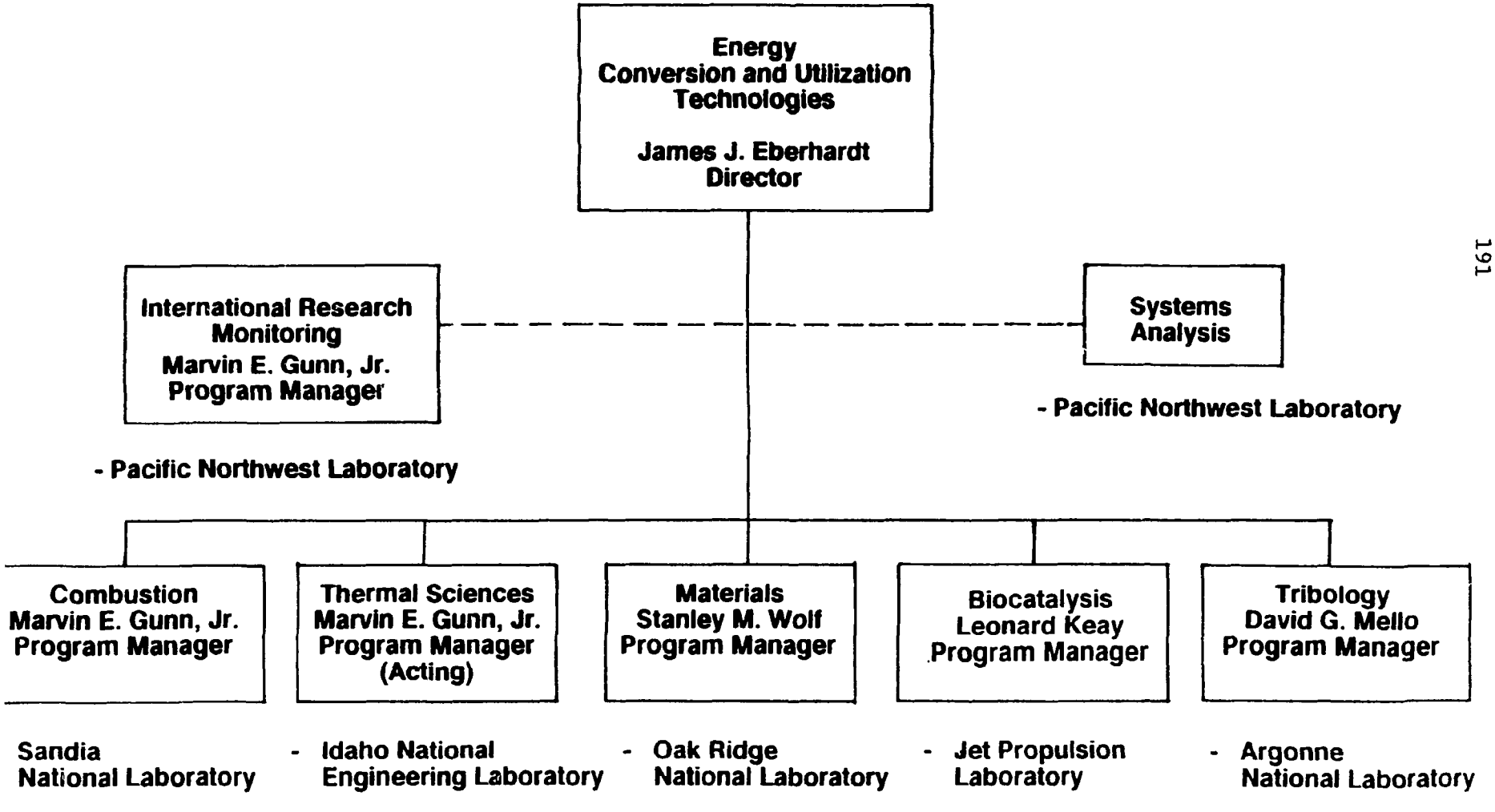
Energy Conservation and Renewable Energy Organization



