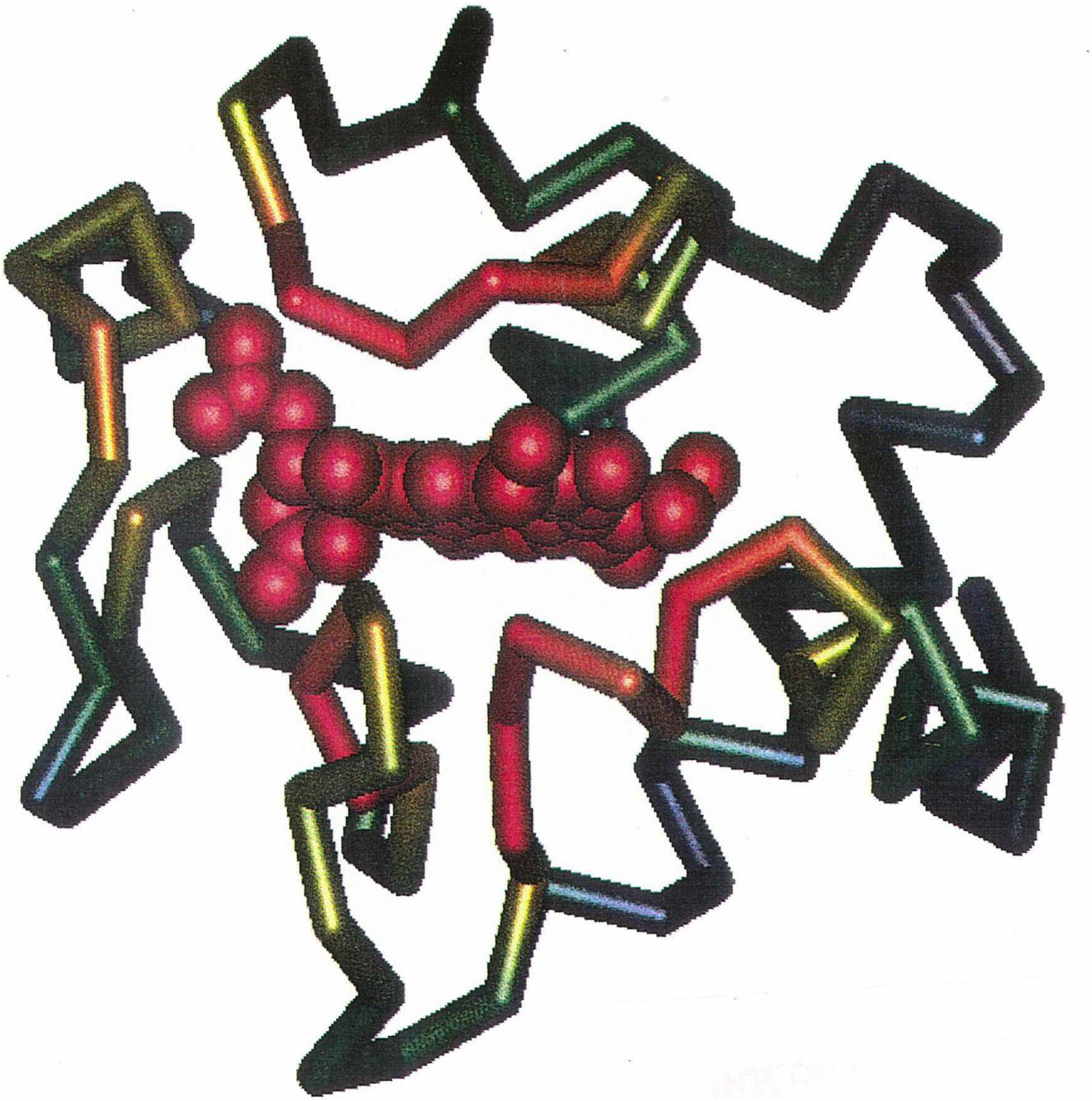
BIOLOGICAL AND CHEMICAL TECHNOLOGIES RESEARCH

FY 1995 ANNUAL SUMMARY REPORT



Office of Industrial Technologies
Office of Energy Efficiency and Renewable Energy

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The Biological and Chemical Technologies Research Program is managed under the direction of Department of Energy Headquarters Program Management with technical support from the National Renewable Energy Laboratory through an agreement (Field Work Proposal EEWIP54) between the National Renewable Energy Laboratory and the Department of Energy.

The Biological and Chemical Technologies Research Program focuses on resolving the major technical barriers that impede the potential use of biologically facilitated continuous chemical production processes and the barriers that restrict the design of new chemical catalysts.

An electronic coupling map is shown for the protein cytochrome b_5. The colors represent the interaction strength between the iron-porphyrin ring (central unit shown as red balls) and each amino acid alpha-carbon in the protein. Strongly coupled amino acids are red and weakly coupled ones are blue. The coupling strengths determine the rate of electron transfer between the iron-porphyrin moiety and other species that might dock with a specific amino acid and transfer electrons to or from the cytochrome b_5 iron-porphyrin in an electron transfer reaction. These simple oxidation/reduction reactions are of vital importance in catalysis, metabolism, and energy transduction. The PATHWAYS software that was used to create this map was developed under DOE Advanced Industrial Concepts funding at the University of Pittsburgh, University of California, San Diego, and the Jet Propulsion Laboratory (Caltech) in the groups of Beratan and Onuchic. This software is in use by hundreds of research groups throughout the world to design and tailor biocatalytic systems. The current release of the software is available electronically from jregan@ucsd.edu.

Abstract

The annual summary report presents the fiscal year (FY) 1995 research activities and accomplishments for the United States Department of Energy (DOE) Biological and Chemical Technologies Research (BCTR) Program. This BCTR program resides within the Office of Industrial Technologies (OIT) of the Office of Energy Efficiency and Renewable Energy (EE). The annual summary report for 1995 (ASR 95) contains the following: program description (including BCTR program mission statement, historical background, relevance, goals and objectives); program structure and organization, selected technical and programmatic highlights for 1995; detailed descriptions of individual projects; a listing of program output, including a bibliography of published work; patents; and awards arising from work supported by the BCTR.

Foreword

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Biological Chemical Research Technology Program
Annual Summary Report
FY 1995

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1.0 Executive Summary

The Biological Chemical Technologies Research (BCTR) program concluded another very successful year of research in FY 1995. With the onset of FY 1996, it now faces new and more severe challenges brought on by deep budget cuts and the de-emphasis of basic-applied research within the Office.

In FY 1995, BCTR program activities received strong industrial support, contributed to the economy through job formation, and achieved scientific and professional success. Corporate support is reflected in EPAct-related cost-sharing of about $2.6M and an additional approximate $3.3M in indirect cost sharing. Overall, industry provided 1.2 dollars for every federal R&D dollar invested through the program. Research activities attracted 11 new partners, bringing the total number of industry partners to 43; which is a net increase of 38% from the FY 1994 total. Economically, BCTR-supported research contributed to the formation of one new small company, Symyx, which has exclusive rights to Lawrence Berkeley National Laboratory (LBNL)-developed technologies. Additionally, BCTR supported employment for over 62 FTEs and leveraged the equivalent of 11.6 FTEs from industry. Scientifically, program participants published 50 peer-reviewed articles and documents, were awarded two patents, and filed two new patents. Professionally, two BCTR investigators were elected to the National Academy of Sciences.

The mission of the DOE BCTR Program is to provide evidence of the technical and economic feasibility of advanced chemical and biological concepts that can improve the energy utilization, operation efficiency, and environmental soundness of U.S. industry process operations. The program combines science with technology to develop novel and revolutionary process concepts; to improve conventional process approaches; and, if the economics and market needs warrant, to facilitate the introduction of these technological advances in the chemicals, petroleum refining, other renewable, and emerging biotechnology industries.

Management of the Program resides with the BCTR program manager in the Office of Industrial Technologies (OIT). Program industrial focus is provided primarily through an industrial review panel that gives guidance relevant to the needs of industry. In addition, technical field assistance is provided in all areas of R&D by the National Renewable Energy Laboratory (NREL). In the area of Chemical Computer Aided Catalyst Design, additional technical field assistance is provided by Los Alamos National Laboratory (LANL). Finally, program support in planning and systems analysis is provided by Pacific Northwest Laboratories (PNL).

The FY 1996 BCTR Program planning budget is $2,100K. The funding profile according to institution has the following distribution: $1,575 K (75%) to the national laboratories (including Sandia National Laboratory (SNL), LANL, PNL, LBL, NREL, Oak Ridge National Laboratory (ORNL), and Ames Laboratory) and $525K (25%) to universities (including, California Institute of Technology (Caltech), Michigan Institute of Technology (MIT), Pittsburgh University, and Howard University).

OIT's Industries of the Future represents a new approach to aligning federal investments in technology research, development, and deployment with the needs and expectations of technology users in the private sector. The emphasis of the BCTR program has always been towards the Chemical and Allied Products (SIC 28) and Petroleum Refining (SIC 29) industries, which are two of the seven industries targeted in OIT's visioning process. The BCTR program fully supports the visioning process and has identified an R&D agenda responsive to their needs. In this regard, OIT's entrance into this R&D perspective is an excellent complement to the way BCTR does business.

2.0 Industries of the Future

OIT's Industries of the Future represents a new approach to aligning federal investments in technology research, development, and deployment with the needs and expectations of technology users in the private sector. It aims to shrink the time required to move innovative industrial technologies from concept to marketplace. A central theme of the Industries of the Future process is helping manufacturers reduce costs and improve efficiency.

Based largely on energy consumption and waste generation, OIT has identified seven industries for its visioning process: Chemicals, Petroleum, Glass, Metal Casting, Steel, Aluminum, and Pulp and Paper. The Biological and Chemical Technologies Research Program pursues research that addresses two of the seven industry visions: Chemicals and Petroleum. Following is an overview of the vision and needs of these two industries.

2.1 Chemicals Industry

The chemical industry's vision of the future is one that provides the building blocks for a modern society and leads the world in the manufacturing and sale of chemicals and related products to meet the need of their customers. The relevance of the chemical industry in the U.S. economy and world marketplace and of issues that confront the industry's ability to maintain its competitiveness is summarized below:

- The Chemical Industry is the top U.S. exporter and one of the nation's most internationally competitive industries. Chemical goals account for one out of every ten dollars of U.S. exports.

- Chemical exports of $52 billion in 1994 yielded a trade surplus of $18.4 billion. But international competition gets tougher every year, and the U.S. share of world exports has declined.

- Technological leadership – new products and new processes – is the key to continued U.S. competitiveness in domestic and world markets.

- Investments in R&D with long term payoffs are made vulnerable by competitive forces, profit squeezes, personnel downsizing, and rising costs.

- Continued U.S. Chemical Industry technological leadership and global competitiveness require continued high levels of R&D investment and the most effective use of R&D resources.

The chemical industry has identified five facets to meet its vision:

Sustainability: Sustainable development means meeting the needs of the present without compromising the ability of future generations to meet their own needs. In this regard, the industry envisions a sustainable future state where: 1) Energy will be more expensive in real terms; 2) Renewable energy sources will play a more significant role; 3) Bioprocessing will represent a major area of new process improvement; 4) Water recycling will be vital; 5) Reduced risk, including reduced emissions, will continue to be a general goal; 6) Prevention of waste will be a major focus of environmental activity; 7) industry will better demonstrate the absence of both acute hazards and long-range, cross-generational effects, or an acceptable means of managing such risks; and 8) The public will accept (or demand) risk analysis, risk management, and application of Responsible Care principles.

Manufacturing and Operations: The industry, by capitalizing on technological advances, employment of a motivated, diverse and technically proficient work force, and working in partnership with government and academia will retain and hopefully expand its position of global leadership by the year 2020.

The industry will meet the challenge of global competition, fulfill its stewardship of the environment and enhance the safety of its operations through: 1) Utilizing to maximum effectiveness technology and automation in manufacturing and operations processes; 2) Expanding current efforts to enhance its manufacturing facilities, practices and policies; 3) Strengthening its focus on customer satisfaction; and 4) Working with government to improve the statutory and regulatory environment in which industry operates.

Supply Chain Management: The chemical industry provides the building block materials for all U.S. manufacturing. Managing the supply chain involves all of the planning and operational decision making involved in the movement, handling, and storage of all materials purchased, processed, or distributed by chemical manufacturers. As such, it involves not only the manufacturers, but also all of their trading partners. Effective management of the supply chain has a direct bearing on the global competitiveness of the U.S. chemical industry. There are three critical elements in supply chain management: 1) responsiveness to rapidly changing customer requirements; 2) cost competitiveness; and 3) protecting the environment and the personnel involved in handling, storing, and transporting chemical materials.

Information Management: The chemical industry has a future vision of a highly efficient and automated manufacturing operation and supply chain. To achieve this vision, data and information must flow in a seamless fashion along the whole supply chain from raw materials suppliers through all the steps of manufacturing operations to the customer. Individuals on a need-to-know basis will have instantaneous easy access to data, information, and decision support tools that will help them do their jobs regardless of the graphical location. There are two major elements to this activity: 1) Infrastructure, which includes database connectivity using interfaces and gateways for a common database; and 2) Business/Enterprise Management, which addresses the need for timely access to information on performance of individual product lines, business units, and plants, such as production costs, inventory levels, profit-and-loss statements, cost of sales, and business volume information.

New Chemical Sciences and Engineering Technology: Chemical science and engineering technology drives new product and process development. Recently, biotechnology has begun to provide alternatives to traditional chemistry, and the application of physical sciences in chemical processes and materials production is advancing the chemical and materials sciences. In a similar manner, measurement and computational technologies have emerged as critical enabling technologies, significantly influencing the conduct and capability of chemical sciences and engineering technology. The intersection of disciplines of chemistry, engineering, physics, biology, computational technologies, and measurement are creating a new technology paradigm resulting in increasing complexity and skills required to develop new products and processes. Noteworthy activities in this area include 1) Chemical synthesis, which includes development of synthesis tools based on combinatorial techniques, computational techniques to guide synthesis by theory and molecular modeling, and molecule-specific measurements; as well as chemistries using "natural" processes such as photochemistry and biomimetic synthesis; 2) Biotechnology, which includes development of improved biocatalysts, (i.e., by increasing yield, selectivity and rate of the bioprocesses, to practice alternate biochemical routes to higher-performance products) and enhanced fundamental biochemical engineering capability and applied engineering skills; and 3) Computational technologies, which address the development of computational technologies tailored to the needs of the industry and access to low-cost, high-performance computing systems and standardized software and database architectures that do not require custom interfaces.

2.2 Petroleum Refining

OIT Refinery of the Future initiative has obtained from the refining industry a list of the core technology areas in which they encourage DOE to become involved. These areas have definite impacts on process efficiency, capital investment strategies, and improving pollution prevention. Improved technologies within the following seven areas will lead to reduced energy usage, better strategies for capital investments that include energy efficient operation, and better strategies to prevent pollution.

The seven areas include catalysis, hydrogen generation, advanced process/controls, environmental technologies, plant reliability, computational fluid dynamics for reacting multiphase flows, and separations.

Catalysis:

> The precompetitive areas in catalyst development in which it would be appropriate for DOE to become involved include developing tools for the screening or design of catalysts; computational modeling of catalyst activities, structure, and lifetimes; and basic investigations of new catalyst types, applications, or functions.

Hydrogen generation:

> About 60% of the merchant market for hydrogen is in the refining industry. Current technology for generating hydrogen is centered on steam reforming of methane gas. Advanced processes for improving steam reforming, new approaches to generating hydrogen, and improved efficiency in hydrogen use and recovery are areas that would be appropriate for OIT/DOE technology to address. This would require some coordination with the hydrogen program within EE. That program has not involved the refining industry and synergism between OIT and Office of Utility Technologies (OUT) in this

area could be useful in addressing the issues of hydrogen generation for the refining industry.

Advanced process controls/instrumentation:

All industrial processes benefit from improved control and instrumentation. Because this industry processes many billions of kilograms of product daily, even a 0.1% improvement in process can significantly reduce energy usage or negative environmental impacts.

Environmental technologies:

The industrial Petroleum Environmental Research Forum (PERF) organization has recognized the capabilities DOE possesses in the area of environmental research. R&D that addresses the long-term future of pollution prevention in this industry is appropriate for OIT involvement.

Plant reliability:

This technology crosscuts all the OIT visions; and DOE has many technology-transfer opportunities based on its extensive work with nuclear hardware and facilities. Refineries have different risks than nuclear plants, but the tools used in assessing the reliability of nuclear facilities could be adapted to the refining industry.

Computational Fluid Dynamics for reacting multiphase flows:

Modeling the complex interactions in the workhorse unit of refiners, Fluidized Catalytic Crackers (FCC), is a multidisciplinary problem that is analogous to many challenges DOE has already addressed. The application of that technology to refineries would be an appropriate activity for transferring that DOE-developed technology into this United States industry.

Separations:

Although this is a very broad area, some separation problems are unique to refinery operations, such as separating multiple component gas or volatile streams. Advanced technologies to address these areas could lead to process efficiencies and energy savings.

3.0 Biological and Chemical Technologies Research Program

The emphasis of the BCTR program has always been towards the Chemical and Allied Products (SIC 28) and Petroleum Refining (SIC 29) industries and has included the use of renewables as feedstock materials in the chemicals industry. The program employs an industrial review panel to provide guidance relevant to the needs of industry. The Chemical Industry and Refinery of the Future initiatives have identified short- mid- and long-term R&D needs germane to these two industries. The BCTR program fully supports the visioning process, and has identified an R&D agenda responsive to their needs. Section 3.1 meshes the BCTR R&D agenda with the applicable technology needs identified in section 2.0 by each of these industries.

3.1 Relationship to OIT's Industries of the Future, EPAct, and the Department of Energy

3.1.1 OIT's Industries of the Future

Chemical Industry of the Future
New Chemical Sciences and Engineering Technology

Chemical Industry Needs	BCTR Program Activities
Chemical Synthesis - Using computational techniques to guide synthesis by theory and molecular modeling	Chemical and biological catalysts including biomimetic catalysts
Biotechnology - Improved biocatalysts for alternate biochemical routes and enhanced biochemical engineering capabilities/applied engineering skills	Biocatalyst development for aqueous and nonaqueous systems, advanced bioreactors and bioprocessing systems for high yields and productivities.
Materials Technology - Prediction of materials properties from the molecular to the macroscopic level including structure property relationships and computational techniques	Computer-aided catalyst design for both chemical and biological materials including biomimetic catalysts
Process Science and Engineering - Reactor design principles/ tools for new, emerging process industries and design principles/tools focused on source waste reduction	Advanced bioreactor design for aqueous and non-aqueous high yield/productivity systems. Process simulation models that identify waste streams from unit operations.
Computational Technologies - Development of computational fluid dynamics tools, user interfaces for molecular modeling, design, kinetics, and thermodynamic properties. Computer-aided catalyst design for both	Multiphase bioreactor predictive models, developed codes for commercial software and user interfaces for design of catalysts (DeCAL, CEMBOS, Cell Multipole Method, etc.)

Sustainability

Chemical Industry Needs	BCTR Program Activities
Dealing with more expensive energy	Analyzed energy requirements of chemicals industry and focuses R&D on biggest energy users
Increased use of renewable energy	Major program element has been integrating renewable feedstocks into chemicals industry infrastructure
Increased use of bioprocessing	Bioprocessing R&D in aqueous and organic phase bioprocessing are major program efforts
Reduced risk (emissions) a major goal	Program efforts in designing better automobile catalysts
Prevention of waste	Improving efficiency for catalytic-based processes to reduce and eliminate unwanted byproducts

Supply Chain Management

Chemical Industry Needs	BCTR Program Activities
Cost competitiveness	Assists in identifying cost-effective technologies in advanced applications

Petroleum Refinery of the Future
Catalysis

Refining Industry Needs	BCTR Program Activities
Tools to design improved catalysts	Computer-aided design of catalysts is a major program area in zeolite, metal-oxide
Modeling of catalysts structure	Computer-aided design of catalysts is a major program area

Hydrogen Generation

Refining Industry Needs	BCTR Program Activities
New processes for generating H_2	Renewables (solar-assisted) technology studied as well as methane coupling which includes hydrogen generation

Computational Fluid Dynamics for reacting multiphase flows

Refining Industry Needs	BCTR Program Activities
Modeling the complex interactions in Fluidized Catalytic Crackers (FCC)	Models of zeolite catalyst systems for kinetics, diffusion, and fluid dynamics. Multiphase models for complex bioreactors

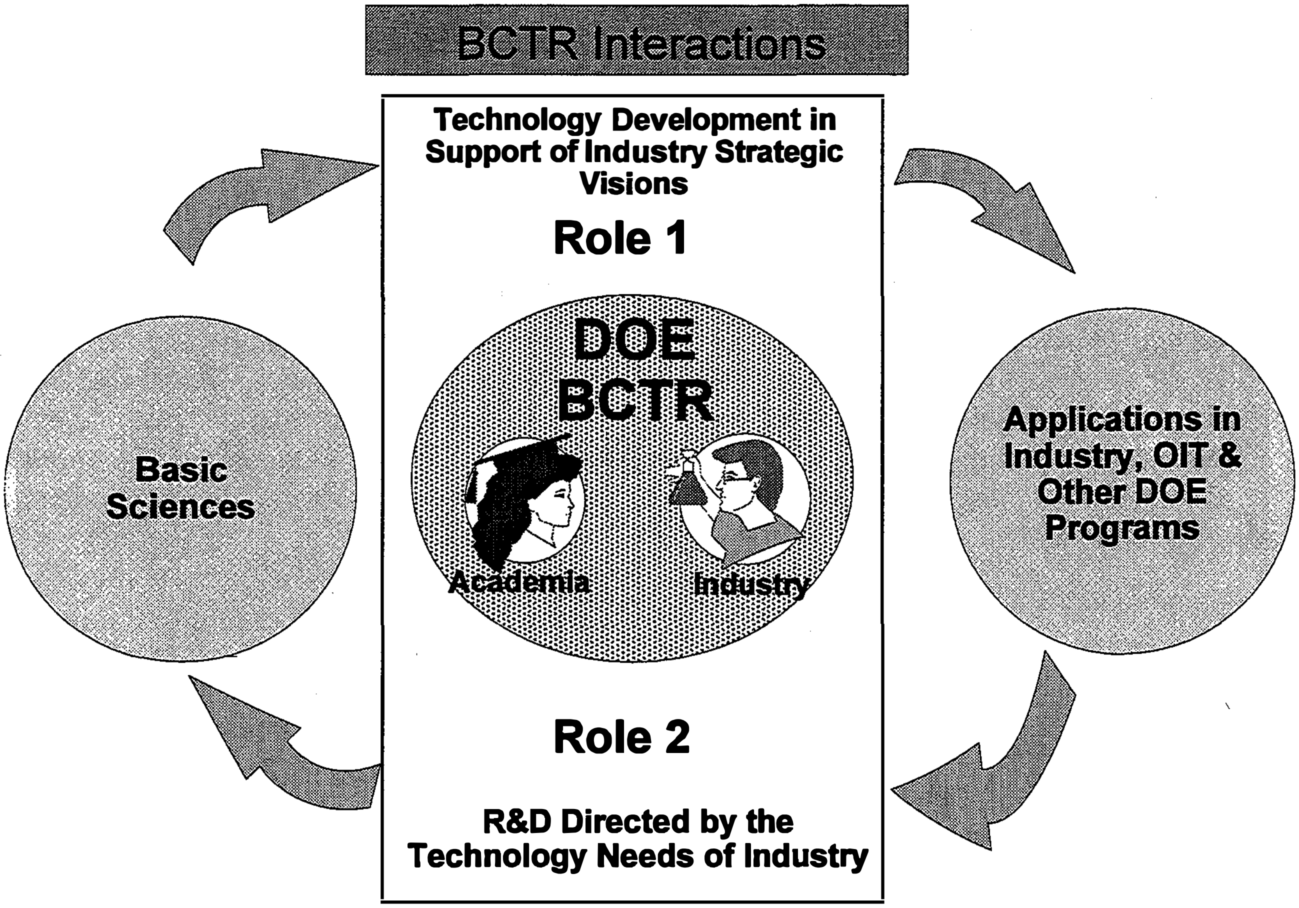
3.1.2 Relationship to EPAct and the Department of Energy

The research efforts of the BCTR Program are centered in six areas: **CD** - Catalyst Design; **PD** - Process Design; **PDV** - Process Development; **BDS** - Biocatalyst Design; **BDV** - Biocatalyst Development; **BPS** - Bioprocess Systems. These R&D activities support EPAct Sections 2103 (Pulp and Paper) via BDV and BPS; 2107 (Improving Efficiency in Energy-Intensive Industries) via all six areas; 2108 (Energy Efficient Environmental Program) via CD, PD, PDV; and 2202 (National Advanced Manufacturing Technologies Initiative), via all six areas. Collectively, these research efforts respond to the Administration's focus on **Global Climate Change** and **Pollution Prevention** through the development of high productivity, low-waste-generation chemical and biological technologies. Of particular interest is the programmatic thrust within Feedstock Process Integration (FPI) to develop chemical and biological approaches that use carbon dioxide as feedstock to manufacture chemicals and materials.

The Department of Energy The Department has five core business areas: Industrial Competitiveness, Energy Resources, National Security, Science and Technology, and Environmental Quality. Of these, the Program directly supports two, Industrial Competitiveness and Science and Technology, through its R&D efforts that address feedstock and energy flexibility and advanced energy and process technology development and deployment. In addition, the Program is responsive to the Core Values of the Department, particularly in regard to being **Customer-Oriented** (through collaborative research with over 30 U.S. companies representing the petroleum refining, chemical and allied products, and renewables industries); in being **Committed to Excellence** (a number of BCTR researches have received meritorious awards for their scientific contributions); and to being a Team and Advocating Teamwork (the Program is a collaborative R&D effort involving over 30 U.S. industries, 7 national laboratories, over 10 universities, various government agencies, and a number of DOE offices).

3.2 BCTR Program Overview

The mission of the DOE BCTR Program is **to provide evidence of the technical and economic feasibility of advanced chemical and biological concepts that can improve the energy utilization, operation efficiency, and environmental soundness of U.S. industry process operations.** The program combines **science with technology** to develop novel and revolutionary process concepts; to improve conventional process approaches; and, if the economics and market needs warrant, to facilitate the **introduction** of these technological advances in the chemicals, petroleum refining, other renewable, and emerging biotechnology industries. Figure 3.1 describes the role of the program in bridging science and technology to industry and other DOE programs to develop useful products and processes.



Two-fold role of BCTR Research Activities

Figure 3.1

3.2.1 Program Goal and Objectives

The overall goal is **to stimulate and nurture the development, testing, and commercial deployment of advanced chemical and biological technologies** that offer the potential to reduce energy use and mitigate environmental impact within the chemicals and petroleum refining industries.

The program objectives, as aligned with the chemical and petroleum industry visions, involved in reaching this goal area are as follows:

1. Develop the technology base required to overcome limitations in the use of biotechnology in the chemical and petroleum refining industries.

2. Pursue development of enabling technologies that offer broad application and use in support of the development of advanced chemical and biological processes.

3. Communicate the results of the program research efforts to OIT end-use programs and the industrial sector for continued development toward commercial deployment.

3.2.2 Strategy

Meeting program goals and objectives involves two strategies as depicted in Exhibit 3.2 and is based on the energy-supply/demand issues of process operations. Strategy 1 - **Boost Domestic Energy Supply** by replacing petroleum feedstocks with indigenous resources and waste material from the industrial, commercial, utility, and residential sectors. Strategy 2 - **Cut Energy Demand** by developing technologies that reduce energy demands for petroleum refining and chemicals and materials processing.

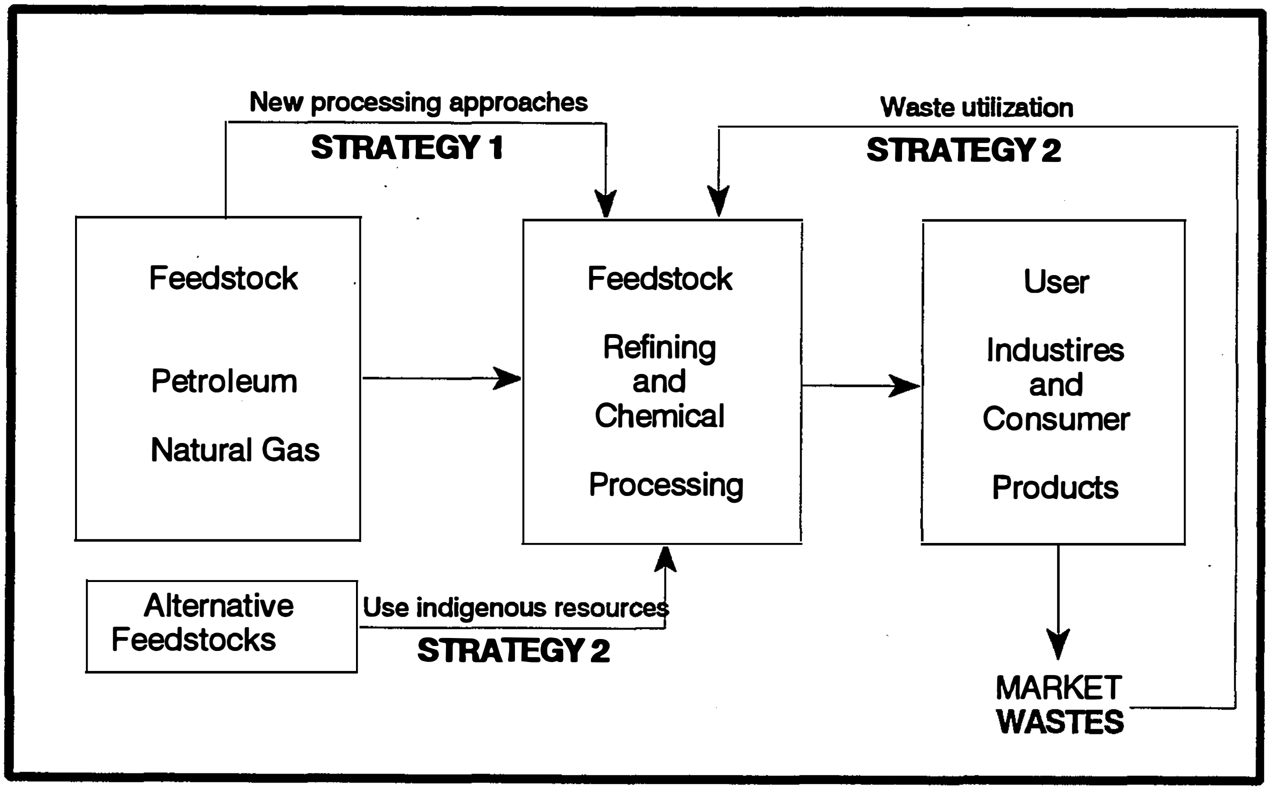


Figure 3.2 BCTR STRATEGIC APPROACH

3.2.3 Metrics

The petroleum refinery industry processes about 22 quads of petroleum-based feedstocks for the transportation industry and about 3.0 quads of petroleum-based feedstocks for the chemicals industry, accounting for about 26% of all energy forms in the U.S. Through the strategy outlined above, the program has identified an accumulated energy savings or petroleum displacement potential of about 2.1 quads by the year 2020. This target assumes a fully funded R&D agenda and is gauged by the primary energy use that could be impacted by successfully deploying innovations developed within the program. Of this total energy savings, 1.8 quads have been identified for the chemicals industry and 0.3 quads in the petroleum industry.

Timely technology development, transfer, and industrial implementation are necessary to achieve the projected 2.1 quads energy savings impact. This energy savings is envisioned to occur through improvements in conventional processing (1.1 quad reduction in energy demand via Strategy 2) and introducing alternative feedstocks, including recycled waste material (1 quad boost in energy supply via Strategy 1). Combined, these two strategies provide a rational approach for optimum resource use combined with energy efficient, cost effective, and environmentally sound process technologies. In this regard, three cornerstone reports by Pacific Northwest Laboratories and

Battelle Memorial Institute identify a potential savings of about 1.1 quads in process and feedstock energy by improving catalyst and process efficiencies. The three reports, Brief Characterizations of the Top 50 U.S. Commodity Chemicals, Characterizations of the Top 12 U.S. Commodity Polymers, and The Top 50 Commodity Chemicals: Impact of Catalytic Process Limitations on Energy, Environment, and Economics provide extensive information on the key processes used in the U.S. chemical industry. Opportunities to achieve the remaining 1.0 quad were identified in a study conducted by the Alternative Feedstocks Program.

3.3 BCTR Program Structure

The program is structured to address the industry needs discussed in Section 2.0. This structure emphasizes two core technological approaches, chemical and biological, as outlined below:

Biological and Chemical Technologies

Chemical Approaches	Biological Approaches
Catalyst Design	Biocatalyst Design
Process Design	Biocatalyst Development
Process Development	Bioprocess Development

3.3.1 Chemical Approaches

Catalyst Design - Objective: create tools to aid in the design of chemical catalysts for chemicals and materials production. Goal: Chemical Computer aided catalyst design (CACD) first generation model development by 1996 and commercial-model applications by 2005.

Process Design - Objective: create process model(s) for intelligent chemical processing of feedstock and waste streams. Goal: First generation model by 2000 and commercial model applications by 2003.

Process Development - Objective: exploratory development of advanced chemical/petrochemical process systems to meet needs identified by these industries. Goal: Complete systems/process analysis of at least one advanced process per year.

3.3.2 Biological Approaches

Biocatalyst Design - Objective: create tools to aid in the design of biochemical catalysts for chemicals and materials production. Goal: Third generation Biological CACD tools/models developed by 1998 and additional commercial model applications by 2000

Biocatalyst Development - Objective: Identify and characterize at least one new biocatalyst per year. Goal: Adapt 5 biocatalysts for commercial application by 2000.

Bioprocess Development - Objectives: Exploratory development of advanced biochemical process systems to meet needs identified by these industries. Goal: Complete systems/process analysis of at least one advanced process per year.

4.0 Fiscal Year 1995 Achievements

In FY 1995, BCTR program activities received strong industrial support, contributed to the economy through job formation, and achieved scientific and professional success. Corporate support is reflected in EPAct-related cost-sharing of about $2.6M. In addition, industry supplied approximately $3.3M in indirect cost sharing. Overall, industry provided 1.2 dollars for every federal R&D dollar invested through the program. Research activities attracted 11 new partners, bringing the total number of industry partners to 43, which is a net increase of 38% from the FY 1994 total. Economically, BCTR-supported research contributed to the formation of one new small company, Symyx, which has exclusive rights to LBNL-developed technologies. Additionally, BCTR supported employment for over 62 FTEs and leveraged the equivalent of 11.6 FTEs from industry. Scientifically, program participants published 50 peer-reviewed articles and documents, were awarded two patents, and filed two new patents. Professionally, two BCTR investigators were elected to the National Academy of Sciences.

Listed below are some of the notable technical and programmatic achievements of the past year by the various program participants.

4.0.1 PACIFIC NORTHWEST LABORATORY AND BATTELLE MEMORIAL INSTITUTE, COLUMBUS

An important study was published entitled "Characterization of the Top 12 Commodity Polymers." This report, together with the previous studies completed last year, provide important baseline data that BCTR and other OIT programs can use to establish research priorities in support of the chemical industry and the petroleum refining industry.

4.0.2 OAK RIDGE NATIONAL LABORATORY(ORNL)

A major technical discovery was made by Oak Ridge National Laboratory team in the area of carbon dioxide fixation. The ORNL team discovered a new single-light photosynthetic pathway for the conversion of CO_2 into fixed carbon in a genetically engineered algal species, *Chlamydomonas rheinhardtii*. This discovery suggests that greater efficiencies in fixing carbon dioxide may be available which would enhance the use of renewables-based processing for chemicals and fuels.

4.0.3 LAWRENCE BERKELEY NATIONAL LABORATORY (LBNL)

Being able to probe the electronic structures of important catalyst types such as zeolites (used in petroleum refining) would allow for better predictions of catalyst performance. Work at LBNL has resulted in obtaining firm answers to electronic and structural properties of Cu-ZSM-5. This will allow use of analytical tools as electron paramagnetic resonant spectroscopy and estimates of thermodynamic data. I addition, estimating diffusion properties within such catalysts is important in characterizing these types of catalysts. LBNL has developed computational tools that can replace cpu-intensive (and hence costly) molecular dynamics calculations that are faster and provide comparable results.

Isolation of carboxylic acids from fermentation broths is one of the costlier unit operations (both energetically and economically) in bioprocessing. LBNL has conducted R&D on improving separations within some of the common fermentation processes. They have found a preferential selectivity for succinic acid over lactic acid. Additionally, they found that lactic and acetic acids can be separated by adsorbing both with Dowex MWA-1 and then vaporizing the acetic acid.

A patent, "Carboxylic Acid Sorption Regeneration Process," was awarded to LBNL in May, 1995.

4.0.4 LOS ALAMOS NATIONAL LABORATORY (LANL)

A major finding in petroleum cracking catalysts was made by LANL during this past year. They have found that hydrocarbon species are not true carbo cations, as has been thought, but have altered chemical properties intermediate between covalent and ionic forms. This has significant implications for the types and use of catalysts within petroleum refining cracking operations. In addition, LANL scientists have found that within zeolite catalysts steric effects alone can not account for the structure-directing effects of these catalysts. This implies that refining operations must pay more attention to other factors in improving their processes

4.0.5 IDAHO NATIONAL ENGINEERING LABORATORY (INEL)

Although the recovery of phosphate from ores by a biological system has been shown, how this process works has not been clear. Without such knowledge, it is difficult to target R&D to improve the energetics and economics of the process. During the past year, INEL, in collaboration with its academic colleagues, has discovered the mechanism for the biomediated phosphate separation process. Additionally, INEL has shown that the microencapsulated bioseparation/application process is feasible using 2 mm beads which include the microbe, the phosphate ore, and the organic binder. Both of these accomplishments have been filed as patents.

4.0.6 ALLIEDSIGNAL CORPORATE R&D

The production of industrial materials used in polymer synthesis on a laboratory scale using immobilized enzymes in organic solvents is important in demonstrating the feasibility of this unusual hybrid chemical/biochemical process system. During this reporting period, AlliedSignal researchers have determined the conditions that raise the activity of an enzyme-catalyzed transesterification of a monomer with a chiral compound in dry amyl alcohol by two orders of magnitude. The conversion was increased 67% (from 20% to 87%) and 10 grams of chiral monomer have been produced, which will be sufficient for further testing.

4.0.7 OAK RIDGE NATIONAL LABORATORY

Increased efficiency in lactic acid fermentations has been a goal of industry intending to employ lactic acid in the manufacture of biodegradable polylactide polymers. Research at ORNL was targeted at showing that the continuous and simultaneous fermentation and purification of lactic acid in bi-particle fluidized bed reactors was more efficient than other bioprocess systems. After short term fermentation trials, the ORNL process could demonstrate productivities of greater than

5g/l/hr, which is 16 times that of a free-cell batch reactor. Improved product recovery from the resin gave concentrations of up to 70 g/L in the fermentation broths.

A partnership with Dow Chemical through a Cooperative research and development agreement (CRADA) to demonstrate bioprocess systems in which chemical streams could be reused rather than undergo waste treatment has been underway during the past year. Dow Chemical scientists have developed the genetically engineered organism that will be used in the ORNL bioprocess system. ORNL is currently analyzing the performance of this organism in immobilized bioreactors for dehalogenation of the selected process streams.

ORNL has also published the summary report, "Bioprocessing in Nonaqueous Media: Critical Needs and Opportunities," as the first step in outlining a systematic technology roadmap for the BCTR program in pursuing the industrial interest in non-aqueous bioprocessing for chemicals production.

A patent, "Process for converting cellulosic materials into fuels and chemicals" was awarded to ORNL in September 1994.

4.0.8 CALIFORNIA INSTITUTE OF TECHNOLOGY (Caltech)

Methodologies employed to screen for recombinant organisms that have been altered in their ability to operate under unnatural conditions such as organic solvents has been used very successfully at Caltech. A company, Recombinant BioCatalysts, have contracted with Caltech to employ these technologies to increase the efficiency of their large scale screening efforts. In addition, Eli Lilly has contracted with Caltech to pursue development of unique enzyme systems using the technology developed by Caltech under BCTR support. These kinds of collaborations mark the evolution of this technology from bench scale techniques to real tools which industry is interested in employing.

4.0.9 HOWARD UNIVERSITY

The use of lignin oxidases, a class of enzymes that help decompose wood, as chemical reagents for biopulping operations has not been feasible owing to the technical challenges in the cost-efficient bulk production of these enzymes. Work at Howard University has demonstrated the enhanced mass-culture production of these enzymes in bacterial hosts rather than in the typical slow-growing fungal species. This discovery opens the door for this technology to be considered in biopulping operations by the forest products industry.

4.0.10 AMES LABORATORY

The development of the DeCAL software package at Ames Laboratory has provided industry with means to analyze metal and multimetallic catalyst systems such as automobile catalytic convertors for the means to replace costly precious metal components. The capabilities of DeCAL have been expanded to include hydrogen adsorption for rhodium and platinum catalyst systems. The model continues to be employed by DuPont Chemical in their analyses of oxygen/metal interactions.

Exxon researchers in collaboration with Ames have shown the structures of some copper palladium clusters with diameters of 40-60 A.

4.0.11 SANDIA NATIONAL LABORATORY, ALBUQUERQUE (SNLA)

The ability to predict catalyst structure must be verified with analytical measurements. Now, four separate methods, x-ray crystallography, resonance Raman spectroscopy, EXAFS, and NMR spectroscopy have verified the structures of metalloprotein structures that were synthesized based on computer-aided molecular design tools developed at SNLA. Peer reviews of this accomplishment have resulted in publication of these results in the open literature. In addition, collaborations with the Caltech Molecular Materials Simulation have led to advances in improving model quality through improving forcefields.

4.1 Fiscal Year 1996 Major Milestones

Submit Report: "Design of Biomimetic Carbon Dioxide Activation Catalysts." **February 1996**

Sandia National Laboratories has developed a model that can design catalysts *a priori* that mimic enzyme systems. The model, based predominantly on force field calculations, is able to predict the secondary and tertiary structure of materials that closely resemble enzyme systems. The model has been applied in the development of carbon dioxide activation catalysts based on porphyrins, a class of enzymes that have the ability to reduce carbon dioxide. The SNL effort was initiated in FY 1991 and has resulted in a model system that has been debugged and used to design materials that show catalytic activity in reducing carbon dioxide.

Submit Report: "Biosolubilization of Phosphate Ore." **March 1996**

Idaho National Engineering Laboratory entered into a CRADA with Simplot, Inc. in FY 1992 for joint development of a bioprocess to solubilize phosphate from low-grade phosphate ores. The CRADA concluded in FY 1995 with bench testing of the concept at the Simplot facilities. This report will summarize the R&D efforts associated with this CRADA as performed by INEL.

Complete Draft of Technology Roadmap for Organic-Phase Bioprocessing. **April 1996**

A major thrust of the BCTR program in FY 1996 is in Organic-Phase Bioprocessing. Several reports have been generated in relation to R&D opportunities in the area, but a roadmap reflecting the information collected has not been completed. A major objective of the BCTR program is to "facilitate the introduction of biotechnology into the chemicals industry." Since 90% of all the chemicals produced in this industry involves organic; media synthesis, bioprocessing capabilities as applied to organic-media synthesis must be developed.

Hold Workshop on R&D Needs and Opportunities in Organic-Phase Bioprocessing. **May 1996**

Several reports have been generated regarding R&D opportunities in the area of organic-phase bioprocessing. ORNL has been commissioned to hold a one or two day workshop involving the industry, academia, and national laboratory scientific communities to obtain input on the R&D needs and opportunities in this area. The workshop is scheduled to be held after the technology roadmap has been generated so that this community can provide comment on the roadmap.

Submit Report: "Theory-Assisted Design of Metal and Zeolite Catalysts." **June 1996**

Lawrence Berkeley National Laboratory (LBNL) has developed a mass transport model to describe and predict molecular movement of petroleum feedstock material through zeolite catalysts. This information is significant to the industry since molecular flow is related to time spent in the catalyst which in turn impacts product distribution from the catalyst. The LBNL effort was initiated in FY 1990 and has produced a model system that has been debugged and applied on a limited basis to show relevance.

Submit Report: "Design and Characterization of Selective Oxidation Catalysts." **July 1996**

Pacific Northwest Laboratory has developed a surface characterization model to describe and predict surface structure and chemistry for oxidation catalysts. Focusing model development on the catalyst vanadylpyrophosphate (VPO), which is used in the production of sulfuric acid and maleic anhydride, the model characterizes the surface structure and composition of the catalyst system to predict and explain the chemistry of the catalyst. The PNL effort was initiated in FY 1991 and has resulted in a model system that has been debugged and validated on the VPO catalyst system.

Conduct Program Review and Guidance & Evaluation Panel Meeting. **August 1996**

This is an annual meeting of the program participants and industry collaborators. The purpose is to review and monitor the technical progress of the R&D efforts.

Submit Report: "Design, Characterization and Testing of Bimetallic Catalyst Systems." **Sept. 1996**

Ames Laboratory has developed a bulk and surface chemistry model to describe the position and distribution of precious metal atoms in a catalyst cluster. The metal atoms investigated include rhodium, palladium, platinum, etc. which are commonly used in environmental applications (e.g., automotive catalysts). The model is able to predict the bulk and surface distribution of the atoms under study, which is of importance to the industry since surface composition is related to catalyst activity and performance. The Ames effort was initiated in FY 1991 and has resulted in a model system that has been debugged and applied on a wide basis to show relevance.

5.0 Project Descriptions

1. Project Title	**Enzyme Catalysts for a Biotechnology-Based Chemical Industry**
Principal Investigators	**Frances Arnold**
Project Site	**California Institute of Technology**

Description:

Improving understanding of the molecular basis of protein stability and enzyme catalysis, combined with the ability to create large quantities of proteins of virtually any amino acid sequence, gives us the ability to redesign natural proteins to fit the requirements of industrial applications. As a result, biotechnologists no longer have to limit themselves to designing processes around natural biocatalysts--designing a biocatalyst to fit the process is gradually becoming an achievable goal. The ability to carry out biochemical syntheses in organic solvents, where solubilities are greatly enhanced and new chemistries are available, greatly expands the scope and potential applications of biocatalysis in the chemical industry. Unfortunately, most enzymes respond unfavorably to transfer to polar organic solvents; they are highly destabilized and their catalytic activities are often reduced by orders of magnitude. The overriding goal of this project is to develop design rules for engineering enzymes at the level of their amino acid sequences to improve stability and catalytic activity in polar nonaqueous solvents. The strategy employed is to use both random and site-directed mutagenesis techniques to alter the amino acid sequence of Subtilisin E, a serine protease with numerous potential applications in organic synthesis and preparation of novel polymers. This research will provide general tools and design rules for engineering stable and efficient biological catalysts. This project seeks to understand and enhance engineering enzyme stability and reactivity in nonpolar and nonaqueous solvents, using the prototype enzymes. Design tools will be provided to guide the selection and efficiency of macromolecular catalysts.

1995 Accomplishments:

- Directed evolution of pNB esterase.

- We have successfully evolved an esterase obtained from E. Lilly to optimize it for the cleavage of the p-nitrobenzyl ester of an antibiotic intermediate in the presence of polar organic solvents. Using multiple generations of random mutagenesis and screening, and more recently, DNA 'shuffling' methods, we have improved the specific activity of this enzyme for hydrolysis of pNB-lorcarbef in aqueous DMF by 16-fold and its total expressed activity by more than 30-fold. The sequences of the mutant enzymes along the evolutionary pathway have been determined, and biochemical characterization has been partially completed. This project has clearly demonstrated the power of directed evolution methods to create novel activities in natural enzymes and to extend their function to nonnatural environments. Eli Lilly has already taken an option to license this enzyme for ß-lactam production. CalTech is currently negotiating to license it for other applications.

Rapid screening method for subtilisin peptide ligase activity.

- A rapid, sensitive method for screening randomly mutagenized protease libraries for peptide ligase activity has been developed and verified using natural proteases: subtilisin and chymotrypsin. CalTech has entered into a joint research agreement with a company to use this technology for large scale screening and evolution of peptide ligases and polymerases.

Protein stabilization by engineered metal chelation.

- A metal-chelating site engineered into cytochrome c by a single amino acid substitution was demonstrated to increase the thermal stability of the protein (Tm) by a full 11°C in the presence of less than 1 mM Cu-IDA. The stabilization free energy is approximately 2 kcal/mole, which represent ~40% of the free energy of folding for this protein.

Research agreement with Eli Lilly & Co.

- CalTech has entered into a joint research agreement fully-sponsored by Eli Lilly & Co. to develop an enzyme for large scale production of a new antifungal agent. The goal in this project is to increase enzyme activity in the presence of organic solvents and acids. An effective, rapid screening method has been developed, and efforts are currently underway to screen random mutant libraries.

1996 Planned Activities:

Random recombination of p-nitrobenzyl esterase by DNA shuffling: improved directed evolution methods.

a. Our first priority will be to complete the publication(s) and patent(s) on this enzyme.

b. This system is now being used to compare two directed evolution strategies: sequential random mutagenesis versus recombination. A convenient recombination method has recently become available. This method may allow the rapid accumulation of beneficial mutations in the evolved enzymes. The goal of our work will be to quantify the extent to which random recombination of genes containing effective mutations can speed the directed evolution approach, either by reducing the number of generations required or by reducing the number of colonies that must be screened.

Subtilisin engineering.

We will continue our efforts to develop subtilisin variants suitable for peptide ligation and polymerization. Much of this work will be done in collaboration with Recombinant Biocatalysis, Inc., which has developed robots and fluorescence scanning systems suitable for large-scale screening. The goal will be to direct the evolution of subtilisin E to optimize it for coupling of amino acids and the production of amino acid oligomers and polymers.

Enzyme for the production of a new antifungal agent.

We will continue to develop the tools and strategies required for directed enzyme evolution and apply them to systems of industrial interest. In particular, we will be continuing efforts to improve the activity of a new enzyme under the conditions favorable for production of a new antifungal agent. In this case, the major goal will be to enhance activity at lower pH. Recombination as well as sequential random mutagenesis approaches will be used.

We are currently developing a joint research project with Thermogen, Inc., the goal of which will be to develop generally-applicable approaches to the stabilization of enzymes.

We will continue to work with Life Technologies, Inc. on the stabilization of specific enzymes by directed evolution and through the use of metal chelation.

Annual Technical Summary Report:

Those of us who wish to use the remarkable catalytic powers of enzymes for applications are constantly stymied by the fact that enzymes have evolved under selective pressure to perform very specific biological functions, and to do so within the context of a living organism. Some of the features required for function in a complex chemical network are undesirable when the catalyst is lifted out of context. Conversely many of the properties we wish an enzyme would have clash with the needs of the biological system, or at least were never required: for example, high stability, ability to function in nonnatural environments or to catalyze reactions on nonnatural substrates.

The explosion of tools that has come out of molecular biology during the last 20 years has made it possible for us to consider 'evolving' proteins for features never required in nature. Using processes of Darwinian-like selection carried out in the test tube, so-called 'directed evolution', we can explore the functions of proteins free from the constraints of a living system. By creating and then screening large libraries of random variants for specific properties, we can step into and explore a variety of futures, futures than can include novel environments or even entirely new functions. Directed evolution offers a unique opportunity for biotechnology: the ability to tailor enzymes for optimal performance in a wide range of applications. Due to the vastness of the potential sequence space, however, explorations by directed evolution must be guided by sound principles and workable strategies. This past year our goal has been to identify the types of problems that are well-suited to a directed evolution approach and to demonstrate two pathways, sequential random mutagenesis and gene recombination, by which enzymes can be directed to evolve interesting and useful new functions.

Evolution of an esterase for large-scale antibiotics synthesis:

We have been working in collaboration with a major pharmaceutical company, Eli Lilly, to engineer an enzyme for use in the production of beta-lactam antibiotics. The goal of this project has been to demonstrate the utility of the directed evolution approaches in creating a specific enzyme of industrial importance. The desired reaction is hydrolysis of the p-nitrobenzyl-protected loracarbef nucleus (LCN). An esterase enzyme which will catalyze the desired deprotection reaction was isolated and characterized as part of the company's effort to develop an enzyme-based alternative to the current chemical process. The enzymatic process, however, was not economically feasible due to the very low rate of hydrolysis in polar organic solvents (required to obtain sufficient solubility of the substrate). We have successfully applied directed evolution methods to obtain significant enhancements of the enzyme's activity under the conditions of a pharmaceutical manufacturing process.

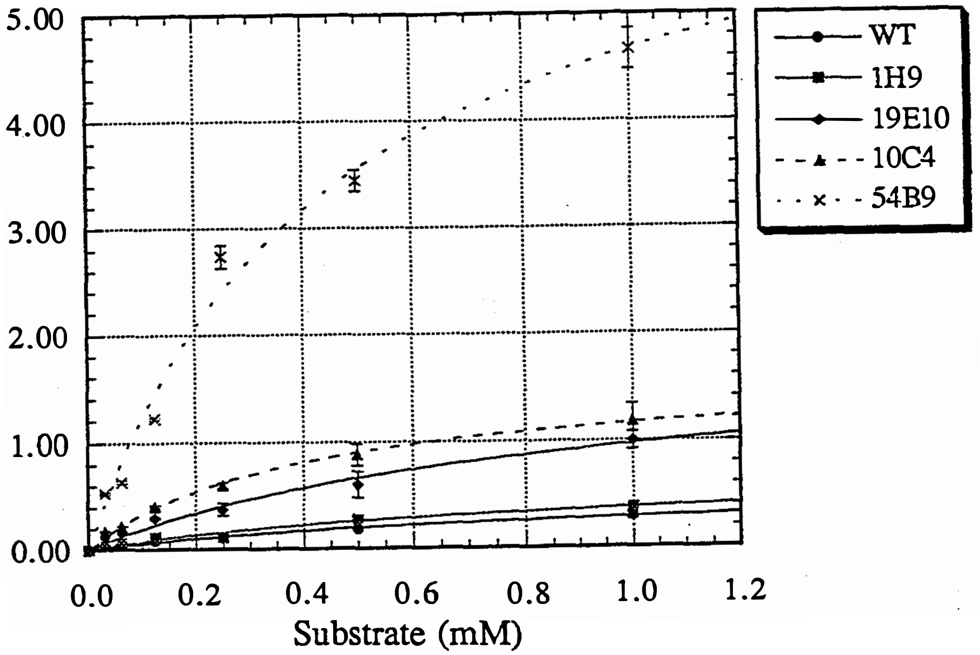
To summarize the results of this past year, the wild-type pNB esterase was subjected to four sequential rounds of random mutagenesis and screening for enhanced activity in the hydrolysis of the p-nitrophenyl ester of the LCN. The validity of this screening approach was confirmed by showing the strong correlation between p-nitrophenyl ester and p-nitrobenzyl ester hydrolysis. The net product of this four generations of directed evolution was an enzyme variant that is 16-fold more active than wild type in 15 % DMF (See Fig. 1a). Sequencing of this and other more-active pNB esterase variants has been completed; the fourth-generation enzyme has six new amino acid substitutions (Fig. 1b). The positions of all the effective amino acid substitutions found in all the more-active enzyme variants studied are indicated on the overall enzyme structure in Fig. 2. Many of the mutations are located in or around the putative active site and substrate binding pocket of the enzyme.

Very recently, we applied a novel 'gene shuffling' method to randomly recombine the genes for the five best mutants from a pool of enzymes from the fourth round of mutagenesis, with the goal of finding the best combination of mutations from these enzymes. The shuffling experiment, which involves digestion of the DNA with DNase enzyme and reconstruction of the gene from the fragments by PCR, has been successfully performed, and ~ 400 colonies from this next pool have been screened for activity in p-nitrophenyl ester hydrolysis. Of these 400, fully eight are significantly more active than the best of the five recombined variants. This frequency of positive variants is more than 10 times the frequency of positives obtained through random point mutagenesis and underscores the utility of this novel recombination strategy.

Subtilisin mutants exhibiting improved activity in organic solvents

In previous work, directed evolution of subtilisin E for increased activity in DMF resulted in the identification of a variant with high activity in organic media and high levels of expression (13M subtilisin E). Subtilisin E and its variants are capable of catalyzing the stereospecific polymerization of interesting nonnatural as well as natural amino acids. However, for peptide synthesis and polymerization using the 'kinetically controlled' approach, the enzyme should be optimized directly for its synthesis capabilities in organic solvent (as opposed to hydrolysis activity). Thus in the past year we have concentrated our efforts to develop a sensitive assay to screen random mutants directly for synthesis activity. The overall strategy is outlined below. One amino acid ester is attached to a filter paper through a flexible linker. Another amino acid ester is labeled with fluorescein, also on a long, flexible linker. Colonies expressing an enzyme able to ligate the amino acids are identifiable on the filter paper through the fluorescent signal. This rapid assay method has been assembled and verified with wild type enzymes. It will be implemented for screening randomly mutagenized subtilisin variants in collaboration with scientists at Recombinant BioCatalysis, Inc.

a



Generation	*0*	wild type
1H9	*1*	*H322–R* *Y370–F*
19E10	*2*	H322–R Y370–F *M358–V*
10C4	*3*	H322–R Y370–F M358–V *L144–M*
54B9	*4*	H322–R Y370–F M358–V L144–M *K267–R* *L334–S*

b

Figure 1: a) Specific pNB esterase activity for hydrolysis of loracarbef-pNB ester in 15% DMF, as a function of substrate concentration.

b) Amino acid substitutions in the enzymes from each generation of directed evolution are indicated

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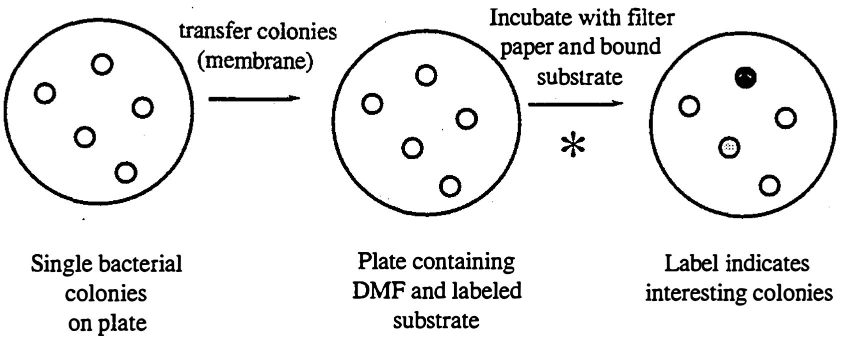
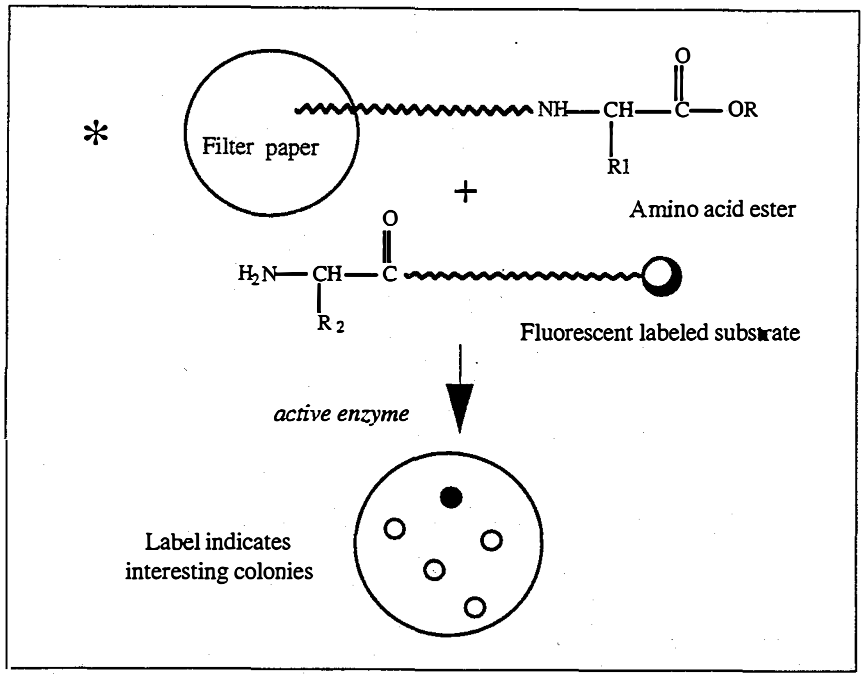
Rapid assay scheme





2.	**Project Title**	**Theory-Assisted Design of Zeolite Catalysts**
	Principal Investigators	**Alexis Bell and Arup Chakraborty**
	Project Site	**Center for Advanced Materials** **Materials Sciences Division** **Lawrence Berkeley National Laboratory**

Description:

The project will develop and validate a hierarchy of theoretical models that could be used by industry for the design and development of catalysts, especially zeolites and metals. Catalyst properties (enthalpies and entropies of adsorption and formation and other thermodynamic parameters) will be calculated and compared with known experimental values. The overall goal is a complete description of various industrially relevant heterogeneous catalyst systems, with models of increasing degrees of sophistication. These models will allow increasingly better prediction of industrially important phenomena, which can be used for the design of these two classes of catalysts.

1995 Accomplishments:

Calculation of the Structure, Electronic Properties, and Preparative Chemistry of Cu-ZSM-5 Zeolites. We have initiated a theoretical study of the properties of copper containing zeolites with a view toward understanding their efficacy in direct NO reduction, a technological problem of immense environmental importance. Previous electronic structure calculations for zeolites in our group have been carried out on materials that do not contain any transition metals. Thus, we performed local density functional (LDA) calculations and employed pseudopotentials to represent the core electrons. The latter approximation proved particularly beneficial because geometry optimizations could be carried out using the Hellman-Feynman theorem which is the least expensive computational method for this purpose. However, in order to treat transition metal containing zeolites (such as Cu-ZSM-5) the use of pseudopotential LDA calculations is inappropriate. LDA calculations cannot be employed because in these systems the spins are not always compensated and spin unpairing is of importance. Thus, local spin density (LSD) calculations need to be performed. The pseudopotential approximation proves to be inadequate because in transition metals the bonding environment is such that there is a propensity for electron promotion from core to valence levels, thus negating the basic premise of the pseudopotential approximation. This issue is particularly important for copper. Thus, we need to perform all-electron calculations. This also implies that the Hellman-Feynman theorem cannot be employed any more to perform geometry optimizations as numerical inaccuracies become intolerable in the core region. In light of the above considerations, in order to study Cu-ZSM-5, we have rewritten our computer code in order to implement LSD calculations with analytical energy gradients for geometry optimization. Our new code is highly vectorized, and runs on the San Diego Supercomputer Center demonstrate its efficacy. Using our code, we have studied various aspects of copper containing zeolites; our study is the first theoretical study of this material. Experimental studies have postulated the existence of Cu^+, $Cu^{2+}O^-$, and $Cu^{2+}(OH)^-$ species depending upon conditions. Extensive EPR studies in our own laboratories have also speculated upon the mechanism via which copper containing zeolites undergo auto reduction during preparation. However, all these issues and the electronic and structural properties of Cu-ZSM-5 were still the subject of much debate. The results of our calculations provide firm answers to many of these questions. We have detailed the electronic and structural features of all the aforementioned copper species in the material of interest.

We find that the copper species always prefers to bond to two lattice oxygen atoms rather than three or one. The spin populations that we calculate allow us to help interpret the EPR data unambiguously, as we can now clearly determine which species are EPR silent. We have also been able to carry out a detailed thermodynamic analysis of the various steps in a proposed autoreduction mechanism, and have shown the plausibility of a mechanism that is consistent with all available experimental data.

Diffusion of Long-Chain Alkanes The effectiveness of zeolites as hydrocarbon separation agents and cracking catalysts is influenced by diffusion of hydrocarbons through the zeolite matrix. Our previous work has shown that for diffusion coefficient for C1-C6 alkanes can be determined from molecular dynamics (MD) simulations. However, for higher molecular weight alkanes, the computational costs of this approach rapidly become prohibitive. As an alternative, we have developed a coarse-grained approach based on Brownian dynamics (BD) and transition state theory (TST). To reduce the number of degrees of freedom a united-atom representation of the alkane molecule is projected onto the a central "wire framework" defined by the centerline of the zeolite channels. Molecules are then represented by the location of the chain head and tail, and by a vector describing the chain conformation or "state". Motion within a state is described by Brownian dynamics and the transition from one state to another is described by TST. The interactions of the diffusant with the zeolite are described by a potential of mean force, which is obtained by over all alkane chains in a given state with chain heads and tails at a given position. The Continuous Configurational Bias (CCB) technique, developed earlier in this work, is used to create a very large ensemble of chains within the zeolite pore volume. Initial application of the BD-TST simulation approach shows that it can reproduce the diffusivity obtained from MD simulations but is nearly two orders of magnitude faster. Of particular interest is the observation that the computational time required to determine a diffusion coefficient by using BD-TST approach scales linearly with molecular weight, and much more rapidly when MD simulations are used.

Thermodynamics of Template-Zeolite Interactions. Organic cations are well known to act as structure-directing, or templating, agents during the synthesis of zeolites, particularly those which are highly siliceous. A central issue of interest is the relationship of template size and structure to the structure of the zeolite formed. To address this question, we have initiated a project aimed at determining the thermodynamics of template occlusion in the zeolite pore volume. Our initial efforts have been devoted to determining the minimum energy configurations of tetraalkylammonium cations in ZSM-5 and ZSM-11. The objective is to establish the effects of template size on the degree of stabilization of the zeolite resulting from template occlusion. These calculations have been carried out using Biosym Technologies software. Our results show that for both ZSM-5 and ZSM-11, the stabilization energy passes through a maximum value as the template size increases. The optimum occurs for tetraproplyammonium cations. As a supplement to these calculations, we are carrying out GCMC simulations to determine the free energy of stabilization of different TAA cations. In this instance we are using the CCB method to insert new chains and the GCMC code developed previously in our group for simulation of hydrocarbon adsorption in zeolites. It is our expectation that it is necessary to examine the stabilization free energy changes rather than the internal energy changes to determine the effects of TAA cation size on the stabilization of a given zeolite.

1996 Planned Activities:

Density Functional Theory Study of Nitrogen Oxide Species in Cu-ZSM-5. We have already initiated a study of the mechanisms via which NO and its derivatives interact with Cu-ZSM-5. We plan to study various species (e.g., NO, N2O, and various adsorbed species), with a view toward clarifying the reaction pathways involved in the decomposition of NO in this material. Once this study is completed, we shall also use information regarding the energetics of interactions that emerge from these investigations as inputs to statistical mechanical methods (a combination of TST and a field theory) to study the dynamics of the diffusion-reaction processes that nitrogen containing species undergo in zeolite catalysts containing copper. We also plan to study cobalt containing zeolites in order to elucidate the intriguing differences between cobalt and copper containing zeolites that have been observed experimentally with regard to their efficacy for NO decomposition.

Prediction the Diffusivities of Long-Chain Molecules in ZSM-5 The BD-TST approach will be used to calculate diffusion coefficients for C6-C20 alkanes. Of particular interest is to establish the manner in which the diffusion coefficient and activation barrier for diffusion decrease with increasing molecular weight. Equally of interest is to establish the extent to which diffusion becomes anisotropic as molecular weight increases. The role of zeolite channel friction as opposed to the barriers to the passage of alkane chain ends from channel section in and out of channel intersections will be examined.