Energy Conservation Organization



ORGANIZATIONAL AND MANAGEMENT STRUCTURE OF ECUT

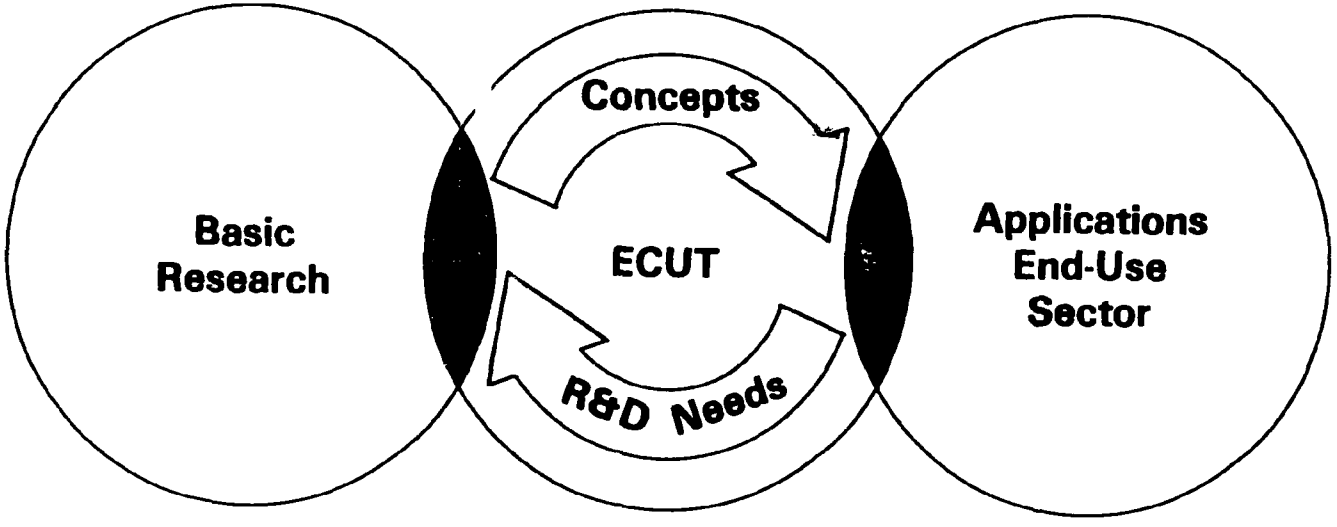


ECUT MISSION

- Monitor and evaluate U.S. (especially that of the DOE Office of Basic Energy Sciences) and International Basic Scientific Research and exploit for energy conservation
- Expand the technology base (i.e., understanding of techniques, processes and materials) generic to energy conservation
- Establish concept feasibility for potentially revolutionary conservation technologies
- Effect technology transfer to DOE End-Use Conservation programs and/or to private industry

ECUT ROLE IN ENERGY CONSERVATION R&D

Spanning the Gap Between Basic Science and Energy Conservation Systems Engineering



Scientific Research Discipline

Inter[illegible]ciplinary [illegible]esearch

Systems Engineering

ECUT ROLE

A Dual Role

- Spanning the Gap Between Basic Science and Engineering for End-Use Applications

- Actively Assessing New or Innovative Concepts for Energy Conservation Applications

ECUT BIOCATALYSIS PROGRAM

ECUT BIOCATALYSIS PROGRAM

Budget History

FISCAL YEAR	TOTAL PROJECT FUNDING ($000)
1980	0
1981	500
1982	800
1983	525
1984	800
1985	2000
1986	2550
1987	3570
1988	3610
1989	4000

ECUT BIOCATALYSIS PROGRAM

Goals

- Develop the technology base to enable private industry to use genetically engineered microorganisms or enzymes (biocatalysts) for the economically competitive production of chemicals.
- Provide a general (not product specific) scale-up and process design data base.
- Develop experimentally verifiable, predictive models for biochemical/chemical catalysis.

ECUT BIOCATALYSIS PROGR[redacted]

POLICY FOUNDATIONS

- Impacts of Applied Genetics: Microorganisms, Plants and Animals (Office of Technology Assessment, April 1981).

- Commercial Biotechnology: An International Analysis (Office of Technology Assessment, January 1984).

- Report of the Research Briefing Panel on Chemical and Process Engineering for Biotechnology (National Academy of Sciences, 1984).

- "Science and Technology Policy: The Next Four Year," George A. Keyworth II, Technology Review, February/March 1985.

ECUT BIOCATALYSIS PROGRAM

POLICY FOUNDATIONS

- "IN ORDER TO MAKE TECHNOLOGY A CONTINUING COMPETITIVE ADVANTAGE FOR THE UNITED STATES, WE NEED TO ...