Interactions of Templating Agents with Zeolite Frameworks The synthesis of highly siliceous zeolites requires the presence of a templating agent, such as a tetraalkylammonium cation, in the synthesis gel. Experimental work suggests that the most effective templating agents are those which exhibit a large degree of non-bonding interaction between the template and the framework of the final zeolite. We will continue our computational effort aimed at identifying the best template structure for the synthesis of a given zeolite. Of particular interest will be to establish the effects of lattice interactions on the internal and free energies of zeolite stabilization by templating cations. The global objective of this effort is to determine the factors dictating maximum stabilization for a given zeolite architecture, so that templating agents can be synthesized for this purpose.

Structure Direction by Organic Cations During Zeolite Synthesis Experimental evidence indicates that structure-directing organic cations are surrounded by silicate anions during zeolite synthesis and that the cations become partially or totally occluded in the synthesis gel prior to the appearance of crystalline zeolites. It has also been established that the rate at which these processes occur is a strong function of the structure and composition of the organic cation. To gain further understanding of the initial processes involved in zeolite synthesis, we will initiate a series of studies aimed at identifying how water and low-molecular weight silicate anions organize in the vicinity of organic cations.

Annual Technical Summary Report:

Electronic and Structural Properties, Preparative Chemistry, and NO Decomposition in Cu-ZSM-5 Zeolites.

A major goal of our efforts is to understand sorption, diffusion, and chemistry in zeolites that are employed in technological applications. Toward this goal, in preceding years we have carried out detailed characterization studies of the acid site in H-ZSM-5. We have also studied the adsorption of ammonia in this zeolite. In FY95, we initiated research aimed at addressing a problem of significant technological importance; viz., the decomposition of nitrogen oxide in zeolite catalysts containing metal cations such as copper and cobalt. The ultimate aim is to guide the synthesis of efficient and inexpensive catalysts for this task, a goal which has important environmental implications. We wish to understand the electronic and structural properties of these catalysts, the chemistry that occurs during their preparation, the mechanisms via which NO (and other species) are decomposed in these zeolites, and the dynamical behavior of these molecules inside the zeolite. To this end, we have investigated the structural and electronic properties of Cu-ZSM-5 and obtained insight into the autoreduction chemistry that occurs during its preparation. We have also initiated studies of the energetics of NO decomposition in Cu-ZSM-5, together with a structural analysis of adsorbed intermediates.

In carrying out the electronic structure calculations for Cu-ZSM-5 we have employed the general framework of Density Functional Theory. Our group was one of the earliest to employ this powerful technique for studying zeolite catalysts. In past years, we used our own code that invoked the local density approximation (LDA), the pseudopotential approximation, and Hellman-Feynman forces to carry out geometry optimizations. However, none of our preceding studies involved transition metal species. LDA calculations are inappropriate for transition metals such as copper where the effects of spin polarization and unpairing are important. Therefore, we have implemented the local spin density (LSD) method into our previous code. Computational studies of copper-containing zeolites are further complicated by the fact that in most bonding environments there is electron promotion from the core levels to the valence levels. This makes it inappropriate to use the pseudopotential approximation and necessitates the execution of all-electron calculations. As a result of performing all-electron calculations, we have had to implement analytical energy gradient evaluations for geometry optimization, because Hellman-Feynman forces are unacceptably inaccurate when core electrons are treated explicitly. Along with these non-trivial changes to our code, we have also rewritten the code extensively in order to make it more efficient on vector machines. By all measures that can be used, we now have a highly vectorized code as evidenced by our runs at the San Diego Supercomputer Center.

Using our new code, we have studied the electronic and structural properties of various copper species in the ZSM-5. Based on experimental evidence, we have focused attention on Cu^+, $Cu^{2+}O^-$, and $Cu^{2+}(OH)^-$ species. We find that each of these copper-containing species binds to the lattice oxygens with two-fold coordination being preferred over three-fold or one-fold coordination. From calculations of the spin populations of each species, we have confirmed that $Cu^{2+}(OH)^-$ should be EPR active and that Cu^+ and $Cu^{2+}O$ should be EPR silent, in complete agreement with recent experimental observations obtained in our laboratories. We have evaluated the thermodynamics of the autoreduction process $2\ Cu^{2+}(OH)^- = Cu^+ + Cu^{2+}O^- + H_2O$ based on our electronic structure calculations and estimates of the entropic contributions. Our calculations indicate that autoreduction can occur at elevated temperatures in the manner written, and that re-oxidation by water vapor should be spontaneous at room temperature. In a related set of calculations, we have demonstrated that Cu^{2+} binds preferentially to the oxygen atoms comprising a five-membered ring containing two Al atoms. However, in the case of a six-membered ring, the Cu^{2+} binds preferentially to one side of the ring.

We have also initiated studies of NO decomposition in Cu-ZSM-5. Several candidate mechanisms have been postulated on the basis of experimental observation. We are currently in the process of determining the thermodynamic feasibility of these mechanisms and the stability of various species that are expected to participate in the decomposition process. In anticipation of the completion of the preceding project, we have also started work on developing a theoretical method for the dynamics of nitrogen oxide species in Cu-ZSM-5. To address the problem of concurrent reaction and diffusion, we are developing a hybrid method that will employ TST and a field theoretic approach. The energetics calculated using the LSD calculations will serve as an input.

Hierarchical Molecular Simulation Approach to the Diffusion of Linear Long-Chain Alkanes in Silicalite

The diffusivity of long linear alkane molecules, such as n-hexadecane, in zeolites of the ZSM-5 family is of great interest in industrial catalytic applications such as catalytic dewaxing and FCC. In FCC, for example, the extent to which a zeolite crystal is utilized for catalytic reaction (effectiveness factor) depends on the relative time constants of reaction and intracrystalline diffusion. As the direct measurement of diffusivity under reaction conditions is impossible, molecular modeling is the only tool for quantifying intracrystalline transport rates.

For alkanes up to C_6 we have successfully employed MD simulation to predict the self-diffusivity. For longer molecules (C_6 to C_{25}), the computational requirements of conventional MD become prohibitive, necessitating the development of new simulation methodology. To circumvent this limitation, we have developed a coarse-grained stochastic model to address the long time scales of the problem. The coarse-graining of configurations invoked in our dynamics work are based on CCB methodology, which we have used successfully to describe the thermodynamics of alkane adsorption in ZSM-5.

An alkane chain is represented by its two ends, which experience a potential of mean force resulting from the interactions of all degrees of freedom of the molecule with each other and with the zeolite. We obtain potentials of mean force through CCB MC calculations - a process that is remarkably efficient. In our coarse-grained representation of dynamics, chain ends move along channel segments as two tethered Brownian particles, while the entry or exit of a chain end from a channel intersection is viewed as an activated rate process. The motion of the chain molecule in the channel system is thus consists of three dynamical processes: (a) Brownian motion of either end segment; (b) exit of an end segment from a channel into an intersection region; (c) entrance of an end segment from an intersection region into a channel. Processes (b) and (c) allow the molecule to "turn corners", changing its overall shape or "macrostate" within the pore network.

All quantities needed in the coarse-grained model (potential of mean force, mobility coefficients, transition rate constants) are extracted from atomistic MC, short-time MD, and three-dimensional TST calculations. By simulating the coarse-grained dynamical model for times long enough to allow substantial displacement of the center of mass, we obtain estimates of the self-diffusivity tensor at various temperatures, including temperatures at which direct experimental measurement of the diffusivity is impossible because the molecules undergo catalytic cracking. Mechanistic aspects of diffusive transport can be elucidated. For example, how easy is it for a chain molecule to "turn a corner" when it comes to an intersection, as opposed to continuing to diffuse along the direction from which it came? If the resistance encountered by bent conformations is much higher than that experienced by straight conformations, it is conceivable that diffusion would occur almost exclusively along the straight or along the sinusoidal channels, a given molecule participating in one of the two modes of molecular traffic but seldom in both. Then, lattice defects or coke deposits obstructing the traffic in one direction would decelerate diffusion pronouncedly. Our potential of mean force calculations have revealed that "compact" configurations, wherein the central section of a long linear alkane is coiled within a channel intersection region, have appreciable probability. An interesting question is whether the "folding" process, whereby such configurations are formed from the much more abundant extended ones, could decelerate the overall transport of the molecule through the zeolite pore network.

Methodologically, the BD-TST approach described above represents a significant step forward in that it establishes a bridge between atomistic simulation techniques that take into account the detailed geometry and energetics, and more coarse-grained modeling methods that can address the long-time dynamics of systems with many degrees of freedom.

Simulations of the Interactions of Tetraalkylammonium Cations with ZSM-5 and ZSM-11

Organic cations are known to facilitate the synthesis of zeolites, particularly those which are highly siliceous, and to be occluded in the intra-zeolite channels. It has been suggested that the occlusion of the templating agent leads to a stabilization of the zeolite framework and that increased stabilization occurs as the non-bonded interactions between the zeolite and the template increase. We have examined this issue in some detail for tetraalkylammonium (TAA) cations in ZSM-5 and ZSM-11. Attention was focused on identifying the separate effects of intra-template non-bonded interactions, template-zeolite non-bonded interactions, and template-template non-bonded interactions on the stabilization energy of TAA cations as a function of their C/N ratio and occupancy in the zeolite lattice.

The zeolites are taken to be purely siliceous rigid frameworks. The interactions between all atoms in the system are (zeolite plus TAA cation) are treated atomistically by using a Class II self-consistent force field. This force field accounts for bonding interactions between atoms and distortions of bond angles and torsion angles. Coulombic interactions are neglected on the assumption that, to a first approximation, these will not be strongly dependent on the size of the TAA cation. Minimum-energy configurations for TAA in ZSM-5 and ZSM-ll were determined by using the BIOSYM Technologies Catalysis software. The methodology for finding the minimum energy consists of three steps. In the first step, molecular dynamics simulations of TAA are carried out *in vacuo*, to create an ensemble of TAA conformations. In the second step, an attempt is made to insert each of the guest conformations into the zeolite lattice. Only those configurations having an energy below a predetermined threshold are considered further. Energy minimization of each crudely docked configuration is performed by using a conjugate gradient algorithm. To handle the case of multiple template cations per unit cell, periodic boundary conditions are used.

We find that occlusion of TAA cations in ZSM-5 and ZSM-11 leads to a stabilization of the zeolite. At low loadings (viz., one TAA cation per unit cell) the magnitude of the stabilization energy increases monotonically with the C/N ratio of the cation. This trend is attributable to the increase in template-zeolite non-bonded interactions as the number of atoms in the alkyl branches increase. At saturation loading, four TAA cation per unit cell, the magnitude of the stabilization energy increases from TMA to TPA, but then decreases for TBA. The lower value of the stabilization energy for TBA is due to the intramolecular energy penalties required to insert four TBA cations per unit cell of either ZSM-5 or ZSM-11. Visualization of the occluded TBA cations reveals that at this loading, one of the four butyl branches on each cation must assume an energetically unfavorable hooked configuration, and the proximity of the methyl groups at the ends of the alkyl branches are close enough to experience repulsive interactions. Reduction of the TBA loading to three cations per unit cell increases the stabilization energy, as a consequence of the reduction in the level of intramolecular attain, but the stabilization energy is still less than that achieved with four TPA cation per unit cell in either ZSM-5 or ZSM-11.

3.	**Project Title**	**Theory of Biocatalysis** **Electron Transfer Reactions**
	Principal Investigators	**David Beratan**
	Project Site	**University of Pittsburgh** **Department of Chemistry**

Description:

The principal project goal is the computer-aided design of enzyme biocatalysts with tailored catalytic rates. The focus of this project has been on electron transfer enzymes, a major class of biological enzymes. In this project, the theory of protein electron tunneling pathway has been developed, implemented as computer models, which connect enzyme electronic structures and their reaction rates.

Specific project objectives include:

1. Develop algorithms to map the key residues in proteins between electron transfer sites that mediate electronic coupling and allow electron transfer reaction to proceed with great speed and specificity;
2. Identify "hot" and "cold" spots with respect to electron transfer in native and modified proteins;
3. Develop an understanding of primary, secondary, tertiary, and quaternary structural effects on electron transfer rates;
4. Use knowledge gained to stabilize energetic charge separated states and to enable the development of semisynthetic/modified protein energy conversion systems.

1995 Accomplishments:

- Tested *ab initio* fragment method for calculating electronic coupling interactions.
- Developed criteria of validity for two-electron transport processes.
- Calculated electronic coupling interactions in large DNA electron transfer systems.
- Initiated studies to design new structures with optimized molecular properties.

1996 Planned Activities:

- Apply new fragment method to protein and DNA electron transfer reactions.
- Expand two-electron theory; initiate application to simple inorganic and electrochemical systems.
- Continue study of inverse strategies for the design of new materials.

Annual Technical Summary Report:

The first two tasks of this project involve the analysis of electron transfer processes in large biomolecules: proteins and DNA. In these two tasks, we intend to calculate the interactions between electron donor and acceptor species bound to large biomolecules. These tasks were the first to be initiated. We have hired the research staff needed to conduct this project and have made contact with the key experimentalists in this area.

In the first quarter of the project we conducted preliminary analysis of electron tunneling interactions in DNA. This was performed by modifying standard extended-Hückel (independent-electron) electronic structure software to allow for: (1) the analysis of very large systems (tens of base pairs) and (2) the computation of Green function matrix elements based upon these Hückel calculations. The computations will be approximate, but will provide tests of whether we can handle systems of the size needed as more precise methods are developed. They will also allow us to gain experience in performing very large scale computations at National Science Foundation Supercomputer Centers (Pittsburgh, Illinois, and San Diego) where we have grants of computer time to conduct this work.

Preliminary analysis at the extended-Hückel level was completed on numerous DNA systems. Qualitatively, electronic propagation in DNA appears similar to that in electron transfer proteins that we have examined previously. In order to make the predictions quantitative, however, we must introduce calibrations associate with electron donor and acceptor electronic binding energies. Unfortunately, this cannot be done completely unambiguously at the Hückel level, so we have begun more advanced self-consistent field computations of the macromolecule electronic structure to provide a more precise analysis of this problem.

Work was begun in the second quarter on the analysis of the role played by multiple tunneling pathways in protein electron transfer reactions. Like the other tasks, this one was begun using fairly simple independent-electron electronic structure methods. We have developed a new scattering matrix method to probe multi-pathway effects in alpha-helix, parallel beta-sheet, and anti-parallel beta sheet electronic propagation. This analysis is being used by us now to dissect interactions into backbone mediated coupling vs coupling interactions that arise from the folded three-dimensional structure of the protein.

Concerning the first two tasks, an important breakthrough was achieved. We are now able to compute Green's function matrix elements in proteins and DNA based upon self-consistent field wave functions, rather than upon (independent electron) extended-Hückel methods. This step was a critical one because it allows us to compute electron transfer rates in proteins and DNA without any major adjustable parameters. We are now exploiting this method in supercomputer calculations of electron transfer rates in DNA and proteins. We hope by the end of the summer to submit a manuscript to a refereed journal describing this new method and our results in DNA. The current work involves applying this method to DNA and proteins. An important part of our current work involves software development so that our code will run on conventional and parallel supercomputers.

We are also beginning to probe the nature of multi-electron electron transfer processes in biochemical reactions in greater detail. A large number of electron transfer proteins transfer more than one electron to achieve their catalytic function. So far, all of our studies have been limited to single electron processes. We are beginning to build simple models for multi-electron redox processes. We hope to be able to determine criteria of validity of regimes where sequential single electron, concerted two electron, and intermediate mechanisms of electron transport dominate.

The preliminary studies associated with the multiple-pathway analysis of protein electron transfer were completed. We are now using the developments of our new method of producing improved Hamiltonians for electron transfer to make this multi-pathway analysis quantitatively accurate as well. Using these improved Hamiltonians, we should be able to dissect the through-bond vs through-space components of the electron transfer interactions contributing to donor-acceptor interactions. This will allow reliable analysis of when single and when multi-pathway contributions to the coupling dominate.

Also related to the analysis of the role played by multiple tunneling pathways in protein electron transfer reactions we have begun to probe the general problem of the "inverse design" of materials. That is, given a prescribed electron transfer rate or other molecular property, what molecule or class of molecules would produce the optimum property. This is a problem of constrained optimization that we are beginning to explore in the context of electron transfer interactions.

4. Project Title **Advanced Bioprocessing Concepts Program**
Principal Investigators **Brian Davison, Eric Kaufman, John Barton,**
John Nghiem, Timothy Scott
Project Site **Oak Ridge National Laboratory**

Description:

This project will advance practical and fundamental knowledge of bioreactor dynamics and immobilized biocatalyst systems. The goals of the project are as follows: development and testing of new bioreactor configurations; understanding and modeling of the kinetic properties of biocatalyst particles and the dynamics of bioreactor systems; and development of enhanced bioreactors for continuous production of industrial products (enzymes, chemicals, etc.).

1995 Accomplishments:

A primary goal of this program is the development and improvement of advanced bioreactor systems that significantly increase the productivity and yield of bioconversion processes. Several of these concepts under investigation utilize biocatalysts which are microorganisms immobilized into or onto particles. We are investigating the use of these biocatalysts in multiphase systems including fluidized-bed, gas-phase reactors, immobilized enzymes and initiating work in organic media.

- Continuous and Simultaneous Fermentation and Purification of Lactic Acid was demonstrated in a biparticle fluidized-bed bioreactor (FBR). The biparticle FBR utilizes microorganisms immobilized in gel beads and adsorbent particles. The feasibility of this approach and its potential advantage over other bioprocessing schemes has been demonstrated in short term fermentation trials. A biparticle FBR with continuous resin addition and without pH control has been operated and compared with a nonextractive control. The biparticle FBR had a productivity of > 5 g/L·h which is 16 times that of a free-cell batch reactor. Improved product recovery from the resin gave concentrations of up to 70 g/L from fermentation broth.

- The degradation of industrial VOCs (pentane and isobutane) was tested in various gas-phase reactor systems (bubble columns, trickle beds, and shake flasks) to confirm and elucidate mass-transfer and kinetic limitations. The trickle bed has operated for a year with rates up to 60 g VOCs/h/m3 using active microbes. A method has been developed to control overgrowth and plugging. Mass transfer coefficients comparable to literature values have been measured for trickle bed which are three times higher than that in the bubble columns. A predictive mathematical model has been developed. These techniques are now being extended to initial efforts using gaseous substrates with the biocatalysts in an organic phase to improve reaction and product solubility.

- Immobilization and reactor design are being investigated for the use of high-efficiency immobilized enzymes for dehalogenation in a CRADA with Dow Chemical. Dehalogenation addresses a direct industrial need to transform a current by-product waste of organic synthesis and allow it to be recycled in the process. The basic organism have been cultured for enzyme production at low yield. Dow Chemical has developed a greatly improved genetically modified organism.

- This project will advance practical and fundamental knowledge of bioreactor dynamics and immobilized biocatalyst systems. The goals of the project are as follows: development and testing of new bioreactor configurations; understanding and modeling of the kinetic properties of biocatalyst particles and the dynamics of bioreactor systems; and development of enhanced bioreactors for continuous production of industrial products (enzymes, chemicals, etc.).

Another part of the increased emphasis in non-aqueous bioprocessing was the completion of a summary report, "Bioprocessing in Nonaqueous Media: Critical Needs and Opportunities." Experimental and modeling tasks have begun in the areas of interfacial phenomena and contacting schemes using enzymes in organic media and in gas-phase reactors.

- The Symposium on Biotechnology for Fuels and Chemicals was partially supported.

1996 Planned Activities:

The program will emphasize multiphase bioreactors. However, the multiphase emphasis will move away from pure aqueous systems to nonaqueous bioprocessing, including liquid organic-phase bioreactors and gas-phase bioreactors. In most cases, there will be at least five elements in the development of each new bioprocessing concept, some of which can be carried out concurrently:

- Evaluation of the primary mechanisms;
- Integrated study of the new concept on a bench scale;
- Development of a predictive mathematical model;
- Establish technical feasibility at an adequate scale;
- Transfer of the new technological concept to a more applied DOE program or to the industrial sector.

Each of these projects is at a different stage of this approach. An effort in the use of biocatalysts in nonaqueous media will begin. Several subcontracts for related work at universities will be administered and support for the Symposium continued.

The investigation of immobilized cells and enzymes for dehalogenation will continue through the Cooperative Research and Development Agreement (CRADA) with Dow Chemical. We will be testing bioreactor configurations, using their improved microorganism and increased enzyme concentrations.

Development of a highly efficient bioreactor for gaseous substrates will continue. The model proof-of-principle system is the removal of industrial volatile organic hydrocarbons by microbes and will be completed. These techniques are now being extended to initial efforts using gaseous substrates with the biocatalysts in an organic phase to improve reaction and product solubility. The initial system is the production of quinones by polyphenol oxidase.

Advanced bioreactors for nonaqueous processing will be investigated in using biocatalysts in biphasic liquid systems. ORNL will help develop and coordinate efforts to continue the program development and identification of needs in this area. Efforts will begin on the optimization of biocatalysts in organic media and use of biocatalysts in supercritical fluids; the majority of these efforts will be carried out at academic institutions. ORNL will emphasize the development of reactor configuration to utilize these biocatalysts. The current experimental system uses peroxidase in the aqueous phase and the substrates and product in the immiscible organic phase. Interfacial effects will be studied along with kinetics.

Annual Technical Summary Report:

The development and use of advanced bioprocessing concepts should result in significant increases in productivity and economic viability of bioprocesses such as those used for the production of fuels or chemicals or for environmental control technology. ORNL has emphasized multiphase bioreactors with continuous operation using high concentrations of active biocatalysts (e.g., enzymes or microbes). ORNL's approach is to use fundamental concepts as the basis for developing these advanced processing systems. After the technical feasibility of the approach is demonstrated, transfer of the technology is established through industrial interactions and publications. These innovative bioreactor configurations are investigated with the aim of demonstrating the process concept and understanding the underlying mechanisms sufficiently well to establish a predictive capability and model for design, scale-up, and economic evaluation. Several such systems are being investigated which emphasize and exploit multiple phases to enhance the overall operation. A new effort is emphasizing non-aqueous systems.

Biparticle Fluidized-bed Bioreactor for Simultaneous Fermentation and Separation - Fluidized-bed bioreactors with immobilized cells can increase the productivity of fermentations for useful products. An additional advantage of the multiphase FBR is the potential to add new phases to perform additional functions beyond those of the solid catalyst, liquid media and gas coproduct. Our approach is to add a fourth phase with extractive capability for the desired product. This will serve to enhance the fermentation by controlling the reactor pH, while simultaneously achieving product separation. The bioreactor consists of a columnar fluidized bed of immobilized microorganisms. A stream of more dense sorbent particles is added to the top of the bed. These particles progress downward through the biocatalyst, adsorb the inhibitory product, and are removed from the base of the reactor. The feasibility of this approach and its potential advantage over other bioprocessing schemes has been demonstrated in short term fermentation trials.

Work was completed on this program in May of 1995, culminating in manuscript in press. The conclusions of this manuscript may be summarized as follows:

- One-week-long fermentation trials using immobilized *Lactobacillus delbrueckii* with sorbent addition demonstrated a volumetric productivity (6.9 g/L·h) at least 16-fold higher than that of a free cell batch fermentation with base pH control (but no in situ product removal) and identical biomass concentration and media composition.

- Regeneration of the loaded sorbent from the BFBR affected a 35-fold concentration of lactic acid compared to original levels in the fermentation broth (70 vs. 2 g/L). Lactic acid concentrations as high at 610 g/L were observed when the loading solution contained 50 g/L lactic acid.

- Due to the fact that the BFBR minimizes product inhibition, expensive "rich" media formulations which contain proteins and buffers to help protect the biocatalyst from product inhibition were unnecessary. Rich media formulations did not seem to increase BFBR performance.

Gas-Phase Bioreactor Systems - Bioreactor concepts that provide for interaction with gaseous substrates are being investigated. ORNL is currently using bioreactors for the degradation and removal of dilute gaseous alkanes from effluent air streams. Advances in this technology hold promise for low-cost alternatives to more traditional energy intensive treatment methods such as incineration or adsorption. Elucidation of engineering principles governing the behavior of gas-phase reactor, such as mass transfer limitations will broaden their applicability. We are extending this effort into the use conversion of substrate vapors by organic-phase enzymes.

A microbial consortia has been enriched which can consume the sparingly soluble alkanes, n-pentane and isobutane as sole carbon and energy sources. Several of the strains in the consortia have been isolated as the responsible agents. We have tested three reactor systems - shake flasks, liquid-continuous bubble column, and a gas-continuous trickle-bed. Sustained degradation of the volatile organic compound has been sustained for 33 months in the bubble column with a maximum rate of ~2 g/h/m^3 and for 19 months in the trickle bed with a maximum rate of ~60 g/h/m^3. A method was developed to control overgrowth and plugging of the trickle-bed biofilter while sustaining high activity by controlling nutrients.

Effective overall mass transfer coefficients were estimated for the reactors. The coefficients were 3 times higher in the trickle-bed than the bubble column. A novel method to estimate mass transfer coefficients was developed for use in active trickle-beds by using temperature to change the intrinsic rate. Mass transfer was determined to be controlling in the shake flasks and bubble columns; at lower temperatures and VOC loadings kinetic limitations were apparent. A predictive mathematical model has been developed.

Biocatalysis in Nonaqueous Media - Part of the increased emphasis in non-aqueous bioprocessing was the completion of a summary report, "Bioprocessing in Non-Aqueous Media: Critical Needs and Opportunities." Experimental and modeling tasks have begun as above and in the areas of interfacial phenomena and contacting schemes using enzymes in organic media. ORNL is emphasizing biphasic liquid system and gas-liquid systems.

These gas techniques above are now being extended to initial efforts using gaseous substrates with the biocatalysts in an organic phase to improve reaction and product solubility. The initial model system is the oxidation of phenol to quinone by polyphenol oxidase in various organics.

ORNL is developing methods and experimental systems to be used in assessing the function and enhancement of enzymes in biphasic systems. Two model systems are being assessed. One is the oxidation of p-cresol by horseradish peroxidase (HRP) with a second immiscible liquid phase, toluene. Kinetics studies indicate that the toluene appears to interact with and protect the HRP against deactivation by hydrogen peroxide. A second system is being developed in which the organic phase will be cyclohexane.

There has also been an effort on the design and mathematical description of experimental devices that will allow examination of effects of liquid-liquid interfaces on enzyme reactions. Two types of devices are being developed for this task: (1) a single droplet suspended in a tapered channel, and (2) a laminar film device. The approach is to have very well defined two-phase hydrodynamics so that slight differences in enzyme behavior that occur due to the liquid-liquid interface can be separated from bulk effects.

DOW CRADA on Dehalogenation - The dehalogenation of process byproducts is being investigated in a confidential CRADA with Dow Chemical Co. ORNL has carried out model reactor evaluations, has cultured the wild-type organism to produce small quantities of enzyme, and has built a continuous reactor system that will allow long-term biocatalyst test to be performed. ORNL has also identified a suitable immobilization gel that will withstand contact with the chlorinated compounds. DOW has developed an over producer of the enzyme.

5. Project Title **Design, Characterization, and Testing of Multimetallic Catalysts For Pollution Control**

Principal Investigators **Terry King and Andrew DePristo**

Project Site **Ames Laboratory**
Iowa State University

Description:

The research goals are to develop and implement sophisticated methods of modeling small, bimetallic particles composed of on the order of 100 to 1000 atoms; to predict morphology and surface properties as a function of composition, reactive environment, and support material using these methods; and to test and validate the prediction experimentally using traditional techniques as well as unique, solid state NMR studies of metals and adsorbates.

The use of supported bimetallic catalysts in industry is extensive in spite of the general lack of fundamental understanding of how the small metal particles interact with the reacting species. The present activity focuses on one very important application, automobile exhausts catalysts, with the intent of generating the scientific basis needed to design the next generation of catalysts in an efficient manner. Consequently the proposed work encompasses basic and applied research that is heavily dependent on a close relationship with researchers at General Motors Research Laboratories. The major challenge for this program, and the reason for the close industrial collaboration, is the application of the modeling and experimental technique developed at the Ames Laboratory to more complex catalysts.

The exhaust from internal combustion engines contains small concentrations of hydrocarbons and CO from incomplete combustion of the fuel, and of nitrogen oxides, NOx, from nitrogen fixation at the high temperatures of combustion. These contaminants are major contributors to air pollution. Federal legislation enacted in 1981 set the maximum allowable levels of pollutants for new cars and trucks (in grams per mile) as 0.41 for hydrocarbons, 3.4 for CO, and 0.4 for NOx. These figures were set on a gradually diminishing scale, and have been modified as the technical difficulties and the costs to remove the pollutants have become better understood. The original figure set for NOx has been particularly difficult to achieve, and was modified in 1982 to 1.0 g/mile. The original figure still remains a research goal. Catalysts used for oxidation of CO and hydrocarbons are based on Pt or Pt in the presence of small amounts of Pd. Pt is more active for oxidation of paraffins, Pd for oxidation of CO and possibly unsaturated compounds. In an oxygen-rich atmosphere Pt on alumina support sinters more readily than does Pd; Pt is, however, more resistant to lead poisoning than is Pd. Pd exists on the catalyst as the oxide, and Pt is more active as the metal, upon which oxygen is chemisorbed.

NOx must be removed by reduction to nitrogen gas. The most active catalyst is Pt, but some NOx are reduced to ammonia, which is not desired. The most effective catalyst for the reduction of NOx to nitrogen is one in which some Rh is added to Pt. Rh is not mined anywhere separately; Rh is obtained as a byproduct of production of Pt and Pd, the ratio being about 0.06 to 0.04 Rh to Pt in South African ores. Thus the optimum catalyst composition will be markedly influenced by market economics which set the price of a byproduct of strictly limited availability.

The potential impact of this activity on technology is large. The driving forces behind the development of improved automobile exhaust catalysts are both economic (i.e. the current catalysts use expensive platinum and rhodium) and legal (i.e. improved performance of emission abatement technology is mandated by the Clean Air Act). There will likely be applications to other technologies that employ supported metal catalysts, particularly in the petroleum and petrochemical industries.

Theoretical (molecular dynamic) calculations have predicted that for both systems, Rh tends to accumulate at the particle surfaces. Experimental verification is made by proton NMR spectroscopy. Hydrogen is adsorbed on the particles, and the relative field shifts of various particle compositions are used to infer the surface compositions. Experimental testing of the particles, using gases of various compositions, is being conducted at GM Research Labs.

1995 Accomplishments:

- The differential heat of adsorption of H on Rh and Pt has been measured for various H coverages using microcalorimetry;

- The DeCAL (Design of Catalysts at Ames Lab) software has been extended to incorporate H-atom adsorption with parametrization accomplished for H/Rh and H/Pt (i.e., the first step in treatment of multimetallic cluster segregation and shape in a reducing atmosphere);

- A collaborative effort has been initiated with DuPont researchers (who perform self-consistent density functional calculations) in order to parametrize O/Metal interactions in the DeCAL model;

- The DeCAL model has been parametrized accurately for both ordered and disordered alloys via a collaborative effort with researchers at the Center for Atomic-scale Materials Physics (at the Danish Technical University);

- A collaborative effort with Exxon researchers (who perform X-ray diffraction experiments) has yielded the structures of CuxPd1-x clusters with diameters of 40-60 C5 and composition range.

1996 Planned Activities:

The major effort is to finish the application software. This will include field testing through the summer of 1996, writing user guidelines, and developing marketing agreements with software companies. At the same time, we will continue work leading to full implementation of the chemisorption simulation capacities including the site dependent energetics of adsorption. We will finish the inclusion of atomic oxygen as an adsorbate and incorporate these capabilities into the software package. This package will be released in September 1996.

Annual Technical Summary Report:

During this year we have devoted considerable effort to finishing the parametrization of the theoretical models, as given in the accompanying tables, and testing them against the small amount of available experimental data. In addition, we have focused on the experimental characterization of supported bimetallic catalysts using 129Xe and 1H NMR and on the experimental determination of the energetics of adsorption of hydrogen on supported metal catalysts.

A major advancement has been our ability to produce more accurate parametrizations of the Bond Order metal Simulation (BOS) model from accurate self-consistent density functional calculations on ordered bulk alloys. Another advance has been the incorporation of H/Rh and H/Pt bonding into the BOS model, thereby allowing for the prediction of cluster structure and composition in reducing atmospheres.

Considerable effort has been expended in developing methods to experimentally validate the theoretical models. It must be noted that a major motivation in pursuing the theoretical studies is that the experimental determination of those surface properties that govern catalytic processes is difficult at best. For example, surface composition of highly dispersed bimetallic particles has been determined reliably in only a few specialized cases. More complicated properties such as surface micromixing have never been determined experimentally.

Table I. Mixing energies (kJ/mole) for bimetallic alloys, AxB1-x, as a function of composition.

	experimental or calculated values (input)							BOS-mixing model (output)[a]				
	composition					T(K)	e/c[b]	composition				
A-B	0.031	0.25	0.50	0.75	0.969			0.031	0.25	0.50	0.75	0.969
Ni-Au	0.65	4.82	7.56±0.42	5.89	0.84	1150	e	0.65	5.10	7.23	5.74	0.84
Cu-Ag	0.59	3.49	4.24±0.21	3.05	0.46	1423	e	0.52	3.10	4.13	3.10	0.52
Ni-Cu	0.10	0.90	1.78±0.42	1.73	0.30	973	e	0.10	0.95	1.71	1.62	0.30
Ni-Pd	-0.26	-1.18	-0.54±1.26	0.48	0.17	1273	e	-0.27	-1.08	-0.47	0.39	0.18
Ag-Au	-0.49	-3.29	-4.65±0.21	-3.59	-0.56	800	e	-0.50	-3.19	-4.39	-3.39	-0.57
	-0.49	-4.10	-5.38	-3.91	-0.56		c		-3.78	-5.27	-4.13	
Pd-Ag	-0.87	-5.06	-5.02±0.42	-2.64	-0.33	1200	e	-0.85	-5.44	-5.26	-2.45	-0.33
Cu-Au	-0.38	-3.00	-5.11±0.31	-4.36	-0.58	800	e	-0.38	-3.11	-4.94	-4.30	-0.58
Pd-Au	-1.07	-7.20	-7.78±0.08	-4.59	-0.59	298	e	-1.11	-6.06	-6.99	-4.43	-0.61
	-1.07	-7.82	-8.32	-5.92	-0.59		c		-8.01	-8.75	-5.11	
Ni-Pt	-1.00	-6.18	-9.26±0.84	-6.71	-0.85	1625	e	-1.07	-5.88	-7.47	-5.32	-0.90
	-1.00	-11.97	-11.75	-8.75	-0.85		c		-9.53	-12.05	-8.54	
Cu-Pd	-1.18	-7.89	-10.70±0.42	-8.93	-1.31	1350	e	-1.18	-7.87	-11.02	-8.66	-1.31
Cu-Pt	-0.90	-6.97	-11.09±0.63	-9.42	-1.61	1350	e	-0.91	-6.45	-11.16	-10.29	-1.57

a Using the parameters in Table II.

b e: experiment; c: calculation.

c Measurement error for mixing energy at x=3D0.50; relative measurement= errors for mixing energies at x=3D0.25 and 0.75 are same as that at x=3D0.50;= relative measurement errors for mixing energies at x=3D0.031 and 0.969 are equal to half of that at x=3D0.50.=20

Table II. BOS-mixing model parameters (kJ/mole) a.

A-B	$\Delta E^{A}_{12,\,A\text{-}B} + \Delta E^{B}_{12,\,A\text{-}B}$	$\Delta E^{A}_{12,\,A\text{-}B}$	$\Delta E^{B}_{12,\,A\text{-}B}$	$\lambda^{A}_{12,\,A\text{-}B}$	$\lambda^{B}_{12,\,A\text{-}B}$
Ni-Au	6.61±1.67	5.84±1.67	0.77±1.67	-0.89±0.31	-0.79±0.31
Cu-Ag	1.38±0.04	2.86±0.04	-1.48±0.04	0.0	0.0
Ni-Cu	0.91±0.37	4.27±0.37	-3.36±0.37	-0.12±0.07	-0.02±0.07
Ni-Pd	-0.41±0.24	2.01±0.24	-2.42±0.24	-0.06±0.05	0.16±0.055
Ag-Au	-1.83±0.31	-4.37±0.31	2.54±0.31	0.09±0.05	0.06±0.05
Pd-Ag	-2.52±0.34	2.52±0.34	-5.04±0.34	0.05±0.05	0.30±0.06
Cu-Au	-1.46±0.33	-2.02±0.33	0.56±0.33	0.08±0.06	-0.02±0.05
Pd-Au	-2.69±0.50	-1.02±0.50	-1.67±0.50	-0.05±0.08	0.19±0.09
Ni-Pt	-1.18±0.67	-6.22±0.67	5.04±0.67	-0.30±0.12	-0.22±0.12
Cu-Pd	-5.40±0.80	-4.31±0.80	-1.09±0.80	0.41±0.13	0.34±0.13
Cu-Pt	-5.22±0.78	-12.06±0.78	6.84±0.78	0.51±0.13	0.19±0.12

6. Project Title	**Biocatalysis by Design**
Principal Investigators	**William Goddard**
Project Site	**California Institute of Technology** **Materials Simulation Center**

Description:

Project is directed to the development of tools for atomic scale modeling and simulation of biological systems, and the prediction of critical parameters for experimental validation of the models. Project will also assess the critical impediments to the widespread application of biotechnology in industry, and will examine possible approaches to overcoming the barriers, using the tools developed at the Materials and Molecular Simulations Center (MMSC). The MMSC was established as a place where academic, government, and industrial researchers can work together on theoretical studies related to catalyst design and structure using molecular dynamics, quantum mechanics, and computer graphics capabilities. The MMSC is a means of technology transfer between the researcher and the end user. The needs of industry therefore direct the project goals and orientation.

Methods and techniques to permit the prediction of the structure and hence function of proteins are under investigation. Project goals include the design of proteins that recognize specific DNA sequences. These proteins would be used to develop regulatory proteins for controlling biocatalysts. In particular, the pseudo-spectral generalized valence bond (GVB) techniques will be used. The current chemical mechanical calculations, the first step in molecular mechanics simulations, are extremely cpu intensive. Consequently, these calculations are restricted to molecular systems that are small (10-20 heavy atoms). However, most industrial applications of interest are much larger in number of atoms of interest and are thus much more computer intensive (in terms of method of calculation) than is practically feasible. Recent developments in the pseudo-spectral GVB techniques, which use ideas originally developed to solve fluid dynamics simulations, have made it more feasible to calculate systems of 100 atoms, which would help substantially ab initio calculations on crystals and systems with periodic boundary conditions. Massively parallel computing capabilities will be necessary to accomplish this goal and to expedite these calculations into reasonable cost/time operations.

1996 Planned Activities:

The Hierarchical Protein Folding Project:

Prediction of tertiary structure from primary sequence. Our major focus in the next year will be to validate our new Boltzmann based Continuous Conformational Monte Carlo (BB-CC-MC) methodology and to use it for de novo predictions of the structures for important biotechnical systems. This will allow entirely new strategies in biotechnology and drug design.

Design of New Site Selective Proteins for Controlling Gene Expression:

The Protein Stitchery strategy is now validated, and we will be helping others in using this strategy to develop proteins for controlling gene expression.

Protein Design Using Modeling and Mutation:

We will continue the process of designing proteins that have modulated redox properties. A particular focus next year will be coordinating these advances with other BCTR projects: [with Beratan (Pittsburgh) on predicting electron transfer and with Greenbaum (ORNL) on photosystem II].

Activation of Ch_4, Alkanes, Co_2:

We will continue the development of catalysts for selective conversion of Ch_4 Co_2 and other alkanes to useful chemical. The Project using porphyrins is in collaboration with Sandia (Shelnutt) and the one using heterogeneous catalysts is with BP Chemical.

Oil Field Science:

We will develop demulsifiers to break up the asphaltene-oil-water emulsions responsible for preventing extraction of oil from some fields. This work will be supported by Aramco, and we are in the process of discussing additional support of this very important project by other companies.

Process Simulation:

We will continue the development of practical supercritical extractions in collaboration with Chevron Petroleum Technology Co. The focus here will be on the predicting the role of additives in promoting extraction of specific compounds for oil fields, from wastes, and form contaminated soils. Our approach is to use interaction energies from theory to modify the equation of state for the supercritical Co_2-additive-extractant system. This same approach will be useful as the basis of phase diagrams in multi-component systems so important in process simulation, and we are discussing new industrially supported projects in this area.

Industrial Collaborations:

We will continue to seek collaborations with industry on problems for which atomistic simulations could play a critical role in solving these problems. It is anticipated that a new collaboration will be initiated with Owens-Corning on surface treatments of glass fibers and with Hewlett-Packard on the process of bubble formation in ink-jet printers.

Commercialization:

We will continue to interact with Schrödinger, Inc. and with Biosym-Molecular Simulations, Inc. to commercialize the quantum mechanical and molecular dynamics software developed at the MSC.

Annual Technical Summary Report:

The Hierarchial Protein Folding Strategy:

Prediction of tertiary (three dimensional) structure from primary sequence. This is the Grand challenge problem in Biotechnology and drug design. We have been working on this problem as a BCTR project for 10 years and have reached some success this past year. Our Hierarchical Protein Folding Strategy (HPFS) involves four parts, three of which were developed in previous years. Over the last two years developed the fourth and final part of this project, a new methodology is called Boltzmann Biased Continuous Conformational Monte Carlo (BB-CC-MC).

We are in the process of validating this methodology for known systems and expect to be ready for *de novo* predictions by 1996.

Design of New Site Selective Proteins for Controlling Gene Expression:

The validation of the Protein Stitchery strategy has been completed and published. It is starting to be used by others.

Protein Design Using Modeling and Mutation:

We have applied this approach to developing a model compound having the spectra of the CuA site of the membrane protein cytochrome oxidase. This model compound has been synthesized and spectroscopic experiments now confirm these predictions. Recent crystallographic studies on native structure proves our approach.

New Processes for Activation of CH_4, alkanes, CO_2:

We successfully developed a mechanism to explain the highly selective conversion to butane to maleic anhydride by VPO catalysts. This catalyst is industrially successful (because of the lack of toxic side products) but the operation has been a mystery. This work is supported by BP Chemical.

Oil Field Science:

We created computer models of the asphaltene molecules responsible for forming oil-water emulsions that prevent extraction of oil from some oil fields. We are developing demulsifiers to break up these emulsions. This work is supported by Aramco.

Co_2 and Ch_4 Activation Using Porphyrins:

This project [joint with Sandia (Shelnutt)] has progressed significantly, with predictions of electronic and vibrational spectra and of structure for these promising catalysts.

Process Simulation:

As part of our new thrust into process simulation, we carried out studies of supercritical extractions in collaboration with Chevron Petroleum technology Co. We are using interaction energies from theory to modify the equation of state for the supercritical CO_2- additive-extractant system.

Industrial Collaborations:

New contracts providing direct support of MSC research have been initiated with 6 companies to complement the 7 already in place.

Commercialization:

The PS-GVB software partially developed at the MSC with BCTR support has now been commercialized by Schrödinger, Inc. This will lead to royalties to CalTech.

Training:

Four additional scientists partially supported by BCTR have finished their PhDs and are working for companies and universities.

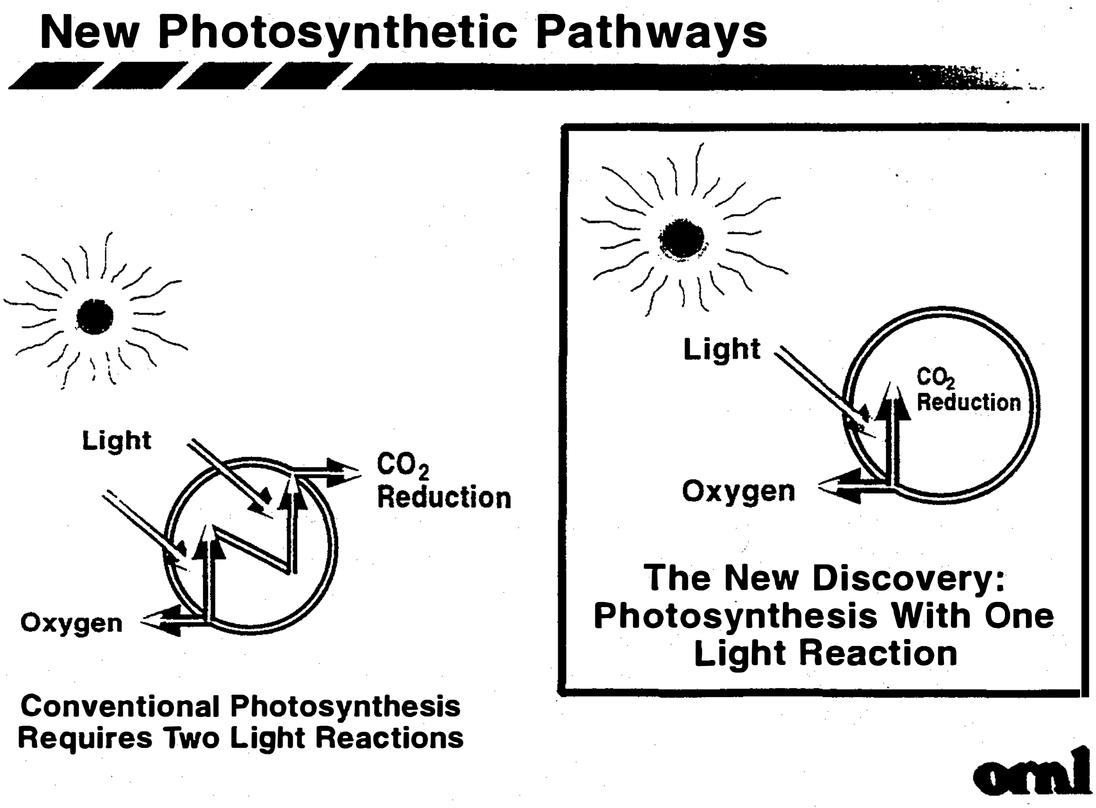
7. Project Title	**Photobiological Reactors**
Principal Investigators	**Elias Greenbaum**
Project Site	**Oak Ridge National Laboratory**

Description:

This mission-oriented research project is focused on the design and development of photobiological reactors for the production of renewable fuels and chemicals using atmospheric CO_2 as the sole carbon growth source and light harvesting and energy conversion by the photosynthetic apparatus of unicellular green algae. It consists of two specific mission areas. The first is the study and development of wild-type and genetically engineered mutant algae possessing unusual properties for the production of renewable fuels and chemicals from renewable resources. The second is the development of the reactors into which the algae are placed. Both aspects of this development program are pursued in parallel. The focus of research progress for the current reporting period is the discovery of a new photosynthetic pathway capable of reducing atmospheric CO2 using a single light reaction of photosynthesis.

1995 Accomplishments:

- Major technical advance in the discovery of a new single-light photosynthetic pathway for the conversion of atmospheric CO_2 to value-added chemicals in the genetically engineered mutants of *Chlamydomonas reinhardtii.* This discovery is illustrated schematically in the accompanying figure.



Conventional Photosynthesis Requires Two Light Reactions

The New Discovery: Photosynthesis With One Light Reaction

- This result is based on a new discovery in the Chemical Technology Division of Oak Ridge National Laboratory utilizing a mutant of the green alga *C. reinhardtii* that was developed in the laboratory of Professor L. Mets, University of Chicago. We have shown that although mutants B4 and 1047 lack the Photosystem I light reaction, they are capable of converting atmospheric CO_2 to reduced carbon compounds. This result is quite unexpected in the context of the conventional model of photosynthesis which envisions two sequential vectorial light reactions operating in series to perform complete photosynthesis. From an applied and industrial perspective, these results are important because they imply a potentially greater efficiency in the conversion of CO_2 to value-added chemical compounds.

1996 Planned Activities:

- The research plan for 1996 follows logically from the important results that have been obtained during the current reporting period. A further investigation of the phenomenon of single-light reaction photosynthesis and the conversion of atmospheric CO_2 to value-added chemicals will be pursued. In particular, the question of photosynthetic and quantum conversion efficiency will be investigated.

- The practical motivation for performing this work is improvement in yields and conversion efficiencies in photosynthesis. The conventional model of photosynthesis utilizes two photons to move on electron from the positive oxidizing potential of water splitting and oxygen evolution to the reducing potential of CO_2 fixation. However, if the same results can be achieved with a single light reaction, a doubling of thermodynamic conversion efficiencies of light energy into chemical energy is expected. In 1996 experiments on the absolute energetic and quantum conversion efficiencies in mutants and wild-type Chlamydomonas will be performed.

Annual Technical Summary Report:

Although mutant B4 of *C. reinhardtii* lacks Photosystem I, it is capable of photoautotrophic assimilation of atmospheric carbon dioxide and sustained simultaneous photoevolution of molecular oxygen and hydrogen. At saturating light intensities, carbon dioxide reduction was stable under anaerobiosis but unstable in air. At lower light intensities, carbon dioxide reduction was stable in both atmospheres. The data indicated that Photosystem I is not necessary for autotrophic photosynthesis. One interpretation of these results is that oxygenic photosynthesis developed as a single-light-reaction process, presumably from a pheophytin-quinone-reaction-center bacterium but became unstable as oxygen in the earth's atmosphere accumulated. Photosystem I was the second light reaction, added to confer stability in oxygen-containing atmospheres. Viewed from this perspective, the well-known Z-scheme of modern photosynthesis is seen as a specialized adaptation for performing low-potential reductive photochemistry in oxygen-containing atmospheres, but is not an irreducible necessity for satisfying the thermodynamic and mechanistic requirements of carbon dioxide photoreduction using water as the source of reductant.

Mutant B4 of *C. reinhardtii,* deposited with the Duke University Chlamydomonas Genetics Center and available as Stock No. CC2933, was obtained following metronidazole enrichment described in detail elsewhere. Analysis of the algal cultures before and after the experiments indicated that this mutant had a chlorophyll fluorescence induction transient characteristic of PSI-deficient strains and lacked the reaction center core (CPI) complex in LDS electrophoresis at 4 C. Complete absence of P700 in B4 samples was also confirmed by EPR and by absorbance spectroscopic measurements. Genetic analysis indicated that the B4 mutation is carried in a nuclear gene and is unable to assemble the functional message for the psaA gene encoding one of the two major apoproteins of the PSI complex.

This property has been described previously for several other nuclear gene mutants of C. reinhardtii that evidently encode components essential for the trans-splicing of this tripartite message. In the absence of the psaA message, the cells were unable to synthesize any PSI complexes.

Mutant B4 was grown photoheterotrophically using minimal growth medium with acetate. The reaction itself was performed in minimal growth medium by successive washings to eliminate all soluble acetate. Data for mutant B4 and the control wild-type 137c were obtained simultaneously in a dual-photoreaction chamber flow system. To maintain anaerobiosis, even during photosynthetic oxygen production, the algal suspension was continuously purged with ultra-high purity O_2-free carrier gas at 50 ml/min.. Therefore, regular respiration or chloroplast respiration play no role in the experiments performed under anaerobiosis, either in helium or CO_2 in helium. The experimental technique for measuring the simultaneous photoevolution of oxygen and hydrogen has been previously described. Even though it totally lacks Photosystem I, sustained simultaneous photoevolution of hydrogen and oxygen was observed, demonstrating that a Photosystem II-driven reaction can span the potential difference between water oxidation/oxygen evolution and proton reduction/hydrogen evolution. Since the steady-state flow stream concentrations (< 10 ppm) of hydrogen and oxygen are far from the standard state, these data place an upper limit on the potential spanned by the Photosystem II light reaction which is less than the standard value. The reversible equilibrium thermodynamic potential for these two reactions is 1.23 V6. Of course, since the reactions do not occur reversibly at thermodynamic equilibrium, the actual potential must be higher, by about 0.2-0.4 V.

With CO_2 present in the carrier gas, there is an initial surge of hydrogen which peaks and then declines to zero while the light is still on. This hydrogen photoevolution pattern for wild-type *Chlamydomonas* has been previously analyzed and reported. Briefly, when the algae have been dark-adapted, the Calvin-Benson cycle is shut off. Consequently, upon illumination all reducing equivalents are shunted through the ferredoxin/hydrogenase pathway. As the Calvin-Benson cycle is activated by light, it becomes the exclusive sink for reducing equivalents. The monotonic decrease in the rate of hydrogen production from its peak value represents the real-time activation of the CO_2 reduction cycle. When the light is turned off, thermally-activated hydrogen evolution is observed. This pattern repeats itself when the light is turned on again. The source of thermally-activated hydrogen is reduced carbon, identified by Klein and Betz as primarily starch, that has been stored during previous photosynthesis. *C. reinhardtii* grows quite well in an anaerobic atmosphere consisting of CO_2 and helium. In a pure helium atmosphere, the hydrogen time profiles are qualitatively different. They climb monotonically to an approximate steady state production rate with the light turned on and return to zero when it is turned off. Since CO_2 is not present in this atmosphere, the CO_2 -reduction cycle is not activated and does not serve as a sink for reducing equivalents. Oxygen-evolving activity is less in helium, presumably due to the absence of CO_2 and the Photosystem II bicarbonate requirement. Irradiation with > 700 nm light (which supports PSI but not PSII) in the wild-type alga results in a low, but significant, rate of hydrogen photoevolution. The source of reducing equivalents for this PSI-dependent hydrogen production is the pool of reduced electron carriers in the electron transport chain linking the two Photosystems. Under strict anaerobic conditions, this pool is replenished by the reserve of endogenous reductants described above. For B4, > 700-nm, as expected, shows no PSI hydrogen production component. Instead, thermally activated hydrogen evolution was suppressed. These control data, in conjunction with the controls described above indicated the absence of PSI before, during, and after the experiments.

Perhaps the most remarkable aspect of the data for mutant B4 for CO_2 in helium is that the Calvin cycle is evidently activated in this system and serves as an electron sink. This is positively demonstrated by measuring the photoassimilation of CO_2 by both mutant and wild-type. It therefore follows that mutant B4 can perform complete oxygenic photosynthesis using a single Photosystem II reaction. At saturating-light intensity, 500 W/m2 (PAR),CO_2 photoassimilation is stable for both mutant B4 and wild-type *Chlamydomonas* in an anaerobic atmosphere. However, only the wild-type alga, containing both Photosystems I and II, is stable in air. Within 24 hours, mutant B4 lost 90% of its photosynthetic activity. Thus, we concluded that PSI is required for stable oxygenic photosynthesis in aerobic environments (the normal condition in nature) but not under anaerobic conditions. Since the oxidant generated in Photosystem II must be more oxidizing than +0.8 V, it follows that a single Photosystem II reaction center has the thermodynamic potential to perform complete photosynthesis as does the energy content of a single photon absorbed by PSII. Thus, although our findings are novel and unexpected, they do not violate known thermodynamic requirements of the photosynthetic process.

A number of observations in the literature have possibly suggested that PSII alone could reduce ferredoxin and thus NADP+. Working with *Chlamydomonas* ACC-1, a mutant that lacked Photosystem I, Klimov et al. observed the photoreduction of NADP+. However, oxygen evolution was not demonstrated, and this was interpreted as a non-physiological reaction, an alternative electron pathway occurring only at high light intensities. Boychenko et al., utilizing a polarographic technique, were able to observe transient evolution of both hydrogen and oxygen from a mutant of Chlamydomonas that was deficient in PSI. Arnon and Barber reported the photoreduction of NADP+ by isolated reaction centers of Photosystem II with 1,5-diphenylcarbazide as the electron source. The reaction centers of this work were not functional oxygen evolution preparations; the key emphasis of the Arnon-Barber paper was the requirement for plastocyanin. More recently, Allakhverdiev and Klimov demonstrated the requirement of manganese for anaerobic photoreduction of NADP+ by Photosystem II of higher plants. The primary electron acceptor of Photosystem II is pheophytin, which has an Em of -0.61 V.

Irrespective of specific molecular mechanisms, our data clearly illustrate a new type of photosynthesis being performed by the Photosystem II light reaction. These experiments also have implications for the evolution and origin of water-splitting photosynthesis. Anoxic photosynthetic bacteria exhibit two different mechanisms characterized by either pheophytin-quinone or Fe-S type reaction centers. Blankenship proposed that "some sort of genetic fusion event took place between two bacteria" producing a chimeric organism. Subsequently, the two systems were linked, and the oxygen evolution system was added. However, no examples of the intermediate stages have been observed. Our data support an alternative hypothesis: a bacterium containing the pheophytin-quinone reaction center developed into the oxygen-evolving PSII type of photosynthesis, with photosystem I being added later for survival when molecular oxygen became a major component of the earth's atmosphere. Ananyev et al have recently demonstrated the photoproduction of superoxide radicals by Phtotsystem II membrane fragments. This may be related to our unsuccessful earlier attempts at observing hydrogen photoproduction by platinized Photosystem II preparations.

From a practical point of view, a single-light reaction implies that the maximum thermodynamic conversion efficiency of light energy into chemical energy, as represented by the Gibbs free energy of molecular hydrogen, can be potentially doubled, from about 10% to 20% since a single photon rather than two is now required to span the potential difference between water oxidation/oxygen evolution and proton reduction/hydrogen evolution.

8. Project Title **Theoretical Studies of Hydrocarbon Catalysis**
Principal Investigators **P. J. Hay and Anthony Redondo**
Project Site **Los Alamos National Laboratory**

Description:

The original goal of this project was to develop computational tools to model chemical transformations of small hydrocarbon molecules within zeolite catalysts. The project has been expanded, and now includes AICD Catalysis-by-Design (CACD) coordination activities of inorganic catalysts, together with an experimental program to provide verified, generic modeling tools for catalysts of interest to industry.

In this project, catalyst research has focused on the details of carbon-carbon bond formation within the pentasil zeolite, ZSM-5. This catalyst has special significance for energy applications; for example, it is used in the "methanol-to-gasoline" process, and has many uses in conventional petroleum cracking. Pentasil zeolites provide unmatched shape selectivity for hydrocarbon molecules; hydrocarbon molecular weight produced within zeolite cannot exceed the limit of light hydrocarbons of gasoline fractions.

Many of the details of the catalytic processes are unclear, however, and warrant fundamental investigations if the yield and selectivity are to be improved, and consequently energy be saved in these processes. These details include the nature of the acid sites responsible for catalysis, and transport properties such as absolute diffusivities. Models of the adsorption and bond formation processes within zeolites will be derived by classical (molecular-dynamics), quantum, and quantum-statistical methods.

Catalyst research will also include modeling and experimental studies of metal oxide oxidations of hydrocarbons. These case studies were suggested by industrial collaborators of the project. Specific project technical objectives include: 1. predict the chemical state and the role of alumina in zeolite catalysis, and verify the predictions by X-ray absorption; 2. use solid-state ionic conducting ceramics to study hydrocarbon oxidations, using new experimental techniques to control the surface free energy. Specific CACD coordination activities include: 1. establish industrial/DOE collaboration programs and workshops; 2. integrate computational tools developed at LANL into other DOE-sponsored projects, to transfer the computational tools to industry; and 3. set experimental standards and protocols for experimental verification of the models.

1995 Accomplishments:

Zeolite catalysis :

We have been examining the structures, energies and reaction pathways of various C5 carbocation species involved in hydrocarbon cracking in zeolites using ab initio and semi-empirical quantum chemical methods. Processes leading to fragmentation of saturated and unsaturated hydrocarbons upon protonation in the gas phase have been investigated using pentane and pentene, respectively, as prototypes for paraffins and olefins. These results have been compared to experimental thermochemical data to benchmark various levels of theory.

The chemistry of species formed from pentane and pentene interacting with acid sites in the zeolite catalyst faujasite has then been investigated using cluster models to represent a portion of the zeolite. We find that pentene can be protonated at the acid site with a low barrier (~ 20 kcal/mol) in a process that is slightly endothermic. Direct protonation of pentane, by contrast, requires a high barrier in a process that ultimately leads to cracking into smaller fragments. Alternative pathways that have lower barriers are currently being pursued. The major finding has been that hydrocarbon species involved in cracking should not be regarded as true carbocations but that they have altered chemical properties intermediate between covalent and ionic forms.

Zeolite synthesis modeling:

Template-zeolite interactions have been studied using various force fields to understand the role of structure-directing organic templates that preferentially lead to specific zeolite formation from solution. The ability to model these interactions in a realistic manner would aid the synthesis of zeolites with desired pore structures using specific organic template molecules. The ability of three different force fields from the literature to predict the unit cell size of the zeolite itself has been investigated. The most successful of these approaches was used to model the organic molecule-zeolite interaction where only steric effects were included (ignoring electrostatic interactions) of several organic amine compounds and zeolites ZSM-12 and SSZ-24. Although a reasonable correlation was found between calculated interaction energies and the preferred zeolite formed, our results show that steric effects alone cannot account for structure-directing effects.

Force fields:

During the past year we have implemented and have begun validating a new procedure for obtaining partial charge distributions for use in molecular mechanics as well as for use in other methodologies that require a rapid charge estimation procedure. The new procedure is called QEq2. In addition, we have formulated a way of estimating resonance energies within molecular mechanics. This will permit screening transition state energetics as well as transition state structures for reactions involving zeolites and hydrocarbon oxidation.

BCTR program coordination.:

The report of the DOE-industry workshop on Computer-Aided Catalyst Design (April, 1994) in Washington, D.C. was issued during the past year and disseminated to participants as well as other interested parties in industry, government, universities and national laboratories. The annual BCTR program review was held in Albuquerque, NM from July 22-24, 1995.

1996 Planned Activities:

Zeolite catalysis:

We plan to continue to map out other cracking and rearrangement pathways of pentane and pentene at zeolite acid sites to cover the major proposed pathways for hydrocarbon cracking in zeolite catalysis. Larger clusters in either density functional or semi-empirical calculations will be used to examine the effects of including more realistic descriptions of the acid site region of the zeolite. Future work should also involve the development of kinetic models for determining molecular products from cracking as a function of initial gas oil composition.

Zeolite synthesis:

We plan to investigate the effects of electrostatic interactions between organic template molecules and the zeolite pore structure using semi-empirical quantum chemistry approaches in order to determine the important factors that determine which zeolite will be formed from solution. Our earlier results indicated that steric effects alone, which basically reflect the relative sizes of the template molecule and the zeolite pore, were insufficient to account for the structure directing activity of the organic template molecules in the case of ZSM-12 and SSZ-24.

Force fields:

During 1996 we will validate QEq2 for zeolitic systems by carrying out comparisons of QEq2 derived charge distributions with charge distributions obtained from *ab initio* electronic structure methodologies. Further, during 1996 we will test the generality of the molecular mechanics resonance term for reactions of relevance to cracking to see if molecular mechanics can correctly predict which hydride transfer processes are transition states and which are stabilized equilibrium structures. Comparison will be made with respect to *ab initio* electronic structure results and experimental data. Finally, during 1996 we will use the validated *ab initio* electronic structure methodology for studying the reactions between hydrocarbons and metal.

Annual Technical Summary Report:

Zeolite catalysis (P. J. Hay, A. Redondo, Y. Guo, LANL)

We have been examining the chemistry that occurs during hydrocarbon cracking in faujasite (zeolite Y), since commercial fluidized catalytic cracking processes in the refining industry employ this catalyst in the production of gasoline and other hydrocarbon feedstocks. According to currently accepted mechanisms, the acid site in the zeolite serves to protonate either saturated (paraffins) or unsaturated (olefins) hydrocarbons, which subsequently undergo chemical reactions leading either to molecular isomers (isomerization), to lighter molecular fragments (cracking), or to heavier products (polymerization).

Pentane and pentene have been chosen as representative paraffin (alkane) and olefin (alkene) molecules to study in this activity. They are small enough where calculations at various levels of sophistication can be carried out on them but large enough to have a sufficiently rich chemistry that is typical of cracking processes. The project has been divided into two parts--gas phase chemistry and zeolite chemistry. The first phase has involved a systematic study of the carbocations produced by protonation of pentane and pentene in the gas phase and the subsequent reactions that occur involving these positive ions. In many cases the thermochemistries predicted by calculations can be benchmarked by comparison with data from gas phase ion experiments or from "magic acid" solution studies.

Gas phase carbocation chemistry. Considering first protonation of the olefin pentene (C_5H_{10}), various $C_5H_{11}^+$ carbonium ions can be formed. One obtains the relative stabilities 3-pentyl ≈ 2-pentyl < 1-pentyl ion as one might have expected since primary ions (1-pentyl) are generally less stable than secondary ions. Two types of reactions of these species have been studied: rearrangement reactions between these species and cracking, or "scission", reactions to form smaller fragments. We have examined the barriers for the former rearrangement processes and find low barriers for interconversion of 1- and 3-pentene (1-2 kcal/mole) compared with higher barriers (~5 kcal/mol) between the two secondary ions. This is consistent with the inability to detect primary ions experimentally. The latter process is shown in Figure 1, where three possible olefin + alkyl cations are formed from the "cracking" reaction. Each of these processes is endothermic relative to the parent cation but requires no additional barrier for reaction beyond the reaction energy.

For protonation of the saturated pentane paraffin species, nonclassical carbonium ions are formed corresponding to protonation of either C-H or C-C bonds. These metastable species can then undergo fragmentation either into carbenium ion + H_2 for C-H protonation or into two hydrocarbon fragments (e.g., $C_3H_7^+$ + C_2H_6) for C-C protonation. The preceding calculations were carried out at the Hartree-Fock level using both small (STO-3G) and accurate (6-31G*) basis sets, at the MP2 level including electron correlation effects, and using the PM3 semi-empirical quantum chemical approach.

Hydrocarbon-zeolite chemistry. The interactions of pentane and pentene with the zeolite were modeled using a $Si_2AlO_{10}H_9$ cluster to represent the immediate vicinity of the Si-OH-Al acid site region. If we denote the zeolite as Z-H, the simplest picture of hydrocarbon interactions would describe the system as protonated pentene (RH^+) interacting with the negatively charged zeolite Z^-. We find that this picture is too extreme, based on calculations of the gas phase carbocation interacting with the Z^- moiety, which place either RH^+Z^- complex 100 kcal/mol higher than the neutral reactants.