  (1) CREATE A SOLID FOUNDATION OF SCIENCE AND TECHNOLOGY THAT IS RELEVANT TO COMMERCIAL USES;

  (2) APPLY ADVANCES IN KNOWLEDGE TO COMMERCIAL PRODUCTS AND PROCESSES;"[1]

- "BIOTECHNOLOGY IS CLEARLY A KEY EMERGING TECHNOLOGY WITH LONG-TERM INDUSTRIAL AND ECONOMIC COMPETITIVENESS IMPLICATIONS" FOR THE PETROCHEMICAL INDUSTRY.[2]

- "A KEY GOVERNMENT ROLE IS TO SUPPORT GENERIC APPLIED RESEARCH ON BIOPROCESSES THAT ARE CURRENTLY CONSIDERED UNECONOMICAL AND THUS DO NOT RECEIVE ADEQUATE RESEARCH DEVELOPMENT INVESTMENT BY PRIVATE INDUSTRY."[3]

---

[1] Report of the President's Commission on Industrial Competitiveness, "Global Competition: The New Reality" January 1985.

[2] U.S. Department of Commerce, "A Competitive Assessment of the U.S. Petrochemical Industry," August, 1982.

[3] National Research Council, "Bioprocessing for the Energy-Efficient Production of Chemicals," April, 1986.

ECUT BIOCATALYSIS PROGRAM

Critical Biocatalysis Technical Issues (*)

- Slow Rates
- Lack of Scale-up Technology
- Lack of Design Tools
- High Water Requirements
- Co-Product Processes

(*From: Cooney, C.L., "Chemical and Fuel Production by Fermentation, "Basic Biology of New Developments in Biotechnology, 1983)

ECUT BIOCATALYSIS PROGRAM

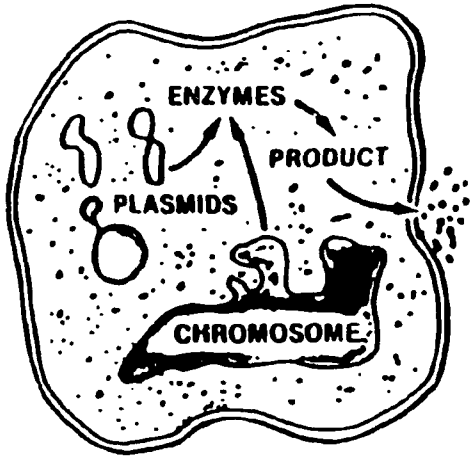
Quantification of Technical Objectives

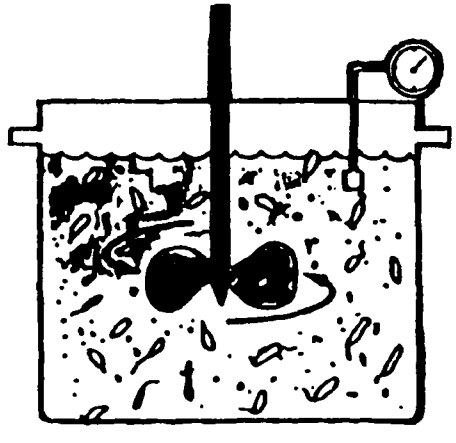
	STATE OF THE ART	PROJECT OBJECTIVE
o BIOREACTOR PRODUCTIVITY (g/l/h)	1 - 10	100 - 500
o CELL DENSITY (number/cc)	10^8	10^{12}
o SEPARATION ENERGETICS (Btu/lb)	10,000	2000 - 3000
o YIELDS (Product Output/Feedstock Input)	> 95%	> 95%