Figure 1: Gas phase chemistry of C_5H_{11}+ carbenium ions

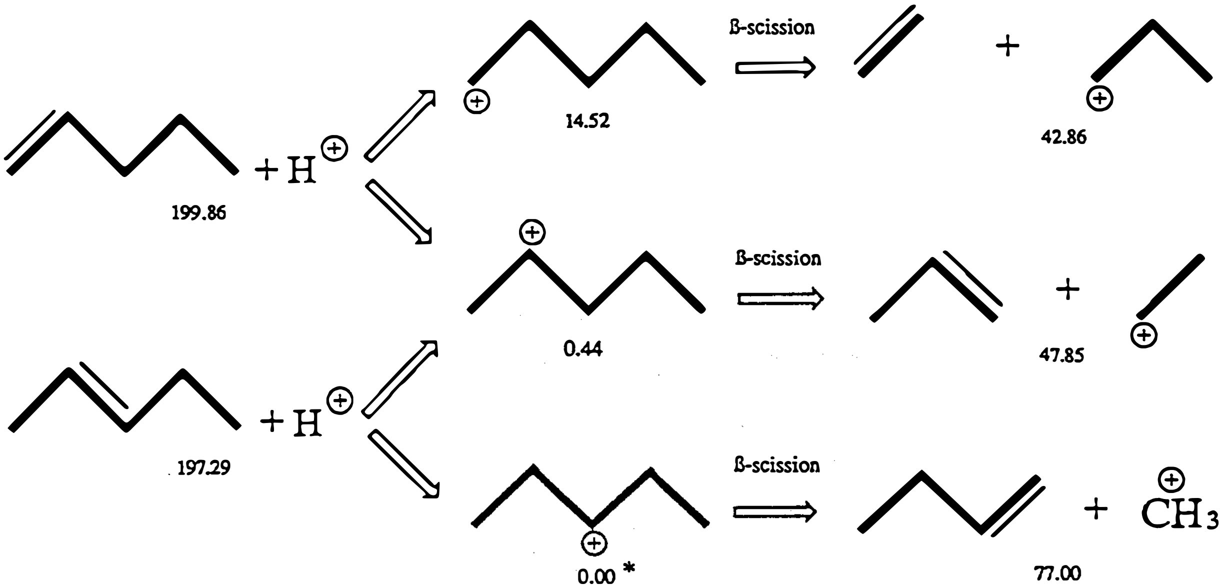
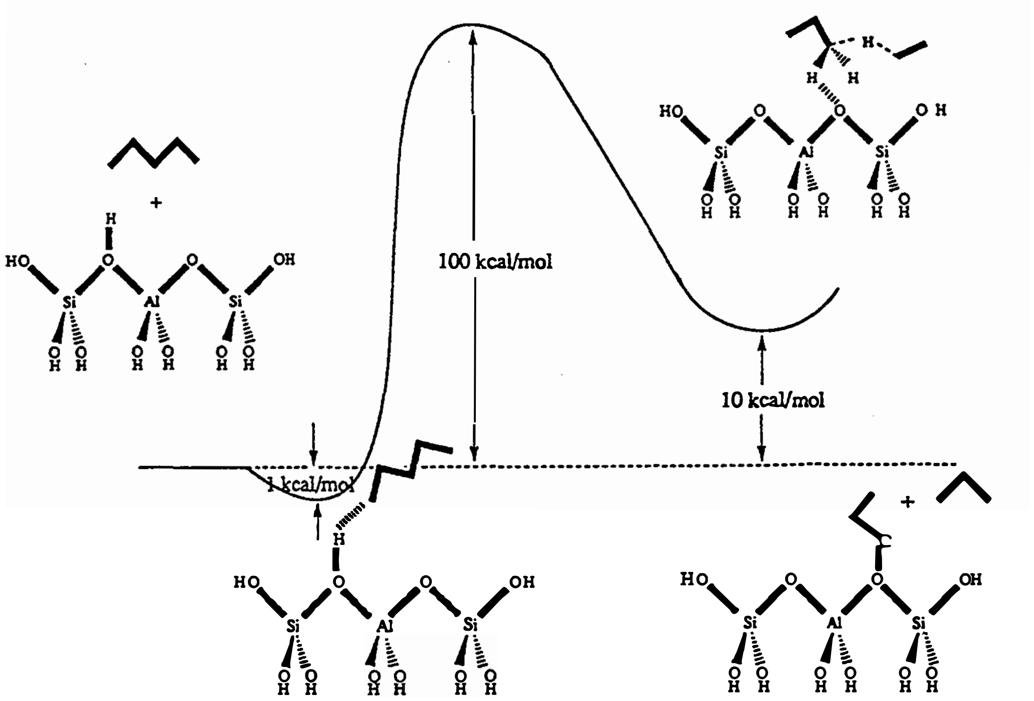
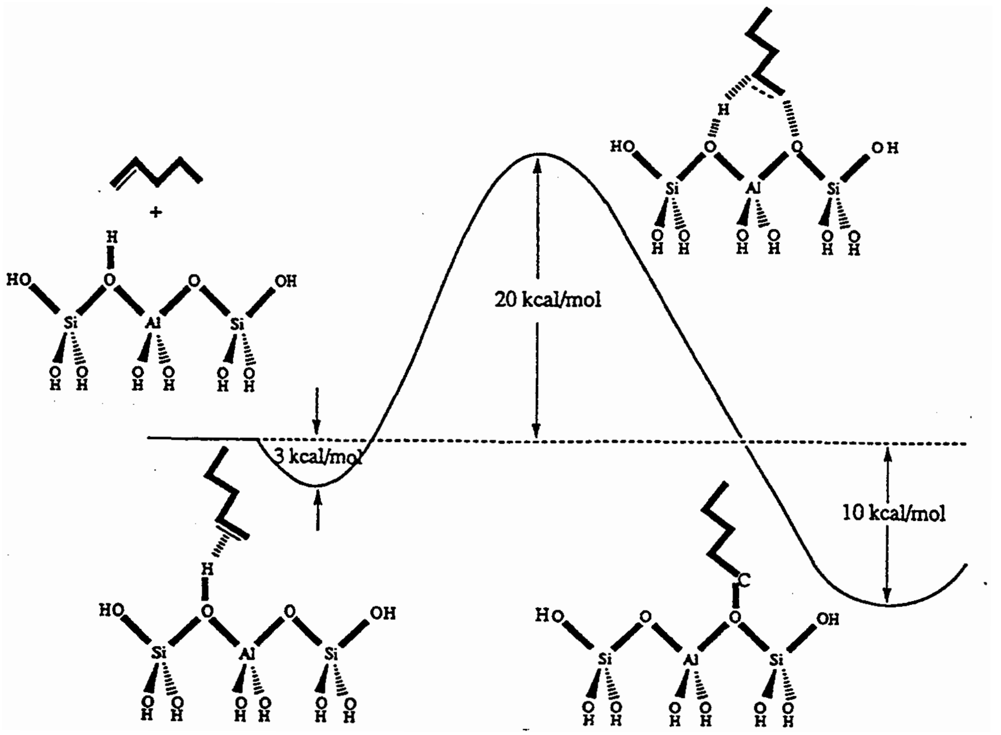


Figure 2: (a) Calculated interactions between pentene and a cluster model of zeolite Y containing 3 T sites.
(b) Calculated interactions between pentane and the same cluster model

a C_5 Paraffin + Zeolite Cluster



b C_5 Olefin + Zeolite Cluster



However, as shown in Fig. 2a, there is a concerted pathway for pentene that involves transfer of the proton and simultaneous formation of a C-O bond with the zeolite with a much lower barrier (~ 20 kcal/mol) and an overall energy for the process that is only 10 kcal/mol endothermic. For pentane, in Fig. 2b, however it appears that the direct transfer of the proton to the hydrocarbon requires a high barrier (as described above for ionic complexes), although ultimately cracking into a C_3 and a C_2 unit does result. We are currently examining alternative pathways for protonation of pentane and subsequent cracking pathways for the olefin-zeolite complexes.

Zeolite synthesis modeling (R. Lobo, A. Redondo, B. Holian)

Zeolites are normally synthesized under hydrothermal conditions using silican, alumina, NaOH, water and an organic molecule, typically a quarternary ammonium ion. The structure of the final product is highly dependent on the size and shape of the organic molecule, denoted the template or "structure directing agent". As an illustration, zeolites ZSM-12 and SSZ-24 are both all-silica one-dimensional large-pore zeolites with pore sizes of 7.4Å and 6.8 Å, respectively (Fig.3a). SSZ-24 has more spherical-shaped pore sizes while ZSM-12 has more elongated pores. In Fig. 3b we show several organic molecules, some of which result in formation of ZSM-12 while others form zeolite SSZ-24 under the same conditions. We have been using classical molecular mechanics and energy minization calculations as a possible strategy for the screening of organic structure-directing agents for the syntheis of novel high-silica zeolites. Three force fields have been used for the classical simulations: the UFF force field of Rappe et al. (see below) developed for inorganic molecules, a force field by de vos Burchard et al. (denoted dvB) developed to model the experimental properties of quartz and other silicate materials, and the cvff91 force field of Hill and Sauer. The first two force fields were examined using the Sirius code of Molecular Simulations and the third force field used the Discover code of Biosym Technologies. Predictions of the unit cell parameters were carried out using these three approaches, under either constant volume or constant pressure constraints.

For template-organic interactions, a combined UFF-dvB force field was employed. For the first phase of this study, electrostatic interactions between the quartenary ammonium ion and the zeolite lattice were ignored. This assumption is equivalent to replacing [Al-O-Si]- linkages by neutral Si -O-Si linkages in the zeolite and treating the positively charged nitrogen by its neutral carbon counterpart. Since the size and shape of the organic molecule are believed to be the dominant factor influencing the pore shape of the resultant zeolite, this study examines the extent to which nonbonded steric interactions control the ultimate structure of the zeolite as it emerges from solution. The neutral organic counterparts to the nitrogen quarternary ions that were used in this study are also shown in Fig. 3b.

The template-zeolite interaction energies obtained from these simulations are summarized in Table 1. In each case the positions of the atoms in the lattice as well as in the organic molecule were relaxed according to the forces in the UFF-dvB potential. If the non-bonded interactions were the dominant factor in structure-directed synthesis of zeolites, the more negative energy would correspond to the observed zeolite. In this particular set of simulations, four out of the five observed zeolites give rise to the lower interaction energy. These results, along with the other simulations that have been performed to date, indicate that steric effects determined by these non-bonded interactions are not sufficient to account for the preference of a template molecule in directing the synthesis of a particular zeolite. We are now in the process of electrostatic effects between the zeolite and the organic template to determine the role they play in zeolite synthesis.

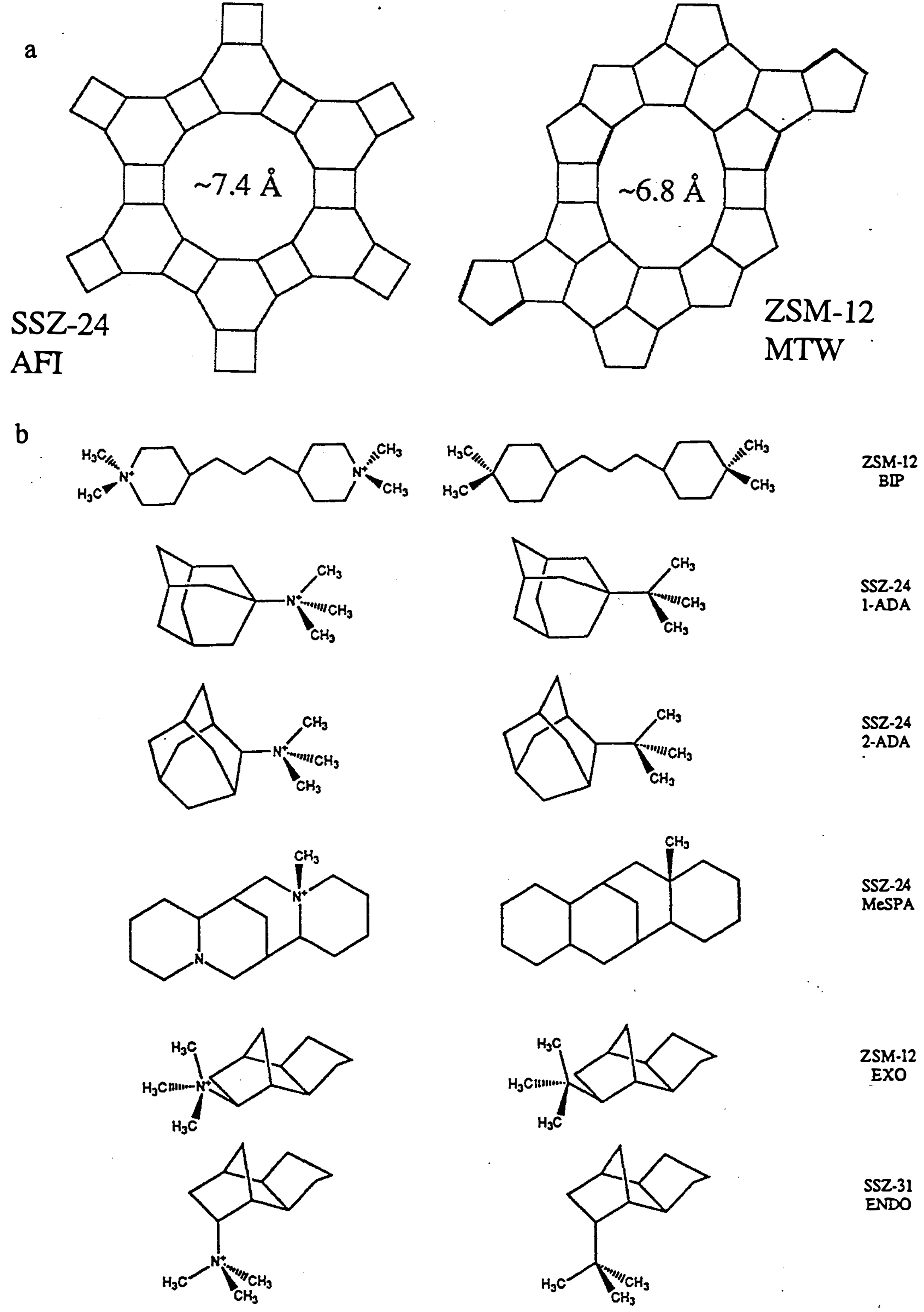


Figure 3: (a) Structures of zeolites ZSM-12 and SSZ-24
(b) Structure-directing organic molecules used in zeolite synthesis (left) and model compounds used in simulations of zeolite-template interactions (right).

New approaches to force fields (A. Rappe, Colorado State University, P.J. Hay, A. Redondo, Los Alamos National Laboratory)

The major developments in the past year have been (1) the implementation of a new force field model (QEq2) for zeolites and other inorganic systems and (2) the incorporation of ionic-covalent interactions and "resonance" effects into classical molecular models.

QEq2 force field development. In order to understand the role that a zeolite lattice plays in controlling the catalytic cracking product distribution, we previously studied the charge redistribution that occured for hydrocarbons when they were placed in a zeolite Y supercage using the QEq charge estimation procedure. We found ground state hydrocarbons to be unperturbed by the field provided by the lattice but that a model transition state for the hydride transfer step of the cracking chain reaction was significantly perturbed by the lattice. This difference was attributed to the hydride transfer transition state being larger and hence bulkier that the ground state reactants---not an unexpected result. However, we also found unrealistic degrees of charge transfer between the zeolite and the hydrocarbons using QEq. This and the relative slowness of QEq led to the development of QEq2 summarized below.

A major remaining weakness underlying current molecular simulation methodologies is the treatment of electrostatics. Current approaches predominantly employ fixed charge distributions and further, generally use an electrostatic moment expansion truncated to either monopoles or dipole moments. While the use of these approximations has led to numerous useful results, improved methodologies are certainly needed. The concept of electronegativity was developed to describe the flow of electrons from one center in a molecule to another. In QEq2 we use Sanderson's concept that as atoms are brought together electrons will flow within a molecule until electron equilibrium has been reestablished. Electronegativites, higher order electrostatic derivatives, and off-center electrostatic effects are used to govern the degree of electron flow as governed by the parameters c and J_{A0}, which denote the electronegativity and self-coulomb integral respectively, that are obtained from ionization potentials and electron affinities. Another parameter has been adjusted to reproduce the dipole moments of H_2O and NH_3. Because of electron cloud penetration effects, coulombic interactions involving electrons are not properly described in terms of 1/R. In QEq and QEq2, electrostatic interactions are described by coulomb integrals.

In QEq2 a quantum mechanical shell model is used for lone pair electron densities. The number of lone pair electrons on an atomic center is based on the number of non-bonded electrons on that center. For example, oxygen in H_2O has four lone pair electrons and nitrogen in NH_3 has two lone pair electrons. For centers with lone pairs the nuclear position is assigned a postive charge of the same magnitude as the number of lone pair electrons. Because lone pairs are electron densities, van der Waals interactions are included at the lone pair positions. The van der Waals term between a lone pair and its nucleus are ignored but terms between a lone pair and other nuclei and lone pairs are included. Conventional atomic van der Waals terms are partitioned between the lone pair positions and the nuclear positions based on the number of electrons in bond pairs and in lone pairs.

In QEq and EEM partial charges are found by equating chemical potentials and applying the constraint that the sum partial charges is equal to the net charge of the molecular system. In order to increase the computational speed of the method and more tightly define the locations of electron densities additional constraints have been added to QEq2. In QEq2 a valence bond bonding model is adopted: electron densities are confined to bond pairs, lone pairs, or delocalized sets of electron density. Within a bond pair, electron density flows to equilibrate the chemical potentials of the bonded centers in the field of the full electrostatic model. Lone pairs of electrons move in the electrostatic and van der Waals field of the other atoms and lone pairs.

In molecular mechanics assigning partial charge distributions to small molecules such as HF, H_2O, and NH_3 is problematic. The QEq2 dipole moments (in debye) for HF (calc. 1.84, expt. 1.826), H_2O (calc. 1.83, expt. 1.85) and NH_3 (calc. 1.47, expt. 1.47) match experiment closely. QEq2, and ab initio ESP partial charges for zeolite models $O(SiH_3)_2$ and $Si(OH)_4$ are collected in Table 2. Given the range of results obtained by ab initio electronic structure the preliminary QEq2 results are quite encouraging.

Ionic-covalent and resonance effects in classical molecular models. As one moves from methane to larger hydrocarbons on an external metal oxide surface or the interior surface of a zeolite, the surface provides a steric block to direct reactivity. For example, if one considers the hydride transfer step of a carbenium ion carrier hydrocarbon cracking process (Fig. 4), the carbenium ion can either be bound to the metal oxide surface, **(1)** or present as a free species, **(2)**. If the carbenium ion is bound to the surface it is difficult to construct a model of the hydride transfer step since the surface will block the backside of the carbenium ion. This suggests that a detailed description of a carbenium ion carrying chain mechanism for hydrocarbon cracking would involve bound carbenium ions **(1)**, in equilibrium with reactive free carbenium ions, **(2)**. This makes computationally modeling such processes problematic. The theoretical methodology used must be able to treat **(1)** and **(2)** with equal accuracy. In order to study the steric role that the surface plays must use a large cluster or the infinite system itself. This suggests that molecular mechanics should be used in conjunction with electronic structure techniques.

One solution to the treatment of molecular processes that can require both ionic and covalent descriptions is to simultaneously use a covalent model and an ionic model and let the geometry and environment determine the proper partitioning between models. This can be accomplished using a valence bond model of bonding,

$$E_{bond} = l^2 cov E_{cov} + l^2 ionic E_{ionic} + 2 l_{cov} l_{ionic} E^x cov, ionic$$

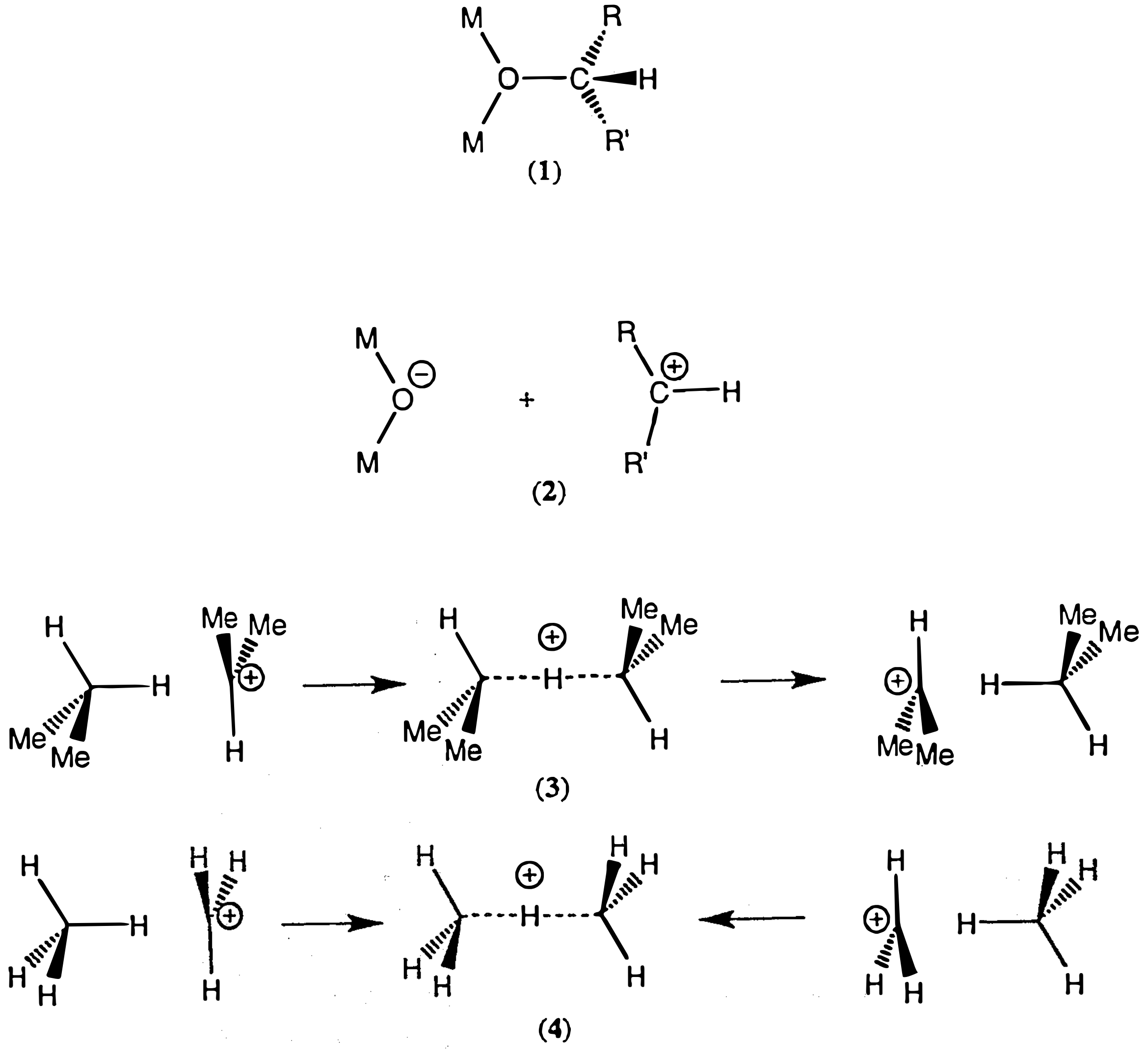
where E_{cov} and E_{ionic} and molecular mechanics representations of covalent and ionic bonding and $E^x cov, ionic$ is the interaction between the two. The l's variationally determined through the solution of a 2x2 determinant.

A related problem arises when one looks at cracking hydride exchange transition states. Recent *ab initio* calculations found the exchange "transition state" for secondary transfer from propane to propyl cation, **(3)**, to be a transition state or saddle point (a single imaginary vibrational frequency). The analogous structure for methane plus methyl cation, **(4)**, is a ground or stationary state with a binding energy of roughly 35 kcal/mol. By molecular mechanics both (isoproyl and methyl) cases are "transition states" if the reactant and product structures are not allowed to interact. As with simultaneously using covalent and ionic models this "interaction" can occur if a valence bond model of bonding is used:

$$E_{bond} = l^2{}_r E_r + l^2{}_p E_p + 2 l_r l_p \, E^x{}_{r,p}$$

where E_r and E_p and molecular mechanics representations of reactant, or first structure and product, or second structure and $E^x{}_{r,p}$ is the interaction between the two. Again, the l's can be variationally determined through the solution of a 2x2 matrix. The difficulty is determining the interaction or exchange terms $E^x{}_{cov,ionic}$ and $E^x{}_{r,p}$. We, in the past, have used a simplified cross term to test whether or not molecular mechanics could reproduce transition state structures. Molecular mechanics results (and in parentheses ab initio results) on a H-D exchange transition state for zeolite cluster model are presented in Figure 5, where the geometric results are quite encouraging.

Figure 4: Structures of species involved in carbocation chemistry (see text)


(1)

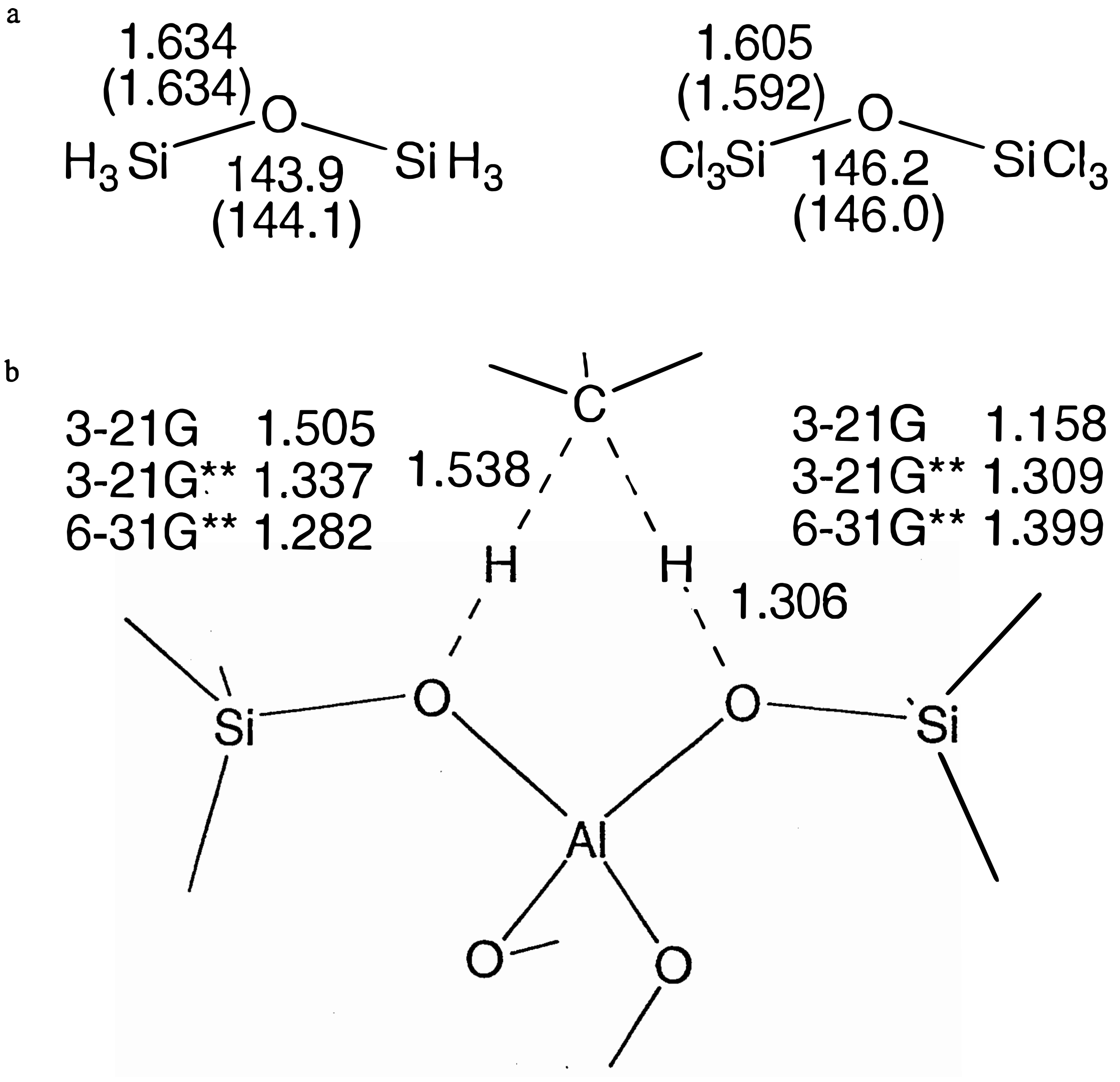
(2)

(3)

(4)

Figure 5: (a) Comparison of QEq2 and experimental (in parentheses) geometries in Si-O-Si compounds (upper panel).

(b) Comparison of transition states involved in methane H-D exchange from QEq2 and from ab initio calculations in 3-21G, 3-21G**, and 6-31G** basis sets (lower panel).



In the past year we have formulated a solution to the cross term problem that may be general—we need to test it for a variety of reactions including the cracking hydride transfer reaction. To date the only case we have studied is benzene, where reasonable values for the "resonance energy" have been obtained from preliminary results.

Table 1. Template-zeolite interactions

Molecule	Zeolite formed	Interaction energies (kcal/mol)	
		ZSM-12	SSZ-24
BIP	ZSM-12	-38.5	-41.7
EXO	ZSM-12	-38.7	-30.2
1-ADA	SSZ-24	.25.8	-27.9
2-ADA	SSZ-24	-27.6	-30.2
MeSA	SSZ-24	-20.2	-38.2
ENDO	SSZ-31	-34.0	-30.5

Table 2. Charge Distributions of $O(SiH_3)_2$ and $Si(OH)_4$

Compound	Atom	QEq2	CHELPG(6-311G**)	MSK	Mulliken
$O(SiH_3)_2$	O	-1.026	-0.565	-1.298	-0.981
	SI	0.895	0.890	1.187	1.101
	H	-0.128	-0.203	-0.176	-0.198
$Si(OH)_4$	O	-0.992	-0.935		-0.711
	Si	2.054	1.763		1.611
	H	0.479	0.494		0.308

9.	**Project Title**	**Periodic Ab Initio Hartree-Fock Theory/Site Isolation in Selective Alkane Oxidations**
	Principal Investigators	**Anthony Hess & M. R. Thompson**
	Project Site	**Pacific Northwest Laboratory**

Description:

The development of experimental and theoretical tools to study heterogeneous catalysis has been the focus of this effort. Research focuses on the development of novel solid-state formalism, its application to zeolites and clays, and experimental validation of molecular-level processes in commercial catalytic reactions. For the study of the molecular-level aspects of homogeneous catalysis, there exists a relatively large tool kit which can be used to probe the catalyst system for information about structure, dynamics, and reaction mechanisms, including vibrational spectroscopy, molecular quantum chemistry, and NMR. However, when studying the molecular-level events in a heterogeneous system with an external or internal surface, the number of useful tools is considerably diminished due to the complexity of the physics. As part of the CACD program of the BCTR program, we are pursuing the development of several tools aimed at facilitating the study of solid-state structure in heterogeneous catalysts. These include the development of periodic *ab initio* Hartree-Fock theory and its application to aluminosilicates and metal oxides, and the formulation of a model to describe the phenomena of "site isolation" in selective oxidation catalysts. Improvements are sought that are clearly revolutionary relative to current practice or understanding and not just incremental.

1995 Accomplishments:

All of the following items were milestones for the reporting period covered in this document. During the past 12 months our effort has concentrated on bringing new quantum mechanical tools on-line that are capable of fully exploiting the power of massively parallel computer architectures.

- Molecular modules of the Gaussian basis density functional code NWdft are currently capable of treating systems composed of 500-600 atoms with double zeta quality basis sets with no symmetry constraints on massively parallel processing (MPP) computer architectures. In addition, analytical first derivatives of the total energy with respect to nuclear coordinates are also completed and operate efficiently on MPP class machines.

- NWdft was integrated into the large scale NWchem suite of codes which provides the advantage of a commercial level user interface and object oriented database tools and allows us access to the established distribution channels associated with the OSC/HPCCI between PNL and many chemical companies

- NWdft/NWchem has been (and is currently being) distributed to industrial collaborators and BCTR program members.

- Symmetry and representation theory modules completed for molecules, polymers, surfaces and bulk solids.

- The initial implementation of 3-dimensionally periodic total energy code has been completed (parallelization of modules well underway). The first generation sequential modules designed to treat systems with 2-dimensional periodicity have been written and are being tested.

- The formal equations defining the analytical first derivatives of the total energy with respect to nuclear coordinates have been completed for 3- dimensionally and 2-dimensionally periodic systems.

- A new numerical integration technology was developed which is 20-50 times more efficient (therefore cost effective) for the same accuracy than the best analogous method in the literature. The approach has been implemented and tested for 3 and 2-dimensionally periodic systems.

1996 Planned Activities:

- Complete the efficient implementation of 3- and 2-dimensionally periodic Gaussian Basis Density Functional total energy codes for MPP architectures. Target efficiency is defined as 93% on 512 nodes on Intel, IBM, Cray and SGI machines. Capabilities of solid state code to match FY95 version of molecular code in FY96.

- To complete the software necessary to evaluate the gradients for 3- and 2- dimensionally periodic systems. Efficiently implement this software on MPP architecures.

- Increase the capability of the molecular modules to the 1000 atom level with no symmetry. To achieve the ~10,000 basis function effectively all "high" scaling components in the code must be removed. Alternatives to matrix diagonalization will be explored.

- Test the new solid state codes by studying chemical transformations at the internal and external surfaces of metal oxides and zeolites.

- Increase our distribution base.

Annual Technical Summary Report:

The role of catalysis in industrial and environmental settings is both widespread and profound. As the quantity and quality of raw materials decline and emission abatement policies increase the chemical, petrochemical and transportation industries are motivated to reduce process and separation energy consumption and produce more selective and efficient per pass catalysts. A key component of this BCTR program involves the development of high performance solid state computational chemistry software, designed from inception to exploit the computational power of massively parallel computer architectures, and the application of such methods to problems in heterogeneous catalysis. In the following paragraphs we briefly report on our accomplishments during the past year and outline several of our goals for the upcoming fiscal year.