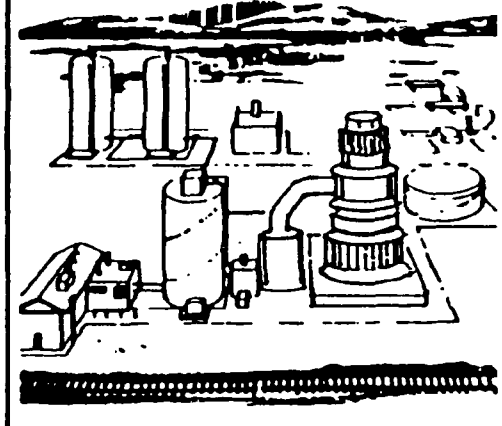
ECUT BIOCATALYSIS PROGRAM

SCALES OF ACTION

CELL 1 μm	REACTOR 1 meter	PROCESS 1 acre
BIOCHEMISTRY AND MOLECULAR BIOLOGY	BIOCHEMICAL ENGINEERING	PROCESS ENGINEERING

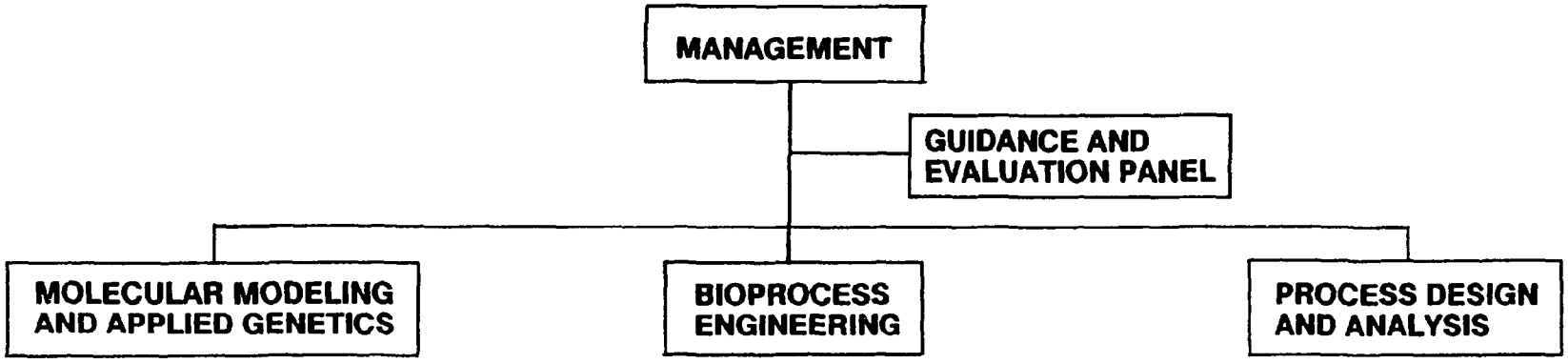






ECUT BIOCATALYSIS PROGRAM

FY 1989 Organization



BIOCATALYSIS BY DESIGN
(W. Goddard-Caltech)

CHROMOSOMAL AMPLIFICATION OF FOREIGN DNA (G. Bertani-JPL)

ENHANCEMENT OF MICROBIAL METABOLISM BY GENE CLONING AND EXPRESSION (J. Bailey-Caltech)

PROTEIN ENGINEERING FOR NONAQUEOUS SOLVENTS
(F. Arnold-Caltech)

OVERPRODUCTION OF POLYPHENOL OXIDASE (W. Dashek-Atlanta U)

BIOLOGICAL SEPARATION OF PHOSPHATE FROM ORE
(R. Rogers-INEL)

IMMOBILIZED ENZYME REACTIONS IN ORGANIC SOLVENTS (A. Klibanov-MIT)

MULTIPHASE FLUIDIZED BED BIOREACTOR
(B. Allen-Battelle)

IMMOBILIZATION CELL SYSTEM FOR CONTINUOUS EFFICIENT BIOCATALYZED BIOPROCESSING
(C. Scott-ORNL)

SEPARATION BY REVERSIBLE CHEM ASSOCIATION (C. King-LBL)

MULTIMEMBRANE BIOREACTOR FOR CHEMICAL PRODUCTION
(M. Shuler-Cornell)

INTEGRATED BIOPROCESSES FOR 2, 3 - BUTANEDIOL (G. Tsao-Purdue)

TWO-PHASE EXTRACTION OF BUTANOL (W. Weigand-U of MD)

PROCESS SYNTHESIS INTEGRATION AND ANALYSIS (J. Ingham-JPL)

ASSESSMENT: BIOREACTORS AND CHEMICAL PRODUCTION
(Busche-En-GENE-er)

ECUT BIOCATALYSIS PROGRAM

Selected Technical Accomplishments

- C.D. Scott - ORNL

  Two of the three goals of the Bioprocess Engineering work element (productivity and yields vs. productivity, yields and separation energetics) quantitatively achieved (2 patents)

- J.E. Bailey - CALTECH

  Genetically engineered E. coli to express a functional hemoglobin gene (2 patents) for more efficient aerobic fermentation.

- A.M. Klibanov - MIT

  Developed enzymatic catalysis in organic solvents and for gas phase reactions (2 patents/2 licenses).

ECUT BIOCATALYSIS PROGRAM

Progress Summary

FY 1980 - 1988

PUBLICATIONS

o	Refereed Journals	51
o	Proceedings/Conferences	24
o	Reports	16
		91

PATENTS 6

LICENSES 4

SOFTWARE DEVELOPED 6

SYMPOSIA 2

SUMMARY

- ECUT is strengthening the link between basic researchers and end-users (design engineers of energy conversion and utilization systems).
  - This is a pioneering effort, a New Role.
- ECUT is filling a critical gap in the technology transfer process.
- ECUT develops analytical tools, data and innovative concepts for end-users.
- ECUT is both guided by and anticipates the needs of the end-user.

ORNL/TM-11054

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