Our work on advanced computational strategies is motivated by the need of our industrial partners to understand the atomic level changes that accompany the transformation of reactants to products during a chemical reaction. Although, the current genertion of software operating on sequential computer architectures can address some of the relavent questions for specific systems significantly more powerful methods are required for computational quantum chemistry to become generally effective tool. Next generation computational tools must be more interactive on the time scale of experimental industrial researchers and provide a cost effect alternative to traditional avenues of investigation. Our approach in designing such next generation software has been to first reduce the formal mathematical order of the theory to its lowest form and adopt a software design statagy whcih fully accounts implement the resulting theory from the onset on MPP systems. The scientific goals of this project are thus to first produce a first principles solid state quantum mechanical tool which can efficiently carry out reaction path following and be used to study and identify reactants, intermediates, and products as well as any associated energy barrier to the overall process. This type of reaction path information provides, at least in part, the necessary information to evaluate the kinetic and thermodynamic properties of the reaction process.

As mentioned above our FY95 research activities have focused on the development of next generation solid state quatum mechanical tools which can take full advantage of massively parallel computer architectures. Our approach provides fully self-consistent solutions of the Kohn-Sham equations represented in a Gaussian basis for either periodic or non-periodic boundary conditions. During the past year we have divided our attention between the optimization and deployment of the non-periodic modules (molecular codes) and on the generation of the first stable solid state algorithms capable of the total energy of systems possessing 3- and 2-dimensional periodicity. The overall goal of this project is to provide computational tools which are approximately 1000 times faster than the current generation of software designed for sequential computer systems. Currently, the molecular modules of NWdft are capable of treating systems composed of 500-600 atoms with all electron double zeta quality basis sets with no symmetry constraints on massively parallel processing (MPP) computer architectures. The overall scaling of the molecular modules as a function of systems size is currently of O(N2.3) . The codes are fully operational on a wide range of platforms (Cray T3D, Intel delta's and paragons, IBM SP2, KSR-2, SGI power challenge's, etc.) and include analytical first derivatives of the total energy with respect to nuclear coordinates (gradients). In addition, during the past year NWdft was integrated into the large scale NWchem suite of codes, also developed at PNL, which provides the advantage of a commercial level graphical user interface and object oriented database tools. Additionally, the merger with NWchem provides us direct access to the distribution channels established through the OSC/HPCCI project, PNL, and several high profile corporations within the chemical industry.

Currently, the 3- and 2-dimensionally periodic codes are undergoing final testing in sequential form and are capable of treating modest sized solid surface and bulk crystalline matrerials. These codes also contain fully developed symmetry and representation theory modules completed for molecules, polymers, surfaces and bulk solids. In addition, we have recently completed the formal mathematics necessary to evaluate the first derivatives of both the 2- and 3-dimensional total energy theories. The solid state gradients will greatly facilitate the structural optimization of bulk materials and provide the oportunity to follow reaction paths and efficiently optimize surface adsorbate complexes at external surfaces. In the inital phases of our solid state development activities we determined that the standard approach to numerical integration stratagies used in the molecular community was inadaqute for our solid state requirements. In response to this we have now developed a new numerical integration technology which is 20-50 times more efficient (and therefore cost effective) than competitive methods for the same accuracy. In additon, the number of operations required to evalute a given integral does not increase with increasing system size after the total system size is beyond a few atoms.

Our principle FY96 goals focus on the implementation of a stable MPP 3- and 2- dimensionally periodic modules for a variety of computational platforms. Target efficiency is defined as 93% on 512 nodes on Intel, IBM, Cray and SGI machines which is approximately the current efficiency of the molecular modules. In addition, we plan to complete the software necessary to evaluate the gradients for 3- and 2-dimensionally periodic systems and to achieve an efficient implementation on MPP systems by the close of FY96. Due to the high demand for the molecular modules we also intend to increase its capabilities to the 1000 atom level without symmetry constraints. To achieve this approximetely ~10,000 basis function goal several "high" scaling algorithms in the code must be improved. In particular, the parallel eigensolvers currently used to diagonalize the secular equations will need to be replaced to achieve a low $O(N<3)$ scaling method. Our final goals focus on the testing of the new solid state codes by studying chemical transformations at the internal and external surfaces of metal oxides and zeolites and increasing the distrubution of the software package.

10.	**Project Title**	**Separations by Reversible Chemical Association**
	Principal Investigators	**C. Judson King**
	Project Site	**Department of Chemical Engineering** **University of California at Berkeley** **Lawrence Berkeley Laboratory**

Description:

Investigate the application of reversible chemical complexation as a means of separation of polar organic compounds from dilute aqueous solutions. Solutes of interest include oxygenated compounds produced by fermentation, such as carboxylic acids, alcohols, and amino acids. These approaches can substantially reduce energy requirements, and minimize the wastes, of conventional chemical separations (distillation, evaporation, conventional solvent extractions) in industry.

General objectives of the project include the measurement of phase equilibria and the rates at which equilibria are established; establish guidelines for the selection of complexation agents; develop suitable methods of process integration (into other unit operations), and to define the most promising applications of the method.

Specific objectives include the identification of regeneration methods for organic acids extracted from solutions; identification of process approaches for the extraction of organic acids at pH above the pKa of the acid; identify suitable extraction agents for glycol and other polyfunctional alcohols.

1995 Accomplishments:

During the past year we have explored three different aspects of recovery of carboxylic acids by amine-based extractants and adsorbents and also investigated means of recovering glycols and sugars by reactive separations.

With regard to carboxylic acids, first, we explored solvent regeneration of amine adsorbents, using Lewis-base solvents. Piperidine, a strong but rather noxious and non-volatile base, provided satisfactory regeneration, but no benign volatile solvent was found. We did identify an effect whereby the presence of water in a solvent strongly promotes adsorption, possibly because there is ionic acid-base interaction at the adsorbent surface in the presence of water, but only hydrogen bonding in an anhydrous system. Second, we investigated competitive uptake of lactic and succinic acids by an amine-based adsorbent (Dowex MWA-1), and found surprisingly preferential uptake of succinic acid, a useful result. As well, we found that lactic and acetic acids can be separated by adsorbing both with Dowex MWA-1 and then vaporizing acetic acid. Third, we developed a sensitive method for measuring small uptakes of glucose by basic polymeric resins and thereby quantitatively measured high selectivities for uptake of lactic acid as opposed to glucose. It appears that these selectivities are superior to those achievable by water-splitting electrodialysis.

With regard to glycols and sugars, we measured equilibria for extraction of propylene glycol with phenylboronate extractant in an ion-pair mixture with a quaternary ammonium organic cation in 2-ethylhexanol diluent. We devised and evaluated a method of regeneration whereby CO_2 is absorbed into the laden ion-pair extractant, thereby releasing propylene glycol for back-extraction into water. CO_2 is then removed from the extractant by stripping, regenerating the phenylboronate ion-pair extractant. Finally, we measured uptakes of glucose by a variety of different activated carbons and confirmed that carbons have substantial capacity, despite the negative deviations from ideality in glucose-water solutions. However, these capacities do not relate to surface areas in any simple way.

1996 Planned Activities:

We are pursuing four different lines of research:

1. Determination of equilibria for, and fractionation among, multiple carboxylic acids with amine extractants and adsorbents. Working in conjunction with Omni Interests, Inc., U. S. Filter/IWT and Advanced Separations Technologies, we will continue to develop technology (optimal adsorbents and operating conditions, possible use of our water-enhanced solvation technology, etc.) for recovering both lactic acid and succinic acid from stillage streams from fermentation ethanol plants. We will measure competitive adsorption and extraction equilibria for mixed carboxylic acids and interpret these in terms of concepts that we have already developed for extraction and adsorption of individual carboxylic acids, plus any new phenomena that may be revealed.

2. Measurement and interpretation of adsorption of sugars and glycols by activated carbons. We will continue to measure adsorption capacities of different carbons for sugars and glycols, interpreting these data in terms of measured physical (surface area, pore size distribution, ...) and chemical (heteroatom content, FTIR spectra, active hydrogen, ...) properties of the carbons. The aim is to determine the mechanisms by which the capacities of carbons for these solutes are generated. With that insight, we should be able to define ways of achieving optimal reversible adsorption of these solutes by carbons and/or define synthetic polymeric adsorbents that are effective for glycols, sugars and related substances.

3. Determination of selectivities between lactic acid and water during extraction with tertiary amine extractants in various diluents. We will measure uptakes of glucose by tertiary amine extractants in chemically different diluents, and will also measure selectivities between lactic acid and glucose. Differences in selectivity will be related to differences in diluent and/or amount of water uptake. The selectivities achievable will extraction will be compared with those that we have found for adsorption.

4. Means for achieving reversible and high-capacity recovery of phenols from aqueous solutions. Earlier research in our group demonstrated that the irreversibilities commonly encountered in adsorption of phenols with activated carbon result from catalysis of oxidative coupling reactions by the carbon surface. We will utilize this insight to seek ways of creating and/or using carbons that will promote reversible adsorption of phenols. We will also examine the use of polymeric adsorbents and amine extractants for recovery of phenols, with particular attention to multi-OH phenols and high-pH conditions.

Annual Technical Summary Report:

Recovery and Fractionation of Carboxylic Acids

Previous Research. In earlier work we measured and interpreted equilibria for extraction and adsorption of carboxylic acids by complexing extractants and adsorbents, notably those that have amine functionalities. We identified the utility of moderately basic (e. g., tertiary amine) extractants and adsorbents for recovery of carboxylic acids at $pH > pK_a$ of the acid, conditions that are typical of many fermentations. We found that aqueous trimethylamine (TMA) is effective for regeneration of those agents without consumption of acids and bases or release of a waste salt. We identified sorbents and extractants of optimal basicity for recovery of fermentation acetic acid in the form of the environmentally benign road deicer, calcium magnesium acetate (CMA). Our research of the last year was directed toward alternate means of regeneration for these moderately basic agents, and to determinations of selectivity among multiple acid solutes and between acids and sugar substrates.

Solvent Regeneration. We explored regeneration of amine-based adsorbents by means of Lewis-base solvents. Piperidine, a strong base with low volatility and rather noxious characteristics, provided satisfactory regeneration. However, no effective benign, sufficiently volatile solvent was found. We did, however, identify a potentially useful effect whereby the presence of water in such a solvent strongly promotes adsorption by a tertiary amine adsorbent (Dowex MWA-1), possibly because there is some extent of ionic acid-base interaction at the adsorbent surface in the presence of water, but only hydrogen bonding in an anhydrous system. The effect is large -- an increase by a factor of 10 to 20 in the equilibrium distribution ratio. These results indicate that solvent regeneration of these adsorbents can be assisted by initially stripping away water that is desorbed from the resin into the solvent.

We performed quantitative calculations of the effectiveness of diluent "swing" regeneration processes for amine extractants laden with a carboxylic acid. Here a diluent that solvates the acid-amine complex well (e. g., octanol) is used in the initial extraction process, in admixture with an inert diluent (e. g., a hydrocarbon). The solvating diluent is distilled from the extract, and the carboxylic acid is then more readily back-extracted into water from the resulting solution of extractant in inert diluent.

Mixtures of Carboxylic Acids. In connection with our Technical Assistance Agreement with Omni Interests, Inc., we investigated competitive uptake of lactic and succinic acids by an amine-based adsorbent (Dowex MWA-1), and found surprisingly preferential uptake of succinic acid. This result is potentially useful for recovering lactic and succinic acids separately from stillage from fermentation ethanol plants. Another industrially important mixture is lactic and acetic acids, which occur together in fermentation processes based upon the pyruvic acid cycle. We found that lactic and acetic acids can be separated effectively by adsorbing both onto Dowex MWA-1 and then selectively vaporizing acetic acid.

Selectivities between Carboxylic Acids and Sugars. It is important to minimize uptake of substrate glucose during extraction and adsorption of carboxylic acids from fermentation media. In addition to the obvious economic and purity reasons, the presence of seemingly small amounts of a sugar in lactic acid causes discoloration, which put the product off-specification for many purposes. We developed a sensitive method based upon high-precision liquid chromatography for measuring small uptakes of glucose by basic polymeric resins. We thereby quantitatively measured selectivities for competitive uptakes of lactic acid and glucose from solutions containing both. The selectivities for lactic acid as opposed to glucose are high and appear to be superior to those achievable by water-splitting electrodialysis, a competing process.

Amino Acids. We carried out a literature survey on methods for recovery of amino acids, which is complicated by the fact that amino acids are zwitterions (both cationic and anionic) over a wide range of pH values.

Recovery of Glycols and Sugars.

Past Research. In earlier work we examined extraction of alcohols by complexation with phenols. We also established that organoboronate anions are effective extractants for *cis*-vicinal multi-OH compounds, such as glycols, sugars and sugar alcohols. We also explored the use of reversible reaction of glycols with aldehydes to form relatively non-polar dioxolanes, as a method of recovering glycols from aqueous solutions. The most effective implementation for propylene glycol was reaction with acetaldehyde, followed by extraction of the resultant dioxolane.

Organoboronate Extractants. This year we continued research on recovery of glycols by means of organoboronate extractants. We measured and interpreted equilibria for extraction of propylene glycol with phenylboronate, used in the form of an ion pair of phenylboronate anion with Aliquat 336 (an organic quaternary ammonium cation) in 2-ethylhexanol as a diluent. Stoichiometric overloading of the phenylboronate extractant was observed, up to about 1.4 moles of propylene glycol taken up per mole of phenylboronate. This unexpected result was shown by nuclear magnetic resonance (NMR) tests probably to be attributable to formation of reverse micelles within the extractant phase.

CO_2 Regeneration. Seeking an effective and economical means of regeneration for organoboronate extractants, we devised and examined a method of regeneration whereby CO_2 is absorbed into the ion-pair extractant, displacing the phenylboronate anion with bicarbonate and thereby releasing propylene glycol for back-extraction into water. CO_2 is then removed from the extractant by stripping, thereby regenerating the phenylboronate ion-pair extractant. A compromise must be made between a stripping temperature high enough to release the CO_2 efficiently and one low enough to avoid excessive thermal degradation of the extractant.

Carbon Adsorption. We measured uptakes of glucose by a variety of different activated carbons and confirmed that carbons have substantial capacity. This is a striking result, since sugars exhibit negative deviations from ideality in glucose-water solutions. Capacities are different from carbon to carbon, and do not correlate in an obvious way with measured surface areas.

11. Project Title **Rational Enhancement of Enzyme Performance In Organic Solvents**
Principal Investigators **Alexander Klibanov**
Project Site **Massachusetts Institute of Technology**

Description:

This project seeks to elucidate the mechanisms of enzyme stability in organic solvents and to apply the knowledge to processes of industrial importance. The project will also investigate gas-phase biocatalysis. The phenomenon of "molecular memory" of enzymes will be explored. Rigid protein configurations in enzymes retain the conformation (imprint) induced by the original ligand/stability factor. The result of this molecular memory is an enzyme with altered catalytic characteristics or a protein with new binding site, which might be able to perform new chemical reactions.

1995 Accomplishments:

The overall goal of this project is to rationally improve the catalytic performance of enzymes in organic solvents. During the project period reported herein, we have focused on (i) the possibility of accelerating enzymatic processes in organic solvents by certain cosolvents; (ii) whether lipase catalysis in organic solvents can be enhanced by introducing interfaces in the reaction medium; and (iii) the structure of proteins suspended in organic solvents.

- We have demonstrated that enzymatic activities in anhydrous solvents can be increased 100-1000 fold by the addition of denaturing organic cosolvents, such as dimethyl sulfoxide and formamide. This striking phenomenon is rationalized in terms of enhanced conformational flexibility of enzymes in anhydrous milieu brought about by the denaturing cosolvents; the latter exert their lubricating effect largely at the interfaces between enzyme molecules in solid preparations, thus easing the mobility constraints imposed by protein-protein contacts.

- We have determined for the first time that, in contrast to the situation in aqueous solution, lipases suspended or solubilized in organic solvents do not adsorb onto hydrophobic interfaces and do not exhibit the phenomenon of interfacial activation.

- We have elaborated and validated a new methodology, hydrogen isotope exchange/high-resolution NMR, to assess the structural intactness of enzymes suspended in organic solvents. This approach has revealed that most organic solvents cause little additional protein denaturation beyond that brought about by lyophilization.

1996 Planned Activities:

- We will continue to address the key issues pertaining to rational improvement and control of enzyme action in organic media. To this end, the following directions of research will be pursued.

- We will aim at pinpointing the specific causes of reduced enzymatic activity in anhydrous media compared to that in water; this understanding should be conducive to making enzymes more active. The effect of such variables as the thermodynamic activity of water in a given solvent and the energetics of substrate desolation as a function of the solvent on the catalytic performance will be systematically investigated. These experiments will be carried out with cross-linked crystalline, rather than lyophilized, enzymes which, apart from being robust practical catalysts, have been shown by us to have the same X-ray crystal structure in organic solvents as in water (thus putative conformational changes can be excluded).

- There are a number of different ways to prepare solid enzymes for subsequent use in organic solvents, e.g. lyophilization, spray-drying, crystallization and cross-linking, acetone precipitation, and immobilization. We will study the effect of the mode of preparation on enzyme structure, as well as on enzymatic enantioselectivity and its temperature dependence in organic solvents. The insights thus obtained should be useful in making enzymes more stereoselective catalysts for preparative transformations.

- In order to utilize whole microbial cells, rather than isolated enzymes, as catalysts in organic solvents the mechanism of solvent toxicity to microorganisms need to be elucidated. We have produced a mutant of *E. coli* which, in contrast to the wild-type strain, is resistant to many organic solvents. We plan to clone the solvent resistance gene. It will then be sequenced, and the protein it encodes for will be identified. This should shed light on the sought-after resistance mechanism. In addition, the mutation responsible for the acquired solvent resistance will be localized and analyzed.

Annual Technical Summary Report:

The overall goal of this project is to rationally improve the catalytic performance of enzymes in organic solvents. During the project period reported herein, we have focused on:

1. The possibility of accelerating enzymatic processes in organic solvents by certain cosolvents;
2. Whether lipase catalysis in organic solvents can be enhanced by introducing interfaces in the reaction medium; and
3. The structure of proteins suspended in organic solvents.

1. The rates of transesterification reactions catalyzed by the protease subtilisin Carlsberg suspended in various anhydrous solvents at 30°C can be increased more than 100 fold by the addition of denaturing organic cosolvents (dimethyl sulfoxide or formamide); in water, the same cosolvents exert no enzyme activation. At 4°C, the activation effect on the lyophilized protease is even higher, reaching 1000 fold. Marked enhancement of enzymatic activity in anhydrous solvents by formamide is also observed for two other enzymes, α-chymotrypsin and *Rhizomucor miehei* lipase. In addition to lyophilized subtilisin, cross-linked crystals of the enzyme are also amenable to the dramatic activation by the denaturing cosolvents. In contrast, subtilisin solubilized in anhydrous media by covalent modification with poly(ethylene glycol) (PEG) exhibits little activation. These observations are rationalized by postulating an enhanced protein flexibility in anhydrous milieu brought about by the

denaturing organic cosolvents. The latter exert their lubricating effect largely at the interfaces between enzyme molecules in a solid preparation, thus easing the flexibility constraints imposed by protein-protein contacts.

2. The question of whether lipases can be activated by adsorption onto an interface in organic solvents has been addressed using *R. miehei* lipase as a model. In aqueous solution, this enzyme undergoes a marked interfacial activation. However, lipase (either lyophilized or precipitated from water with acetone) suspended in ethanol or 2-(2-ethoxyethoxy)ethanol containing triolein exhibits no jump in catalytic activity when the concentration of triolein exceeds its solubility in these solvents, thereby resulting in formation of an interface. To test whether the lack of interfacial activation is due to the insolubility of the enzyme in organic media, lipase has been covalently modified with PEG. The modified lipase, although soluble in nonaqueous media, is still unable to undergo interfacial activation, regardless of the nature of the interface. This inability is due to the absence of adsorption of lipase onto interfaces in organic solvents, presumably because of the absence of the hydrophobic effect (the driving force of lipase adsorption onto hydrophobic interfaces in water) in such media. The uncovered lack of interfacial adsorption and activation suggests that the short α-helical "lid" covering the active center of the lipase remains predominantly closed in nonaqueous media, thus contributing to diminished enzymatic activity.

3. The structure of a model protein, bovine pancreatic trypsin inhibitor (BPTI), in organic solvents has been examined using hydrogen isotope exchange/high-resolution NMR methodology. When lyophilized deuterated BPTI is suspended in acetonitrile, tetrahydrofuran, ethyl acetate, or butanol, each containing 1% ^1H_2O, several protein amide protons that are buried and strongly hydrogen bonded in aqueous solution are found to exchange with the solvent significantly within 24 h. In contrast, in water most of these portions do not exchange appreciably even after a week under otherwise similar conditions. The isotope exchange rates of the corresponding amide protons of BPTI are similar in these nonaqueous solvents. When solid BPTI is prepared by different methods, such as rotary evaporation, acetone precipitation, or lyophilization from a dimethyl sulfoxide solution, and subsequently suspended in acetonitrile containing 1% water, the exchange intensities of the amide protons vary greatly among the preparations. These data combined suggest that the structure of BPTI in the four aforementioned organic solvents is partially unfolded, but not more so than in lyophilized powder, i.e., that these solvents cause little additional protein denaturation beyond that brought about by lyophilization. Using the same methodology, the BPTI structure also has been studied in several protein-dissolving solvents containing 1% water. In dimethyl sulfoxide, dimethylformamide, or methanol, the same amide protons exchange almost completely within 24 h, while in glycerol (known to stabilize proteins and to function as a water mimic) they do not. These results demonstrate that some protein-dissolving organic solvents strongly denature BPTI and that intermolecular contacts in the suspended protein are important in maintaining the protein conformation in organic solvents. Our findings, if general, explain the considerable but much reduced (compared to water) enzymatic activity in nonaqueous media.

12.	**Project Title**	**Biological Separation of Phosphate from Ore**
	Principal Investigators	**R. D. Rogers**
	Project Site	**Industrial Biotechnology and Process Engineering** **Idaho National Engineering Laboratory**

Description:

The project is aimed at bioseparation of insoluble phosphate complexes from ore matrix. To achieve this, selection and culturing of microorganisms capable of dissolving and concentrating insoluble phosphate forms have been made. The mechanism by which microorganisms dissolve the phosphate complexes are being investigated. Promising bioreactor configurations will be tested at the bench scale.

1995 Accomplishments:

- Provided technology transfer to CRADA partner. Planned "teaming" activities have been carried out with the J. R. Simplot Co.

- Discovered biomechanism responsible for the phosphate separation process. We have isolated the gene responsible for the biosolubilization of phosphate ore. This has lead to the development of a working hypothesis on the part microorganisms play in the solubilization process.

- Tested a model "micro encapsulated" bioseparation process. Studies have shown that the biomechanism of solubilization can be maintained in a 2 mm bead containing the requisite microorganism, phosphate ore and an organic binder.

- Patent application submittal. Two applications were submitted to the U.S. patent office. Subjects of the patents were the genetic pathway responsible for solubilization and encapsulation technology.

1996 Planned Activities:

Research and engineering efforts aimed at overcoming identified hurdles have been focused in the development and testing of prototype biobeads. Efforts which were started during FY 1995 will continue to be applied to overcoming these hurdles in order to commercialize a viable biosolubilization process. It should be noted that completion of the proposed work is contingent on obtaining sufficient funding. The FY-1996 proposed project will:

1. Develop a model micro encapsulated bioseparation process.

    a. Devise a method for encapsulation of the component parts of the biosolubilization process (microorganism, carbon source, RP) into 2-3 mm beads for the production of slow release phosphate fertilizer products (>80% of processed phosphate is used for fertilizer).

b. Investigate methods for developing an encapsulation matrix (what material and methods are best suited for the binding of the component parts while maintaining microbial viability).

c. Develop or adapt a method for the production of beads.

e. Conduct a marketing investigation to determine the best application for the bead technology.

f. Conduct an engineering evaluation of the process to determine the quantity of phosphate ore that will be diverted to the new technology and the calculated unit costs including savings resulting from pollution prevention.

2. Develop process for the *in situ* mining of phosphate.

   a. Adapt methodology for maintaining microbial activity in a phosphate ore body.

   b. Adapt methodologies for injection of microorganisms into an ore body and recovery of soluble phosphate.

   c. In conjunction with Government and industrial partners conduct a field demonstration of the *in situ* methodology.

   d. In conjunction with partners conduct an engineering evaluation of the process to determine, based on projected use of the in situ technology, the quantity of phosphate ore that will be diverted to the process and calculated unit costs including savings resulting from pollution prevention.

Annual Technical Summary Report:

The concept of bioseparation of elements from their ore matrix is the first step in producing solutions from which the freed element can be subsequently purified and concentrated. Such a technology lends its self to bioprocess engineering principles for development from bench scale processes to scaled up processes suitable for industrial use.

The importance of microbial solubilization of phosphate was demonstrated 45 years ago. Numerous studies have since been conducted on the effectiveness of microbes in solubilizing insoluble forms of phosphorous. The literature reports that 1-33% of the phosphate contained in rock phosphate (RP) can be released by microbial activity in batch tests. Using a naturally occurring microorganism selected from screening over 800 isolates, researchers at the Idaho National Engineering Laboratory (INEL) showed that a selected microorganism was capable of solubilizing over 85% of available RP. Enhancement was also maintained with increasing RP concentrations when the process was carried out under continuous conditions with a bench scale bioprocess unit.

Because of the positive potential of the proposed method a large scale continuously stirred tank (CTR) bioreactor system was developed for the processing of RP. During the development of the CTR technology (which has had a patent issued) it became apparent that there were two other potential applications of the bioprocess which could accomplish the same end goal of producing a "green" phosphate product. Results of the testing suggest that the bioprocess could be applicable to the development of a slow release, pelletted fertilizer bead and to the *in situ* separation of phosphate from ore at the mine site. Of these two applications the bead technology has already progressed to the development of a model process for production.

Therefore, one envisioned application of the technology will be in devising a method for encapsulation of the component parts of the biosolubilization process (microorganism, carbon source, RP) into 2-3 mm beads for the production of slow release phosphate fertilizer products (patent applied for). The incremental decrease in the cost of pollution management from use of this methodology alone would increase the profitability of the industry. It is expected that the US industry would also be able to compete more effectively with an increasing supply of non US product currently being sold in the country.

Initial work on the encapsulation process has used alginate as an encapsulation matrix. Various studies using this material showed that is was possible to maintain activity RP solubilization when the microbial component E-37 and a supply of RP were incorporated into alginate. Data on the rate and quantity of RP solubilization were comparable to those for the process run under bioreactor conditions.

The current encapsulation methodology is being used as a model system to test the encapsulation concept. At this time the beads are not being coated to prevent the "leaking" of E-37 into the surrounding media, nor has a usable carbon source been incorporated. Bead coatings, carbon sources, and encapsulating matrix continue to be subjects of the project.

Work has been progressing on validating the encapsulation concept. Prototype beads containing RP and E-37 have been manufactured and used in three greenhouse plant trails. Barley was used in all studies because it has been reported as being a suitable indicator plant for showing signs of phosphate stress. At the initiation of each trail, several barley seeds were planted in individual pots. After germination the plants were thinned to three plants per pot. Silica sand (20 mesh) was used as the medium for growth in the first two studies. The sand was washed and sterilized before use. After the requisite formulations of biobeads (treatment and control formulations) were mixed into individual quantities of sand the material was placed into 250 mL polyethylene jars. The third trial was conducted with soil in 800 mL pots. In the latter two studies plants were allowed to grow to maturity (until headed out). In all the trials plant growth was documented by weekly photographs and by height measurements. Plants were harvested at the end of the growth period to determine dry weights. In these trials glucose was the carbon source for the treatments and was supplied at the beginning of the study.

Results from the first two trials (third one still ongoing) showed that plants grown in association with beads containing RP and E-37 had better growth (overall height) than those plants provided only with an initial amendment of a phosphate solution. Based on these results it appeared that the concept of the biobeads being able to provide a source of phosphate for plant growth was validated.

The source of carbon which can be used to stimulate the activity of E-37 has also been investigated. It has previously been determined that glucose is the preferred carbon source to simulate phosphate solubilization by E-37. We have shown from a molecular and genetic basis that the metabolism of glucose by E-37 via the direct oxidation pathway utilizing glucose dehydrogenase results in the production of gluconic acid and protons. They are essential to the solubilization process. The use of glucose to drive the process on a commercial scale, however, is not cost effective. Therefore, an economically viable source of glucose has been required. An alternative that has been investigated is starch (polyglucose). Starch can be hydrolyzed to glucose by alpha amylase enzymes. We have been evaluating the ability of these enzymes to providing the necessary glucose for phosphate solubilization.

Preliminary work allowed us to select an amylase enzyme which was effective in hydrolyzing potato starch (waste potato starch is available in large quantities due to the potato processing industry) to glucose. In subsequent shake flask studies we have compared the RP solubilization activity of E-37 in the presence of glucose and starch amended with alpha amylase. Comparable amounts of soluble phosphate were obtained from use of the two carbon sources. This was conclusive evidence that potato starch could be used. The next step was to determine if the starch could be used in place of glucose in prototype biobeads. Studies validating this concept have been completed. Test data showed that biobeads containing E-37, RP, and starch exposed to a solution containing alpha amylase produced soluble phosphate in quantities comparable to the same type bead (only without starch) exposed to glucose. These very encouraging results prove a justification for continued development of the bead concept.

13. **Project Title** **Chemistry and Immunology as Tools for the Rational Design of Stable Active Enzymes**
Principal Investigators **Peter Schultz**
Project Site **University of California at Berkeley**

Description:

Recently it has been shown that the diversity and specificity of the immune system can be tapped to produce highly selective catalysts. Because antibodies[1] can be generated that selectively bind almost any molecule of interest, this new technology offers the possibility of generating tailor-made catalysts for applications in chemistry and biology. The specific objective is to develop methods for generating antibodies that bind substrate and a reactive cofactor[2] in a catalytically productive fashion. The project engineers antibodies that contain metal binding sites in order to carry out redox and hydrolytic reactions with antibodies. A combination of theoretical modeling and recombinant DNA methodology is being used to design and introduce the metal binding site. A second class of cofactors of interest are reducing agents such as metal hydrides which are involved in industrially important reactions such as carbonyl reductions. Antibodies are being generated to catalyze metal hydride-dependent stereospecific carbonyl reduction. Molecular modeling is used to understand the structural and electronic features of hapten and antibody required for enzymatic activity.

1995 Accomplishments:

Our program addresses two approaches to catalyst design: the development of tailormade enzymelike catalysts based on catalytic antibodies and the development of a combinatorial approach to the development of new solid state catalysts. The first ongoing program aims at tapping the diversity and specificity of the immune system to produce highly selective catalysts. Because antibodies can be generated that selectively bind almost any molecule of interest, this new technology offers the possibility of generating tailor-made catalysts for applications in chemistry and biology. Ongoing projects include: (a) The generation of catalytic antibodies that carry out important chemical transformations that are difficult or impossible to achieve via existing chemical approaches. Three classes of reactions are being pursued: the stereo and regioselective reduction of carbonyls, selective oxidation reactions, and selective carbonyl addition and hydrolysis reactions. These reactions are widely used in industrial processes. The ability to carry out such reactions with high selectivity, few byproducts and in water at room temperature would represent a significant advance in the development of environmentally compatible catalysts. We will begin to develop the necessary technology to use catalytic antibodies in large scale chemical processes. (b) We are also pursuing the generation of antibodies that hydrolyze cellulose to glucose feedstocks. The first step in this direction is the generation of antibodies with glucosidase activity. Once rules are developed for hydrolyzing these simple substrates, we will synthesize haptens to elicit antibodies that hydrolyze cellulose.

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[1] Antibody: an immunoglobulin present in the serum of an animal and synthesized by plasma cells in response to the invasion by an antigen, conferring immunity against later infection by the same antigen.

[2] Cofactor: a small, non-protein molecule, that associates with the protein portion (apozyme) of an enzyme and is essential for the enzyme to function.

A new initiative will focus on the development of a more systematic, cost effective and time efficient approach to the discovery of new solid state catalysts. This will be accomplished by developing methodology that allows the parallel synthesis and analysis of new solid state materials with novel catalytic properties. Such methodology would allow one to synthesize and characterize materials approximately 10^3 to 10^5 fold faster than the current rate. The ability to rapidly synthesize and analyze large libraries of materials should lead to new and exciting catalysts composed of previously unexplored combinations and stoichiometries of elements. Moreover, the synthesis and analysis of large numbers of diverse chemical catalysts, when coupled with modern theoretical methods, should add significantly to our understanding and ability to correlate the structural and chemical properties of catalysts.

1996 Planned Activities:

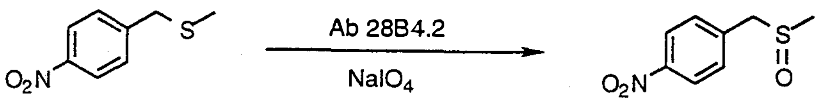
- Generate and assay aliphatic N-oxide antibodies for their ability to catalyze stereoselective ketone reduction.
- Generate and characterize ability of porphyrin specific antibodies to catalyze alkane/alkene oxidation.
- Solve structure of oxidase antibody 28B4.2.
- Characterize properties of antibodies designed to catalyze tertiary ester hydrolysis.
- Synthesize new generation glycosidase haptens.
- Fabricate and test multihead inkjet device for generating a small library of zeolites.
- Engineer improved sulfur oxidase antibody using protein engineering methods.
- Assay next generation of glycosidase antibodies.
- Generate second generation antibodies (porphyrin and NaCNBH3 dependent) aimed at commercial reactions.
- Develop methods for assaying zeolite libraries. Detection methods will focus on characterizing the physical properties of each member of the library (e.g., crystallinity using a scanning Raman detector in the search for chiral zeolites) as well as the catalytic properties (e.g., a scanning capillary mass spectrometer to look at product distributions and rates or temperature sensitive detectors to measure catalytic activities).

Annual Technical Summary Report:

Heteroatom oxidation

An antibody elicited against aminophosphonic acid hapten **3** was shown to catalyze the sodium periodate-dependent oxidation of prochiral sulfide **1** to sulfoxide **2**. The reaction catalyzed by antibody 28B4.2 displayed saturation kinetics consistent with random-binding mechanism. A family of Lineweaver-Burk plots was constructed at six fixed concentrations of $NaIO_4$ with varied concentrations of sulfide **1**. The slopes and y-intercepts obtained from this analysis were replotted as a function of the sulfide or $NaIO_4$ concentration to give a true maximum velocity (V_{max}) of 49.0 μM s^{-1} and a catalytic constant (k_{cat}) of 8.2 s^{-1}. Michaelis constants (k_m) of 252 μM and 43 μM were obtained for $NaIO_4$ and sulfide **1**, respectively. The antibody-catalyzed reaction was fully inhibited by the hapten. A dissociation constant (K_d) of 52 nM for hapten **3** was obtained by fluorescence quenching. The enantioselectivity of the reaction catalyzed by several different antibodies was characterized by extracting the products into 2-butanone and analyzing their composition by chiral HPLC (Chiralpak AD column Daicel Chemical Industries, hexanes: isopropanol gradient).

The enantiomeric excesses listed in Table I represent the average of two experiments. To our surprise, the antibodies displayed lower overall selectivities



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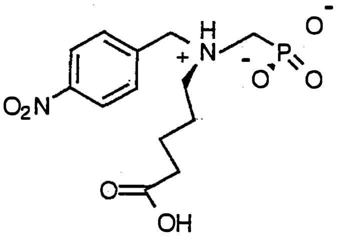

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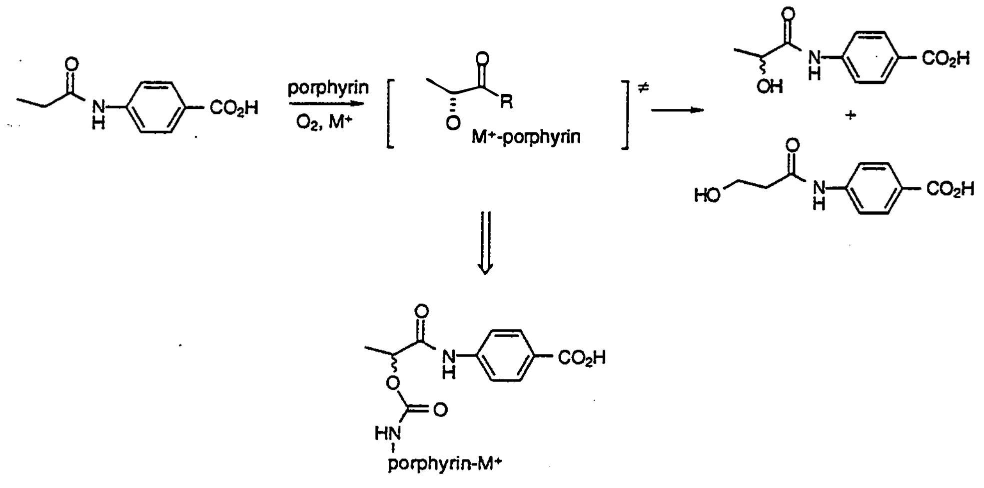
Table I.

Antibody	% Enantiomeric Excess
36D3.1	65.3 = 1.2
6D2.1	41.0 = 1.0
26D6.10	44.8 = 2.5
38A6.2	<5
32A5.5	82.4 = 0.5
27A5.7	57.3 = 1.1
19C4.7	94.5 = 1.5

compared to previously characterized antibodies catalyzing ketone reduction (96% ee). We are currently crystallizing these antibodies with their respective haptens to clarify the relationship between hapten structure and selectivity. We have generated crystals of antibody 28B4.2 which diffract to 1.8 Å resolution. There is one antibody per unit cell. We expect to shortly have the structure in hand using the method of molecular replacement. This structure should provide important insights into the design of new antibody catalysts that use abiological cofactors.

Alkane hydroxylation

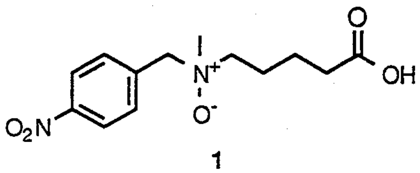
Regio- and stereoselective hydroxylation of alkanes is an important process in many chemical processes. Our goal is to establish a simple model that will generate antibodies which can catalyze this process. If successful, the strategy can then be applied to other biological chemical systems. The strategy we are pursuing is illustrated below.



The requisite hapten was synthesized by linking a porphyrin (the redox catalyst) site-specifically to a substrate analogue. The proximity of cofactor and substrate is expected to generate antibodies which can both catalyze and direct the reaction to the desired position. The substrate was synthesized by one step coupling of 4-aminobenzoic acid and propionic chloride. The synthesis of products is in progress. When the products are in hand, the background rate will be measured. If products can be detected with normal analytical methods, the hapten will be synthesized by coupling one product enantiomer to an N-amino porphyrin via a carbamate linkage and subsequently coupling the complex to carrier protein. If the background reaction is found to be too slow for aliphatic C-H hydroxylation, an allylic group will be introduced into the substrate to facilitate the reaction.

Aliphatic ketone reduction

Catalytic antibodies have been used to accomplish highly selective functional group transformations unattainable by current chemical methods. Recently, antibodies raised against amine-oxide hapten **1** were shown to catalyze the stereo- and regioselective reduction of aryl alkyl ketones **2-4** and diketone **5** in the presence of sodium cyanoborohydride. Antibodies specific for this hapten were expected to stabilize the tetrahedral transition state resulting from nucleophilic attack on the carbonyl group. This simple hapten design should provide a versatile strategy for the reduction of a broad range of compounds, since amine-oxide haptens are readily synthesized by oxidations of the corresponding amines. With this goal in mind, we have targeted the enantioselective reduction of 3-octanone. Since the catalyst must discriminate between ethyl and pentyl side chains, this reaction is difficult to accomplish using conventional chemical reagents. The corresponding amine-oxide hapten **6** was synthesized by reacting mono-methyl glutarate with N-ethylmethyl-amine in the presence of EDC to form the amido-ester.



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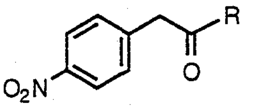
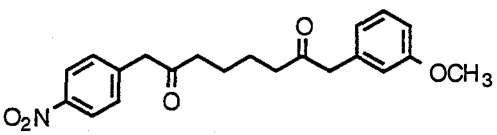


2: R = CH_2CH_3
3: R = $CH(CH_3)_2$
4: R = CH_2Ph



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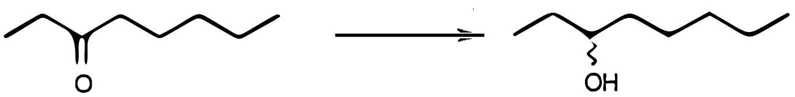

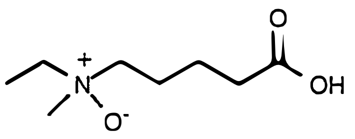

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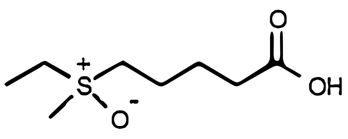

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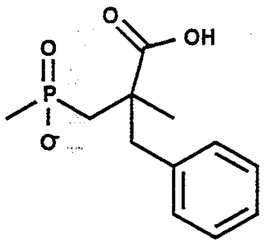
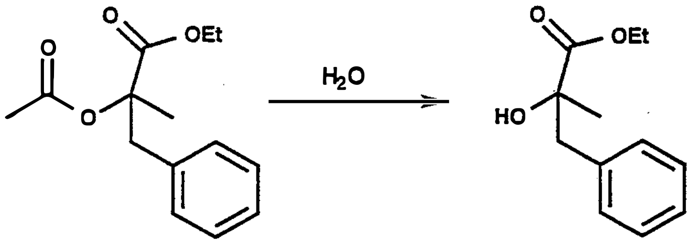
Reduction of the amide with borane gave the amino-ester, and saponification of the ester followed by mCPBA oxidation gave hapten **6**. Since hapten **6** was synthesized as a mixture of enantiomers, both (R)- and (S)- reductases are expected. Twenty antibodies are being generated, purified, and assayed at pH 5.0 in the presence of $NaCNBH_3$ by gas chromatography (GC). In addition, three antibodies specific for sulfoxide hapten **7**, an isoelectronic analog of hapten **6**, were purified and assayed for the same reaction. Unfortunately, a significant antibody catalysis was not observed. We are in the process of generating a larger pool of antibodies against these haptens.

Tertiary ester hydrolysis

As part of our continuing effort to expand the repertoire of antibody catalysis, we have targeted a number of reactions which are difficult to accomplish in aqueous solution or are not catalyzed by any known enzyme. Hydrolysis of tertiary esters falls into the latter category, and is potentially amenable to the approaches which have previously generated antibodies which hydrolyze alkyl and aryl esters. The phosphinate hapten shown below was synthesized in order to elicit a catalyst for the corresponding tertiary ester hydrolysis, also shown below. The tetrahedral phosphinate mimics both the geometry and charge distribution of the reaction's transition state. The hapten was coupled to both BSA and KLH (epitope densities of 25 and 21 respectively) as the activated NHS ester. Swiss Webster, Balb-c, and AJ mice were immunized using standard protocols; the optimum immune response was given by Balb-c mice, and we are currently in the process of generating monoclonal antibodies.



Hapten

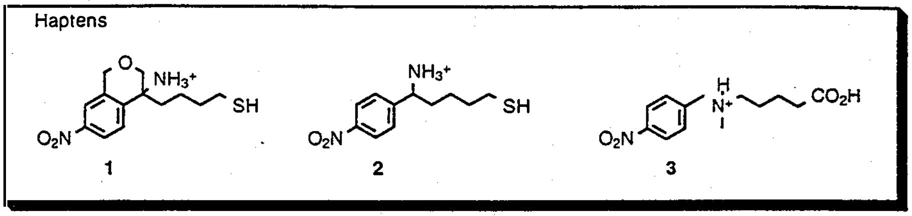


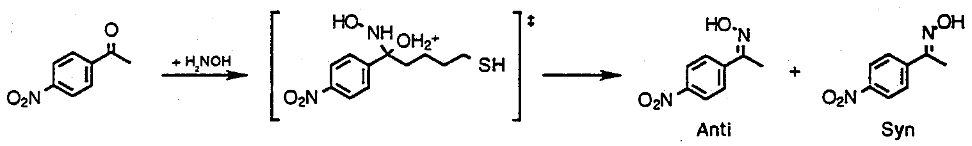
Reaction

Antibody-catalyzed stereocontrolled oxime function

Oximes are an important class of compounds as pharmaceuticals or agrichemical intermediates. Often it is necessary to synthesize one of the two possible isomers, *syn* and *anti*, selectively because they have different physical and biological properties. Because there exists no selective chemical catalysts capable of this transformation we again viewed this as an important opportunity to demonstrate the power of the catalytic antibody approach. We have demonstrated that antibodies elicited against ammonium haptens **2** and **3** catalyze oxime formation with various degrees of stereoselectivity and that some yield the unfavorable *anti* isomer as a major product. However the stereoselectivity was not absolute, most probably due to lack of stereochemical consideration in the hapten design of **2** and **3**. Therefore, we designed a new ammonium hapten **1** containing a cyclic ether moiety that mimics the hydroxyl group of the hydroxylamine moiety in the hope that antibodies elicited against this hapten will yield as the major product unfavorable *anti* isomer.

Our first attempt involved the synthesis of the hapten from isocromanone. Isocromanone is not commercially available and it was synthesized from ethyl o-toluate via five steps by known methods. Unfortunately, some of the reactions proved difficult (such as selective nitration and oxidation) despite several attempts, and an alternative synthetic strategy is currently being pursued. Once the hapten is prepared, characterization will be straightforward because the well-established assay methods previously employed can be used.





Antibodies with glycosidase activity

We have generated and characterized antibodies specific for haptens **10** and **11** for their ability to

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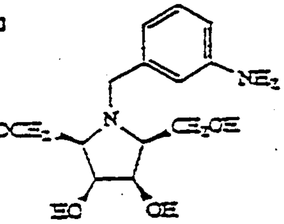

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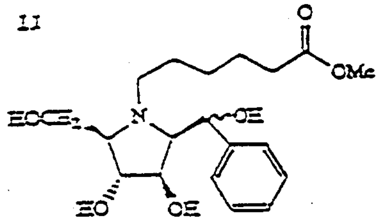
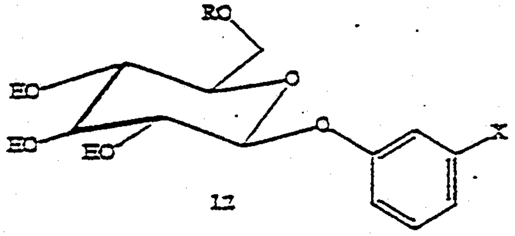


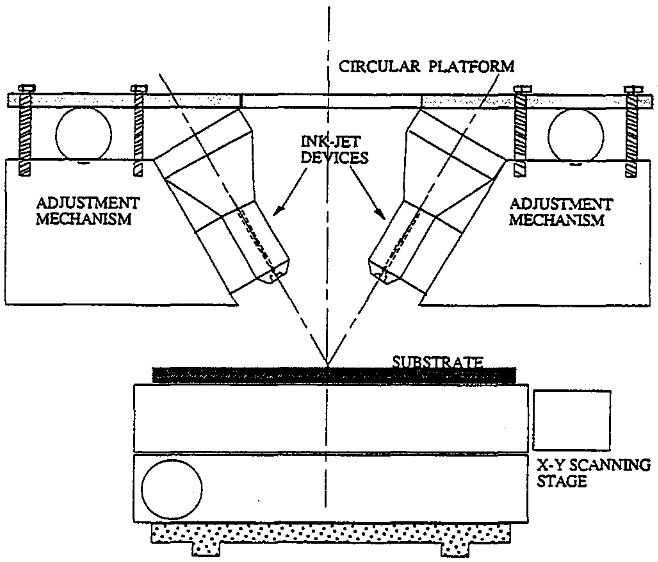
hydrolyze ß-glycoside **12**. Unfortunately, the antibodies obtained were found not to catalyze the



hydrolysis of the corresponding glycosides. This may be a result of the relatively low affinities of these antibodies for the haptens. We are now regenerating hybridomas against derivatives of **10** and **11** containing additional carbohydrate/alkyl groups in the hope of generating antibodies with higher affinities.

Design and preliminary fabrication of an inkjet system for generating zeolite libraries

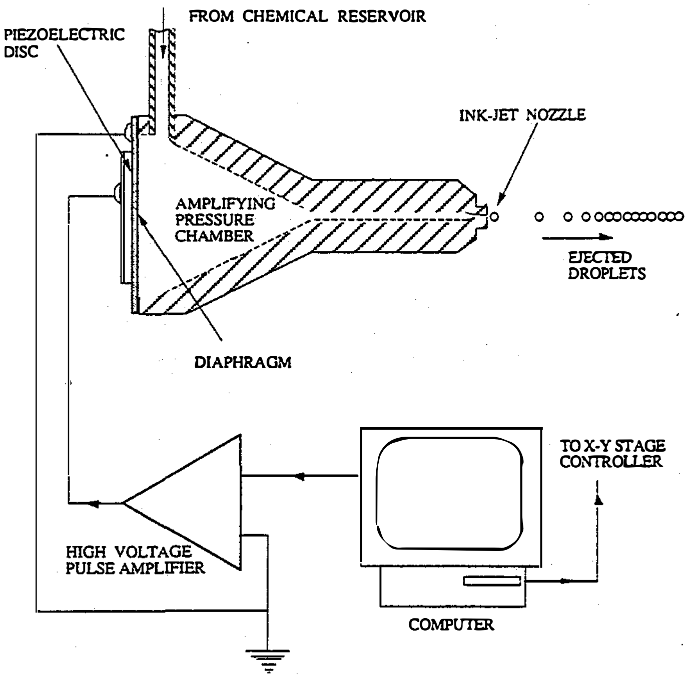
Metal oxide catalysts (zeolites, amorphous metal oxides) are typically synthesized from solution precursors. Consequently, we will develop a solution based inkjet delivery system for generating combinatorial libraries of these materials. The ink-jet technique has a number of attractive features for solution-based combinatorial synthesis. Ink-jet devices can deliver droplets of chemical solutions as small as a few picoliter per droplet in a "drop-on-demand" fashion. Up to a few thousand droplets per second can be delivered to reaction "vessels" with pin-point precision. Variation of the composition and stoichiometry can be easily automated with computer controlled ink-jet devices. Superior to traditional robot controlled piston-operated micropipettors in speed, accuracy and low cost automation, a piezoelectric inkjet delivery system is ideal for accurate pipetting of small quantities of solutions of various precursors to be used in the synthesis.



Multinozzle inkjet delivery system

We have designed a multi-nozzle ink-jet system for simultaneously delivering up to eights different precursor solutions. In this design, the key consideration is the integration of eight single-nozzle ink-jet devices (each connected to its own reservoir) such that we can deliver variable amounts of any given precursor to a single site on the library substrate, simultaneously. Our design consists of eight single nozzle devices, containing eight different chemical precursors, mounted aziumuthally on a circular framework. By fine adjusting the orientation of each single device, all eight jets can focus onto a single spot/reaction vessel on a substrate. The substrate is mounted on a scanning X-Y stage controlled by a computer. The library will be synthesized by scanning the stage and activating the ink-jet devices so that precursors are delivered simultaneously to each and every site with the desired composition and stoichiometry. A laser optical sensing system will be used to monitor droplet delivery. Intermediate evaporation steps can be carried out if desired.

From the above discussion, one can see that the performance of an individual ink-jet device is critical to the overall performance of the system. For the system to work, the jet from each nozzle should be very straight, single streamed and resistive to slight air disturbance at least within a few centimeters of the nozzle. After testing many different designs, we have successfully developed a high performance single-nozzle, "drop-on-demand" ink-jet device. As shown below, the ink-jet device is composed of three parts: the reservoir, the pressure chamber and the nozzle. If an electric pulse is applied to the piezodisc, the diaphragm is displaced and recovered, ejecting one droplet of the solution out of the pressure chamber. Our current ink-jet device is capable of ejecting very small drops (typically 50 picoliters) whose flying trajectory is extremely straight within 2 cm. The ejection rate is up to 2000 droplets/second which very likely can be increased further. Fluctuation in the volume of the droplets is typically within 3%. The performance is very reliable and extended tests have been conducted to check reliability. The components have been coated with chemically resistant materials. By gating the number of pulses applied to the ink-jet device (thus the number of droplets delivered) any desired stoichiometry can be achieved. The first prototype of the multihead inkjet system has been fabricated and is being tested. A computer controlled optical monitoring system is currently being developed.



Individual inkjet device

14. **Project Title** **Computer Aided Molecular Design of Biomimetic Catalysts**
Principal Investigators **John Shelnutt**
Project Site **Sandia National Laboratories**

Description:

The goal of the research program is to develop generic computer-aided molecular design (CAMD) methodologies and apply them to the design of biomimetic catalysts. Considerable progress has been made this year, especially in the characterization and testing of CO_2 catalysts. Global warming resulting from the greenhouse effect has given new impetus to chemical storage of solar energy. Further, CO_2 conversion using solar or electrochemical energy would provide a renewable source of clean fuels and feedstocks. Therefore, the CAMD effort will continue to focus on catalysts that activate and convert CO_2. The ultimate goal of this part of the research program is a process for solar-driven reduction of CO_2. However, other biomimetic reactions of commercial interest are also targeted in our CAMD efforts, particularly asymmetric oxidation of olefins. Application of the biomimetic CAMD methodology, coupled with experimental studies of the designed catalysts, results in an essential knowledge base consisting of structure-reactivity relationships that are required for rational catalyst design. Sandia is uniquely qualified to carry out this catalysis research because of its extensive CAMD and experimental facilities, expertise in designing C-H bond and CO_2 activation catalysts, and past work in solar-driven chemistries.

Elements of the research are:

- CAMD of catalysts that mimic CO_2-activating enzymes and olefin oxidation hemoproteins,
- structural studies of the enzymes,
- synthesis, characterization and testing of designed catalysts,
- integration into a solar-driven process, and
- development of improved molecular modeling techniques.

Industrial partners are participating in enhancement of molecular modeling methods (Biosym) and development of CAMD methodology, and in utilization of the resulting CO_2-activation catalysts and processes (DuPont). A CRADA with DuPont for developing a catalyst for production of anhydrous formaldehyde has been completed and awaits DOE approval.

1995 Accomplishments:

Our accomplishments for FY95 are primarily in the areas of testing the designed catalysts, synthesis of advanced catalyst designs, and structural characterization and validation of the CAMD methods.

- Catalyst Testing. Most important to the ultimate success of this program is the demonstration of catalytic activity of the cobalt porphyrins in CO_2 conversion reactions and the development of reliable testing protocols for evaluating incremental improvements in new catalyst designs. We previously demonstrated catalytic activity for the production of CO, H_2, and other products at low potentials (less negative than -1.0 V vs. Ag/AgCl reference) using a gas-diffusion electrode system. We are now concentrating our research efforts on improving this electrochemical testing cell and testing procedures. The gas-diffusion electrode cell has now been improved so that smaller amounts of porphyrin catalyst can be tested and the reproducibility of activity measurements for the same electrode is good. Cobalt-porphyrin catalysts on electrodes were tested for CO_2-reduction activity using various gas flow rates, electrolytes, electrodes and cell configurations. We are also working toward better reproducibility in the preparation of electrodes, primarily in the deposition of the Co-porphyrin catalyst. The testing cell modifications include the replacement of the hydrogen-oxidation electrode used in the earlier design. With this improvement we now get almost no hydrogen at the CO_2-reduction electrode, suggesting that diffusion of H_2 through the cell to the C_2O electrode was occurring. With the new cell we have obtained CO_2 conversion efficiencies to as high as 50% of the CO_2 gas stream; previously our highest conversion efficiencies were about 5%. We have also now compared our electrodes prepared with several of the cobalt-porphyrin catalysts (for example, Co dodecaphenylporphyrin, Co phthalocyanine, and Co tetraphenylporphyrin) with commercially-available cobalt-porphyrin electrode preparations

- Synthesis of advanced catalyst designs. With regard to the design of new Co-porphyrin catalysts, we have also completed structural characterization studies of hydrogen bonding to the some of the newly designed catalysts that have H-bonding functionality. We have found resonance Raman spectroscopy to be most useful for these studies. Although we have not yet synthesized the most promising of these H-bonding catalysts, we have demonstrated H-bond formation with H-bond acceptors like CO_2. At present, weak H-bonds are formed between H-bond acceptors in solution and H-bond donor groups on the porphyrin. These structural studies continue to validate the molecular modeling and design methodologies that we are using and suggest new improvements in the catalysts. Additional Co-porphyrin catalysts with two H-bond donor groups attached to the porphyrin are being synthesized. These catalysts should have much higher affinity for CO_2 and will orient the carbon atom of CO_2 over the cobalt ion. There has been some concern that the affinity for CO_2 might become too great when additional H-bonding functional groups are added, but based on the weakness of the hydrogen bond observed so far this is probably not a problem.

- Structural Characterization and Validation of the CAMD Methods. Predicted catalyst structures were verified by X-ray crystallography, resonance Raman spectroscopy, EXAFS and NMR spectroscopic studies. Several manuscripts which verify the use of our molecular models for predicting metalloporphyrin structure have been published in Journal of the American Chemical Society and Inorganic Chemistry. One of the new papers is a result of the new collaboration with Prof. Bill Goddard, initiated to improve our force field for POLYGRAF molecular mechanics calculations by using a method developed by Sidharth Dasgupta and Goddard.

- Cynthia Lundgren, an electrochemist at DuPont, and John Shelnutt have completed a CRADA to design, synthesize and test catalysts for CO_2 conversion to anhydrous formaldehyde. The CRADA document is currently awaiting DOE approval.

1996 Planned Activities:

- Test New CO_2 Catalysts. Prof. Josè Colucci-Rios at the University of Puerto Rico will be at Sandia this summer to help in the testing of the designed catalysts. Prof. Rios now has two graduate students working in our labs on the project and details on electrode preparation have been transferred to Prof. Rios' lab. We expect this will greatly accelerate the testing of the catalysts.

- Currently available CO_2-reduction catalysts will be tested and compared for activity and stability. These are primarily the cobalt derivatives of already synthesized porphyrins as well as catalysts synthesized during the next year. (Specific catalysts are described in the discussion of synthesis in this and the preceeding sections.)

- Both current-voltage performance of the cells and cyclic voltamograms will be obtained for each catalyst. Methods for detecting products other than CO will be developed and utilized to determine product selectivities.

- Finally, under CRADA work with DuPont, catalysts will be screened for selectivity in the formation of formaldehyde and electrode systems will be investigated for the possibility of anhydrous operation.

- Design and Synthesize CO_2-Activation Catalysts. In the molecular modeling area, we expect to complete the development of a spectroscopically accurate force field within the next year. This spectroscopic force field should immediately aid in the characterization of catalysts by helping with the interpretation or Raman spectra. The prediction of molecular structures may also improve with the inclusion of force constant cross terms.

- Design and Synthesize Chiral Catalysts. In the area of asymmetric oxidation catalysis, we will synthesize a chiral dodecaphenylporphyrin or pyridyl analog in collaboration with Medforth and Smith at UC Davis.

- *Ab Initio* modeling of Catalysts. We will complete geometry optimization calculations of NiP in various starting conformations using both LDF and HF methods. We expect these calculations to determine the accuracy that can be expected for more elaborate porphyrin structures and also to determine which methods give the best results. We will investigate the ability of these quantum methods to predict the low energy conformers (local minima) that we have found previously using molecular mechanics methods and confirmed experimentally using spectroscopic methods.

- Characterization of New Catalysts. We will complete the structural characterization of metal derivatives of the new porphyrin catalysts with resonance Raman spectroscopy, NMR spectroscopy, UV-visible absorption spectroscopy, and X-ray crystallography. These studies will aid in determining the effects of electron withdrawing substituents, conformation differences, and hydrogen-bonding on the activity and stability of the catalysts. Specifically, we will fully characterize the metal mono- and di-amino-OEPs, hydroxymethyl-OEPs, formyl-OEPs, chiral porphyrins, and metal octapyridylporphyrins.

- For the porphyrins expected to form hydrogen bonds with CO_2, we will carry out spectroscopic studies of the binding of CO_2 to the porphyrins.

Annual Technical Summary Report:

Catalyst Testing. Experimental testing of the designed porphyrin catalysts for CO_2 reduction is continuing. We have now found electrode-electrolyte systems in which our catalysts carry out CO_2 reduction. It is our immediate goal to use our molecular design methodologies to develop catalysts that selectively produce a desired CO_2 reduction product at the minimum potential beyond what is thermodynamically required and with the greatest current efficiency possible.

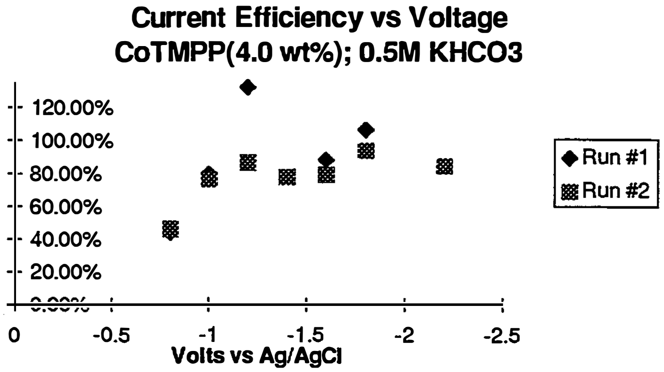
A modified ASTRIS Quickcell is currently being used. The CO_2 electrode consists of an Alupower gas diffusion electrode modified with the Co-porphyrin catalyst. The Co-porphyrin catalyst is added by soaking the gas diffusion electrode in a pyridine or CS_2 solution of the catalyst. Product (CO) concentration is determined by gas chromatography. No CO product is observed from the cathode in the absence of the Co-porphyrin catalyst. Previously, CO, H_2, and unreacted CO_2 were the products detected in the exiting CO_2 stream. In the modified cell which replaces the H_2 gas-diffusion anode with a platinum foil which oxidizes water, only a small amount of H_2 has been detected in the CO_2 stream, indicating that there was substantial crossover of H_2 from the anode. However, H_2 is produced for electrodes with low coverages of Co-porphyrin catalyst.

Replacement of the H_2 electrode may also contribute to the higher conversion efficiencies obtained with the new testing cell. Current at the CO_2-reduction electrode was limited by the H_2 oxidation rate at the hydrogen electrode. This limitation is removed in the present cell design. Up to 50% conversion to CO has been observed the the current cell. Prevously, no more than 5% of the CO_2 gas stream was converted to CO. A more controllable gas flow rate is partly responsible for the higher conversion efficiency.

No significant difference is observed for electrodes prepared from pyridine and CS_2.

The cell has also been modified so that a smaller area electrode can be utilized. This means that smaller amounts of the porphyrin catalyst can be tested, and, therefore, more electrode tests can now be conducted, without resorting to large scale synthesis of catalysts. This is important since much smaller expenditures of resources are required and environmental, health, and safety concerns are reduced. In our standard catalyst test, 0.68 μmoles of catalyst are loaded onto the 5-cm2 electrodes, providing roughly 0.14 μmole of catalyst/cm2, or 8 μg of cobalt/cm2. When ten times this amount of catalyst is loaded on the surface, no more than double the activity is observed, which indicates we are using the lower loading of catalyst effectively, but that additional loading is not available. For comparison, in other gas-diffusion electrode applications using platinum, the lowest catalyst metal loadings used are 20 μg/cm2, and it is clear that higher loadings should be feasible. In order to utilize more of the surface area of the gas diffusion electrode, we are exploring alternate catalyst loading techniques using other solvents and immersion of the electrodes for longer periods of time.

We have found that a commercially prepared electrode [Electrosynthesis Corp., Lancaster, NY] loaded with CoTMPP for fuel cell applications yielded nearly a 100% current efficiency for CO_2 to CO conversion. In their preparation the cobalt porphyrin is loaded onto the carbon particles prior to making the gas diffusion electrode, so that there is a uniform distribution of catalyst throughout the electrode, which results in a higher number of catalytic sites than does loading catalyst onto a preformed electrode. It is also found that lowering the CO_2 flow rate below 16 ml/min starves the electrode of reactant; so in spite of enhanced residence times, lower CO production is achieved.



We have also completed an initial study of a series of our designed catalysts (the CoDPPs in which peripheral hydrogens were replaced by fluorines) to explore the effect of a shift in the redox potentials of the catalysts. At present the results are confusing because of the $CO_2/HCO_3-/CO_3=$ equilibrium with pH and other problems. These issues are being addressed.

Detection of Substrate-Catalyst Hydrogen-Bonding Interactions. Aside from the testing results, the most important progress made this year is the demonstration of hydrogen-bonding between substrate molecules and H-bond donor groups attached to the catalyst. H-bonding between substrate molecules and the -NH_2 group of 5-amino-octaethylporphyrin (5-NH_2 -OEP) has been demonstrated by resonance Raman spectroscopy. The molecular structure of 5-NH_2-OEP is shown in Figure 1 and its resonance Raman spectra in the C-N stretching region of the amino group for various solvent conditions are shown in Figure 2. (Figure 2 also shows the spectrum of NiOEP (unsubstituted) for comparison.) H-bond formation is detected in the resonance Raman spectrum by the upshift in the frequencies and changes in relative intensities of several Raman lines in the region of the C-N stretching vibration of the amino group. No other significant differences are observed in the spectra, indicating negligible effect of H-bonding on the porphyrin macrocycle. Of particular interest is the upshifts in the lines at 1260 and 1382 cm-1 and the decrease in the intensity of the latter line. The hydrogen bonding is weak since fairly high concentrations of the H-bonding solvent is required. At one atmosphere of CO_2, no H-bonding was observed, as is to be expected for such low CO_2 concentrations.

Hydrogen bonding to substrates with H-bond donor groups also may have been detected for 5-monoformyl-octaethylporphyrin (5-CHO-OEP). However, in this case the interpretation is complicated by the presence of two conformers--one for which the formyl group is perpendicular to the plane of the porphyrin and another for which the formyl is parallel to the porphyrin ring and conjugated with the ring. These conformers also differ in the degree of nonplanarity, adding further complexity to any interpretation in terms of H-bonding. The metal derivatives of 5-mono-hydroxyethyl-octaethylporphyrin (5-CH2OH-OEP) have also been synthesized and investigated for H-bonding to donors and acceptors.

Because of these promising results we are now synthesizing the 5,15-diamino-, 5,15-diformyl-, and dihydroxyethyl-OEP derivatives; molecular modeling suggests much stronger H-bonding to this derivative. At this point, we can say that hydrogen bonding to at least the NH_2 group does occur and probably is strong enough to promote substrate binding and conversion, but not so strong as to interfere with catalytic reactions occurring at the metal site. The cobalt derivatives of the 5-amino, 5,10-diamino, and 5,15-diamino derivatives will be synthesized in large enough quantities to test in electrochemical CO_2-reduction reactions.

Synthesis of Advanced Catalyst Designs. Dodecaphenylporphyrin Derivatives. Synthesis of the iron and cobalt derivatives of the fluorinated dodecaphenylporphyrins (DPP) has been completed in quantities suitable for catalyst testing. This required the scaled-up re-synthesis of the entire series. The compounds are: CoDPP, CoDPPF8, CoDPPF20, CoDPPF28, and ComDPPF36 (also CoDPP(MeO)20). The synthesis of all but ComDPPF36 is complete. These halogenated porphyrins are of interest because of their (1) high stability, (2) the presence of a CO_2-binding cavity, and (3) their novel electronic properties, which will extend the range of redox potentials for this series in the negative direction. Efforts are still underway to synthesize more highly fluorinated versions of CoDPP via the Susuki reaction.

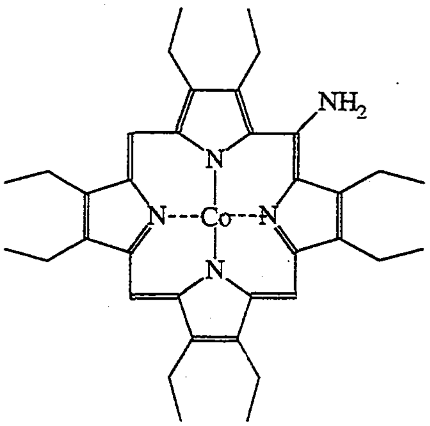


Figure 1. Cobalt 5-amino-octaethyl-porphyrin. The amino group is an H-bond donor and acceptor.

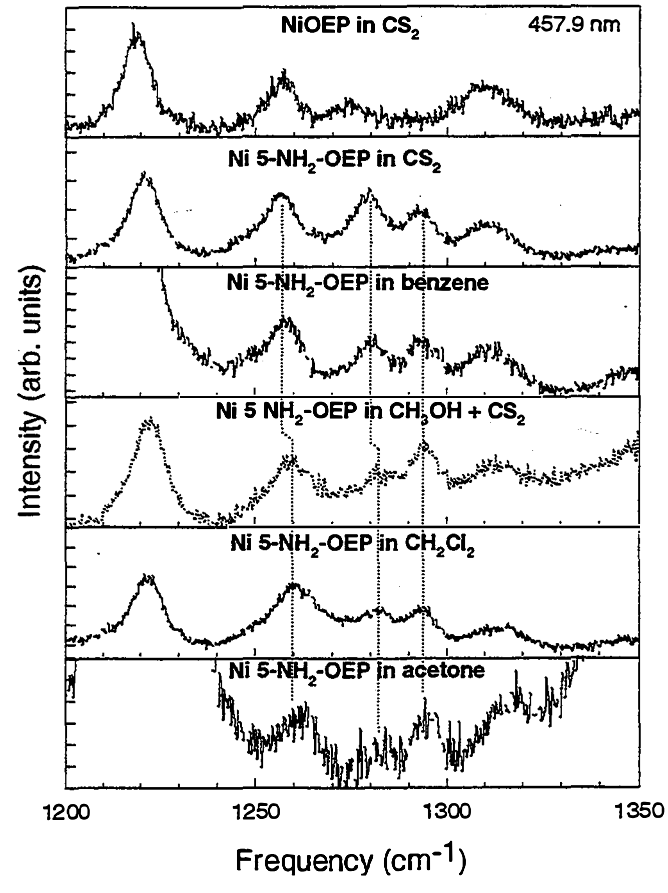
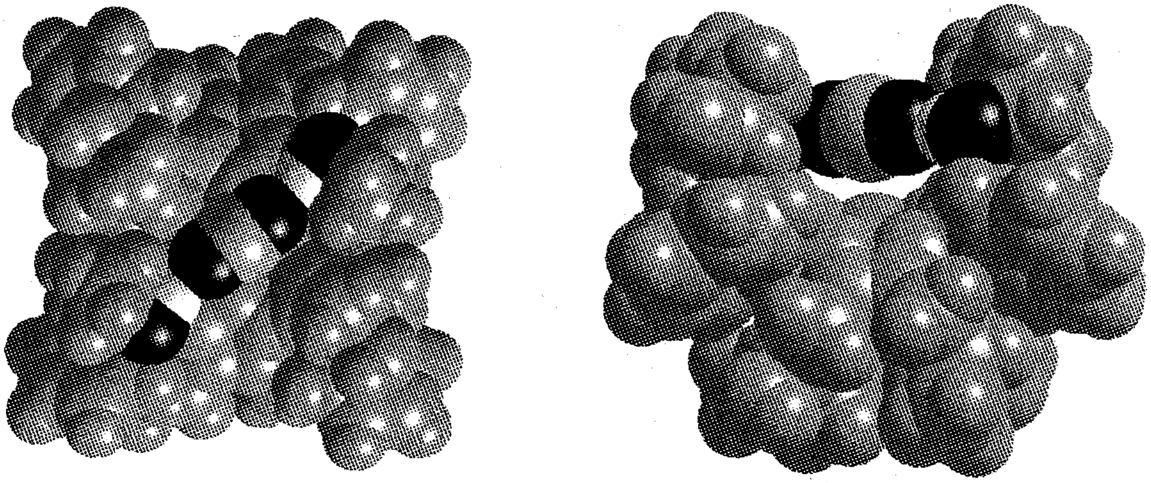


Figure 2. Resonance Raman spectra of nickel(II) 5-NH₂-OEP (and NiOEP for comparison) in various solvents. This region of the spectrum contains the C-N stretching vibration which normally occurs in the 1260-1330 cm^{-1} range for primary aromatic amines. H-bonding sovents (bottom three spectra) cause intensity and frequency changes in this region of the spectrum and in no other porphyrin Raman lines. Solvent lines obscure some porphyrin band in some cases.

We are continuing our efforts aimed at the synthesis of porphyrins with chiral hydrogen-bonding functionality. These catalysts will serve dual purposes as chiral alkane and olefin oxidation catalysts and as CO_2-reduction catalysts, depending on whether Fe or Co are incorporated into the porphyrin. As enantioselective oxidation catalyst the H-bonding functionality gives a stronger interaction with the substrate which can be used to direct the approach of the substrate to the catalytic metal center. The probable result for a H-bonding substrate molecule is higher enantiomeric excesses than can be attained in the absence of H-bonding groups. Synthesis considerations have forced us to change our original target catalyst design. The most promising design in now that in which the chiral hydrogen-bonding functionality is attached at one of the pyrrole-carbons via the Susuki reaction. The result is a porphyrin with eight chiral substituents attached.



Top View Edge View

Figure 3. Chiral octasubstituted derivatives of cobalt tetraphenylporphyrin. These catalysts are expected to be active and stable CO_2 reduction catalysts with hydrogen-bonding functionality provided by the OH (or NRH, αvδ NH_2) groups of the chiral substituents(in this case $CH_2C^*HOHCH_3$. For the conformer shown, the OH groups (red and white) are located at each end of the CO_2-binding cavity; CO_2 is red and gray.

The desired effect of the chiral substituents is to place two hydrogen-bond donor functionalities (the -OH groups shown in Figure 3) in the groove in which CO_2 is bound. There is another advantage of this molecular design over that suggested previously in that the chirality of the cavities on the top and bottom of the porphyrin are identical.

Model Validation: Tetraalkylporphyrin Derivatives. We have completed a comprehensive study of Ni derivatives of a series of meso-tetrasubstituted porphyrins including one that is a potential CO_2-reduction catalyst. These include tetraphenylporphyrin (TPP), tetramethylporphyrin (TMP), tetraethylporphyrin (TEP), tetraisopropylporphyrin (TIPP), tetra-t-butylporphyrin (TtBP), and tetraadamantylporphyrin (TAdP). Besides these tetraalkyl derivatives, two chiral derivatives were included in the study. One of these, a cyclopropyl derivative, has H-bonding capabilities. Meso-substitution invariably gives ruffled, as opposed to saddle, distortions of the macrocycle. The idea is to identify characteristic spectroscopic signatures for the two types of distortion. This is important from the standpoint of designing a rigid cavity for CO_2. This model validation work is reported in a long manuscript in press in the J. Am. Chem. Soc. This is probably the most fundamentally important work to come from our molecular modeling of metalloporphyrin catalysts. It provides the entire framework necessary for discussion of porphyrin distortions and substituent effects on molecular properties. This new insight should greatly aid in the design of new materials based on the porphyrins, including catalytic materials.

Development of New Modeling Methods. We have been using Molecular Simulations' new CERIUS2 Version 1.6 and Bill Goddard's research version of POLYGRAF, which we obtained in a recent working visit to CalTech. The latter program has quite a bit of functionality that is not in the commercial version. Some of these new capabilities are vital to our goal of developing a molecular mechanics force field for porphyrins that accurately predicts not only the conformation of the molecules but also the energies and vibrational spectra of the various conformer (local minima). We have completed the computer-optimization of the our molecular mechanics force field to the experimental data. We have also re-optimized the force field because of a minor error that has been in POLYGRAF from the beginning. This new force field corrects a minor problem in treatment of the metal in earlier versions and uses a periodic cosine function for the angle term of the metal. This improved force field was used in the calculations reported in J. Am. Chem. Soc. manuscript mentioned above. This new force field has now been converted to a format acceptable for the new CERIUS2 1.6 program, and we are beginning to use this program for our routine molecular modeling and design work.

15.	**Project Title**	**Energy, Environment and Economics Characterizations of Commodity Chemicals and Polymers**
	Principal Investigators	**Anna L.Tonkovich, Edward Lipinsky and Joyce Widrig**
	Project Site	**Pacific Northwest Laboratory and Batelle Memorial Institute**

Description:

Pacific Northwest Laboratory supports the mission of the Biological Chemical Research Technology program by providing systems analysis to identify industrial R&D needs in the chemical industry with the potential to offer greatest impact on economics, environment, and energy.

1995 Accomplishments:

Work during FY '95 focused on quantifying the impact of energy, environment, and economics on the catalytically produced top 50 U.S. commodity chemicals, and developing a companion report to the Brief Characterization of the Top 50 U.S. Commodity Chemicals on the Top 12 polymers. The Top 12 polymers were characterized and ranked based upon production volume, energy intensity, total energy consumption, price, revenue, and capital intensity. This effort resulted in two reports: The Top 50 Commodity Chemicals: Impact of Catalytic Process Limitations on Energy, Environment, and Economics and Characterizations of Top 12 Commodity Polymers.

1996 Planned Activities:

PNL plans to continue to support the BCTR program by using the data generated during the previous studies to develop initiatives that identify opportunities for non-incremental changes in process technology and the alternatives for more environmentally benign processes. With.the guidance of our headquarters program manager, David Boron, our industrial guidance and evaluation panel, and reviews with other industrial representatives, we will select and develop at least two new initiatives for consideration by the BCTR program. Possible initiative areas include:

- Advanced polymerization concepts that facilitate conversion to end products
- Develop online computer assistance on polymer processing for decision making and real time control
- Alternative routes to feedstocks
    - Based on natural gas
    - Based on biomass
- Integrated Catalyst/Reactor/Separator systems

- User-site manufacture of chemicals/polymers
- Oxidation Systems based on water or air.
- Develop an integrated approach to the production of higher value hydrocarbons, fuels, and/or hydrogen by conversion of methane/natural gas

Additional data gathering and synthesis will be done in support of selected initiatives.

Annual Technical Summary Report:

Production of the Top 12 Polymers account for a large percentage of the output of the Top 50 Commodity Chemicals. This relationship led to an evaluation of process limitations for both the polymers and the chemicals that are their feedstocks. In addition, an evaluation of the conversion processes by which the polymers are converted into useful objects was performed.

The production processes for the top 50 U.S. commodity chemicals consume excess energy, generate unwanted byproducts, and require more than a stoichiometric amount of feedstocks. The excess energy use was about 0.83 quads/year, which is about one percent of U.S. energy consumption. Approximately 54 percent of the energy loss was a result of low per-pass yields that require separation and recycle of unreacted feedstocks. About 20 billion pounds of carbon dioxide is the major unwanted byproduct. The cost of the wasted feedstocks is about $2 billion/year. Process limitations resulting from unselective catalysis and unfavorable reaction thermodynamic constraints are the major contributors to this waste. Several advanced concepts are presented in the report that address these problems in an integrated manner

Many commodity chemicals are used to produce large volume polymer products. Of the energy and feedstock wasted during production of the commodity chemicals, nearly one-third and one-half, respectively represents chemicals used as polymer precursors. Approximately 38% of the carbon dioxide emissions are generated producing polymer feedstocks.

The production volume for the Top 12 Polymers exceeds 76 billion pounds per year. These materials are energy-intensive, accounting for about 3 quads/year of energy consumption. The revenue derived from sales of these polymers is about $45 billion. Considerable value is then added by the users of the Top 12 Polymers who convert them into fibers, films, and many molded items. These conversion processes (e.g. injection molding and extrusion) also consume substantial energy, mostly in the form of electricity.

Production volume, energy intensity, total energy consumption, price, revenue, capital intensity are the six criteria that are used to characterize each polymer. The top polymer for each criterion could be an important topic for BCTR's attention. They are polyethylene (production volume, quads per year, and revenue), nylon-66 (energy intensity and price), and PET (capital intensity). When the polymers are ranked on the basis of the four independent criteria, eliminating the redundancy of production volumes, a different perspective arises in which nylon and PET dominate the list.

Despite the great progress made by the producers of the Top 12 Polymers, the processes are far from perfect. Production of many of the Top 12 Polymers is not optimized because of such drawbacks as slow reaction times, operation at close to product degradation temperatures, multiple passes at low yields per pass, and the need for expensive control systems to prevent runaway reactions. Conversion processes frequently have one or more of the following drawbacks: massive equipment is needed to apply high pressures, constraints on product thickness, consume significant amounts of electricity, prolonged cycle times, and narrow range of processing conditions to avoid degradation. Most of these drawbacks can be traced to the need for enhanced heat transfer and mass transport in polymerization and conversion operations. Several initiative ideas to address these issues are presented in the report.

16. Project Title | **Over Production of Lignocellulosic Enzymes by Genetic Engineering Methodology**
Principal Investigators | **Arthur Williams**
Project Site | **Howard University**

Description:

The project seeks to understand the biological and chemical processes involved in the secretion of the enzyme polyphenol oxidase (PPO) by the hyphae, the basic unit of the filamentous fungus *Coriolus versicolor*. These studies are made to determine rational strategies for enhanced secretion of PPO, both with the use of recombinant DNA techniques (Howard University), and without (Clark-Atlanta). This effort is done in concert with work at Clark-Atlanta University but focuses on recombinant DNA techniques to enhance enzyme production.

The major thrust of this project was two-fold: (i) to mass produce *C. versicolor* tyrosinase (polyphenol oxidase) by genetic engineering as well as cultural manipulations; (ii) to utilize polyphenol oxidase (PPO) as a biocatalyst in the processing of lignocellulose as a renewable energy resource. Wood-decay within forests, a significant renewable photosynthetic energy resource, is caused primarily by *Basidiomycetous* fungi, i.e., white-rot fungi. In the case of the white-rot fungus, *C. versicolor*, its degradative capacity results from the ability to elaborate extracellular cellulolytic and ligninolytic enzymes. With regard to the latter, at least one of the enzymes, PPO, appears within the external milieu in a highly time-dependent fashion when *C. versicolor* is cultured in a defined growth medium.

In this study, the assessment of genomic and cDNA recombinant clones with regards to the over-production of PPO continued. During antibody (anti-tyrosinase) screening of genetic clones, enhanced production of polyphenol oxidase in several variants was observed. Further, immunoprecipitation and ELISA protocols were employed to detect and quantify tyrosinase(PPO) during cultural manipulations. Catechol and tyrosine appeared to stimulate the production of PPO in bulk cultures. In the attempt to over produce PPO, it was noticed that the activity of catechol oxidase (CTO) was higher than PPO. Summarily, owing to the fact that tyrosinase complex possesses multiple activities e.g., monophenol oxidase, PPO, CTO, the employment of it as a biocatalyst to process lignocellulose will be discussed.

Wood-Decay in the Forest - Renewable Energy Resource:
Wood-decay within Forests, a renewable photosynthetic energy source, is caused primarily by Basidiomycetous fungi, e.g., white-rot fungi which possess the ability to degrade lignin, cellulose and hemicellulose, the main polymers of wood. In the case of at least one of these fungi, *C. versicolor*, this ability results from the fungus' capacity to elaborate both extra-cellular cellulytic and ligninolytic enzymes. synthesis and secretion of these enzymes can occur *in situ* and in vitro. With regard to the latter, at least one of these enzymes, PPO makes its appearance within a culture medium in a highly time-dependent fashion. Presumable, extracellular PPO originates via secretion of intracellular PPO. However, this presumption requires verification. Because PPO, an enzyme capable of converting diphenols to diquinones and oligomerizing the lignin derivative, syringic acid, appears to be inducible, it is conceivable that the c. versicolor culture system could be a model

for achieving over-production of enzymes, a stated mission of the DOE-ACID Project. In addition, the system, which can be scaled-up to industrial levels of enzyme production, seems be one in which the regulation of synthesis and/or secretion of both cellulolytic and ligninolytic enzymes can be investigated separately; i.e., experiments can be designed whose results may lead to the independent control of synthesis and/or secretion.

Lignocellulose - a Forest By-Product:
Besides its presence within wood, lignocellulose often constitutes an unwanted component in the paper-pulp industry and also renders certain agricultural commodities less digestible to ruminants. Thus, an available supply of lignocellulolytic enzymes could be marked industrial value. To maintain an adequate supply, substrate induction coupled to hyphal 'batch culture' and/or genetic engineering techniques could be employed to over-produce these enzymes.

The cellulases and ligninases that are secreted by *C. versicolor* can degrade cellulose and lignin, the main organic polymers of wood. *C. versicolor* can be grown in 'batch culture,' which makes the over-production and the enhanced secretion of interest, commercially and biologically.

Objectives

1. Detect and quantify the expression of PPO in recombinant clones of *E. coli*.
2. Detect and quantify the over-production of PPO during cultural manipulations.
3. Mutagenize cloned DNA inserts harboring the PPO gene.
4. Assess the expression of recombinant clones carrying mutagenized DNA inserts for their abilities to over-produce PPO.
5. Perfect the PPO system as a model for producing large scale quantities of industrial enzymes.
6. Characterize the catalytic properties and activities of the PPO complex.
7. Utilize PPO as a biocatalyst in wood bioprocessing, e.g., lignocellulose utilization.
8. Initiate the development of a course curriculum impacting upon the environment with heavy emphasis pertaining to biotechnology, manufacturing, and bioprocessing.

Methodology

Part I Genetic Engineering of the PPO Gene

1. Construction of PPO recombinant clones
2. Screening of recombinant clones of PPO
3. Trafficking of PPO in *E. coli* cells

Part II Biological Processing of the PPO Gene

1. Enzymatic catalysis of PPO.
2. Enzymatic assay of PPO.
3. Bioprocessing of PPO.

1995 Accomplishments:

- Generated several PPO recombinant clones of *C. versicolor* by means of cDNA approach.
- Assessed the expression of the PPO gene in bacterial cells as well as a unique yeast variant.
- Quantified PPO production via antibody probing.
- Enhanced PPO production via cultural manipulations.
- Detected other enzymatic activities associated with the PPO complex.
- Initiated the utilization of PPO as a biocatalyst in the processing of lignocellulose.

1996 Planned Activities:

- Market the mass production of PPO via genetic engineering and cultural manipulations
- Resolve other enzymatic activities associated with the PPO complex.
- Utilize multiple activities of PPO complex in the bioprocessing of lignocellulose.
- Demonstrate other industrial applications of PPO.
- Generate a patent of PPO proteins.

Annual Technical Summary Report:

Results

During the past year, the assessment of genomic and cDNA recombinant clones with regards to over production of PPO has continued.

During antibody, anti-tyrosinase, screening of genetic clones, enhanced production of PPO in several variants was observed. That is, immunoprecipitation and ELISA protocols were employed to detect and quantify tyrosinase, i.e., PPO, during cultural manipulations. Cultural filtrates and tissue extracts were concentrated whereby standardized aliquots were tested in the ELISA protocol. On the other hand, corresponding aliquots were subjected to SDS-polyacrylamide gel electrophoresis subsequent to immunoprecipitation. Based upon these results, one major protein band was detected from the tissue extracts while no visible band was displayed from the filtrates during the 15 day growth period.

Other recombinant clones were generated by means of the cDNA approach. Specifically, two methods were employed to separate poly (A+) mRNA from bulk RNA and the polyA Tract System proved more efficient. An aliquot of the poly (A+) sample was subjected to formaldehyde-agarose gel electrophoresis to confirm its presence. In order to determine whether the poly (A+) mRNA was functional, samples were subjected to the BRL in vitro translation system and two protein bands, approximately 40-60 kDA, were visible. These protein bands were transferred to nitrocellulose paper, using the Sartobolt apparatus and probed with fluorescein-PPO antibodies.

Additionally, the enzymatic profile of activities associated with the tyrosinase complex was determined. Catechol and tyrosine appeared to stimulate the production of PPO. The activity of CTO was even higher than PPO. Summarily, owing to the fact that tyrosinase complex possesses multiple activities, e.g., monophenol, PPO, and CTO, its employment as a biocatalyst to process lignocellulose is currently being tested.

Significance of Biological Processing of PPO

The white-rot basidiomycete, *C. versicolor*, secretes PPO as well as other lignocellulolytic enzymes *in situ* and *in vitro*. These enzymes degrade cellulose and lignin, the principal polymers of wood. Wood is a component for trees which constitutes a renewable forest energy resource. Also, these enzymes may play a role in the recycling of waste from lignocellulose degradation.

To date, environmental biotechnology has a direct and fundamental role in bioremediation, biorestoration, waste treatment, environmental diagnostics and analysis, the preservation of biodiversity, and the development of environmental risk assessment technologies. Some examples of recent developments in toxic organic chemicals and others that emit visible light signals when they encounter specific chemicals in the environment.

Collectively, modern bioprocessing technology is able to achieve economically efficient manufacture of many desirable end products by using renewable resources and biocatalysis that minimizes energy consumption and reduces waste products. Biological processes require lower temperatures and pressures than traditional chemical processes and are inherently more energetically efficient. Applications of biotechnology to energy production and use are closely related to bioprocessing and are likely to be an important part of our economic future by promoting production of fuels from renewable resources.

17. Project Title **Immobilized Enzymes in Organic Solvents**
Principal Investigators **Haya Zemel**
Project Site **AlliedSignal**

Description:

This project will determine the mechanisms of enzyme activity and stability in nonaqueous media and derive microscopic models of the mechanisms. The models will be used to explain observed enzymatic action, and to predict conditions for optimum catalysis by (modified) enzymes in nonaqueous media. The application of enzymes to organic synthesis in non-aqueous media is an emerging technology with tremendous promise for the chemical industry. It might very well be the key to legitimizing biocatalysis and transforming it into a widely practiced commercial chemical processing technology. The need for this transformation is becoming increasingly obvious, as environmental issues of toxic waste generation are forcing the industry to take action aimed at reducing the production of pollutants. Enzymes, in their specificity and efficiency can contribute immensely towards cleaner and safer chemical production processes. One of the obstacles to utilizing enzymes in a commercial setting is the fact that most industrial chemicals are insoluble in the enzymes natural aqueous environment. Thus the adaptation of enzymes to organic media is a major requirement. Moreover, the use of organic solvents as the enzymatic reaction's medium offers many other advantages as well. For example, the need to covalently immobile the enzymes is eliminated as they are insoluble in the organics. So are many enzyme cofactors; these will be retained with the enzyme on the surface of a support in contact with the solvent, and will not wash off. Such consideration will provide a simpler and less costly bioreactor. Other advantages of enzymes/organic-solvents systems include: reduced product and substrate inhibition, simplified separation and reversal of thermodynamic equilibria.

The potential of the technology is great. What is needed in order to realize this potential is the understanding of the phenomenon on a molecular basis so that the parameters, which affect the efficiency and stability of such systems, can be intelligently manipulated to afford the practical scale-up of enzyme-catalyzed chemical production processes in non-aqueous media. The objective of this project has been to investigate the physical chemistry of immobilized enzymes operating in organic solvents, and improve the efficiency of an enzyme reactor performing trans- or interesterifications in organic media.

1995 Accomplishments:

- We have greatly improved the performance of an immobilized protease bioreactor for production of an acrylic monomer in a dry organic solvent:

  The initial rate for trans-esterification of vinyl acrylate with (R,S)-sec-(2-naphthyl)ethanol in dry amyl alcohol catalyzed by subtilisin Carlsberg has been increased by two orders of magnitude. The conversion level was improved from 20% to 87%, and over 10 grams of chiral monomer have been produced. The major part of the improvement was achieved by protecting the enzyme during pretreatment, before utilization as the biocatalyst in the non aqueous media. The protection was provided by the addition of a lyoprotectant and by the proprietary fabric or fibers support. Additional rate acceleration was obtained by better controlling the water content of the support and reaction mixture.

- Enhanced performance of proprietary supports was demonstrated.

  We have demonstrated that immobilizing the enzyme on a proprietary polymer, in either a free fiber configuration or as a component of a non-woven mixed polarity fabric provides a significant boost in enzyme activity over particulate supports. This was demonstrated for both wet and dry non-aqueous systems.

1996 Planned Activities:

The remainder of the year will be devoted to completing experiments with the lab scale reactor for enzymatically catalyzed production of (R,S)-sec-(2-naphthyl)ethylacrylate. As a part of this effort we will conduct the following work:

- We will continue investigating the proprietary fiber as a potential bioreactor enzyme support. Three more fiber compositions, obtained from our Fibers Business unit, will be tested in order to understand the positive effect that this fiber has.

- A circulating batch reactor has been constructed and will be used to examine reactor design parameters such as, shear sensitivity, packing density, fluid dynamics, residence time and temperature effects. These operating parameters will be examined and optimized. At the end of the period enough material will be produced for polymerization.

- Submit a final report.

Annual Technical Summary Report:

The overall goals of this project are to investigate the critical factors that limit commercial scale applications of enzymes in organic solvents, and to scale-up a process for the production of a precursor to a specialty polymer. The overall performance of an immobilized enzyme can be influenced by its intrinsic structure and changes in this structure, as well as by external factors such as water content, support identity and morphology, reactor design, etc.. In the past we have investigated the interrelation between support morphology and water content and its effect on overall enzyme performance. We looked at particulate and fabric supports in wet as well as dry non aqueous environments. In the wet organic solvents, we found mass transfer issues to dominate the enzyme performance. Thus particle porosity, as well as the use of a particular non-woven fabric as an enzyme support, provided an advantage. In dry systems, where mass transfer is generally not an issue, the particulate supports did not provide any benefit, while the non-woven fabric greatly enhanced the rate of the enzymatic reaction. We believe that this particular fabric with its hydrophilic fibers acts as a lyoprotectant in the process of drying the enzyme.

This year we have investigated the fabric support in more detail. This was done using a model reaction: trans-esterification of vinyl acrylate with (R,S)-sec-(2-naphthyl)ethanol in dry amyl alcohol catalyzed by subtilisin Carlsberg. We have obtained several non-woven compositions from Kimberley Clark and tested these as enzymatic supports in a dry non-aqueous environment. None of the fabrics was satisfactory. In fact, the enzyme immobilized on most of the fabrics showed very little activity as compared to the AlliedSignal mixed polarity fabric. Since the reaction system is a dry one, mass transfer issues are not dominant. Thus the non-woven mixed polarity structure of the AlliedSignal fabric is not as important as the fiber composition and its lyoprotecting effect. In addition, the fabric is an experimental one, and as such is too expensive for future commercial use. (The use as an enzyme support does not provide a sufficiently large market to lower the cost). Therefore, we have chosen to examine the fibers themselves. The fibers, which are block copolymers, are available in a variety of compositions. The support could then be "tuned" to provide the best environment for the enzyme.

The enzyme support was prepared by cutting the fibers to 0.5" lengths, washing them to remove dyes and additives, immobilizing the enzyme on the fibers, and packing them into the reactor. The length of the fiber pieces was selected to provide an open structure with no clumps. The enzyme was immobilized by wetting the fibers with a minimal volume of a solution containing the enzyme dissolved in a preferred buffer. The wet enzyme loaded fibers were first dried in a vacuum desiccator at 4°C and then lyophilized. The trans-esterification reaction was carried out at 45°C, and was followed by HPLC using a Phenomenex Chirex Phase 3007 column. Enzyme loading on the fibers was selected as a compromise between rate and cost, and provided 3.3 mg enzyme per ml of reaction mixture. A concentration of 0.5M vinyl acrylate and (R,S)-sec-(2-naphthyl)ethanol was used. The solvent, amyl alcohol, was dried on molecular sieves before use.

The enzyme performance was optimized in batch experiments where an immobilized enzyme was compared to a free suspended enzyme. One fiber composition was tested so far. The results are summarized in Figure I. The major effect on enzyme performance results from the pretreatment, i.e. lyophilization step. The addition of a lyoprotectant, polyethyleneglycol (PEG), to the enzyme solution before freeze-drying, brought about a 100 fold increase in initial rate and a 4.3 fold increase in conversion. This suggests that most of the activity is lost before the enzyme is contacted by the organic solvent. The organic solvent, in contrast to water, does not allow the renaturation of the damaged (unfolded) enzyme. The positive PEG effect is true for both a free and an immobilized enzyme. These results are in agreement with other reports.

The trans-esterification reaction is very sensitive to water, even at very low concentrations (.1%), as the water competes with the alcohol for the acrylic acid. The hydrophilic fibers retain a significant amount of water even after freeze-drying and, therefore, affect the reaction. In addition to competition, the presence of water lowers the stability of the enzyme since it facilitates its unfolding. This might explain the small drop in activity from the free enzyme to the immobilized enzyme. Mass transfer limitations might play a role as well. We have been able to further improve the immobilized enzyme's performance by adding molecular sieves to the reaction mixture (see Figure I). A more rigorous drying regime is also planned. The water promoted destabilization is probably the cause of decreased performance of a recycled enzyme. After 150 hours in the organic solvent at 45°C the initial rate is down. However, it is still higher than that of a free enzyme lyophilized without PEG.

Three more fiber compositions will be tested in order to understand the positive effect that this fiber provides. A circulating batch reactor has been constructed and will be used to examine reactor design parameters such as, shear sensitivity, packing density, fluid dynamics, residence time and temperature effects.

In 1994 we examined the economics of a lipase catalyzed synthesis of AlliedSignal's new product, VEctomer 4010. The enzymatic synthesis has the potential to provide a cleaner route to a better performing material. We have demonstrated that the product can indeed be made enzymatically in a non-aqueous environment, but the rate of VEctomer production was very low and significant quantities of by-products were formed. The economic assessment suggested that the enzymatic route will not be cost effective even with major predicted improvements. Therefore we discontinued working on this particular reaction.

18. Project Title	**Pollution Assessment and Prevention Software for Chemical Industry Process Simulators**
Principal Investigators	**Brian Volintine**
Project Site	**Department of Energy - Headquarters**

Description:

The project is conducted through an Inter-Agency Agreement between the U.S. EPA and the DOE. EPA has responsibility for managing the project, and for selecting an appropriate subcontractor to conduct the work, to be established by a cooperative agreement with a non-profit institution. The project will provide pollution assessment modules for commercial chemical process software.

1996 Planned Activities:

Anticipated 5-year, $1 M effort. DOE's participation will be reviewed on an annual basis, and will be reviewed annually by an industry panel evaluating the course of the development.

Annual Technical Summary Report:

Through a competitive solicitation, the Michigan Technological University, through its Center for Clean and Innovative Technologies (CenCITT) was selected to be the prime subcontractor for the project, September 1995. Michigan Tech will lead an industry consortium for the project development, to begin October 1995.

19. Project Title **Biocatalyst Modeling**
Principal Investigators **Robert Meglen and Gene Petersen**
Project Site **National Renewable Energy Laboratory**

Description:

Both random and site-directed mutagenesis techniques have been successfully used to alter the primary sequence of enzymes to improve stability and catalytic activity in nonpolar solvents. Since enzyme activity is in part due to the three-dimensional structure of the enzyme, one may infer that substitutions in primary sequence induce secondary and tertiary structural changes that affect enzyme activity. Successive application of random mutagenesis provides a valuable database for exploring the relationship between structural modification and enzyme activity. However, if a "rational design" technology is to emerge from enzyme modification experiments, it will be necessary to develop data analysis tools for converting these databases into information that relates specific structural changes to enzyme activity and permits predictive capacity. The goal of this project is to use existing data from random mutagenesis experiments to develop quantitative structure activity models that can be used to assist in the selection of potential enzyme mutation strategies.

1995 Accomplishments:

- Selection, acquisition, and testing of hardware and software of 3-D structural modeling

  We have acquired a Silicon Graphics Indigo2 workstation to serve as the platform for the structural modeling portion of this project. Molecular Simulations Incorporated Quanta/CHARMM software will be used to derive 3-dimensional structures for potential mutations not present in the existing model system database. Tests of (MSI) CHARMM minimization were performed. Preliminary results indicate that significant hysteresis exists in the process of estimating structures for double substitution variants. However, additional testing has indicated that path-dependence disappears when molecular dynamics (heating and equilibration) is used to perform minimization. This indicates that synthetic (model generated) mutations must be energy minimized using dynamics calculations.

- Selection and acquisition of a test-model construction database

  While preliminary feasibility tests of the modeling concept have been performed on two systems, staphylococcus nuclease and subtilisin E, these enzymes have limited data for testing and validating the 3-dimensional structural component of the modeling. Therefore, T4 lysozyme has been chosen as a validation system for modeling. The Brookhaven National Laboratory (BNL) Protein Data Base (PDB) currently contains primary sequence data for 176 lysozyme variants. The x-ray crystallographic structures for 75 of these variants are known and have been obtained from Brookhaven National Laboratory Protein Data Bank (PDB). The measured activities and stabilities for these variants and 100 additional variants, for which the structures are not know, have been added to our test-model database.

- Preprocessing software has been written to convert 3-D coordinates into model-relevant parameters.

  Software was written to convert the atomic coordinates PDB data files into average residue coordinates for use in structure/activity modeling.

  Software was written to compute the nearest neighbor residue interactions for use in structure/activity modeling.

  Software was written to compute 3-dimensional autocorrelation functions for use in structure/activity modeling.

- A database from which amino acid properties will be incorporated into activity models has been acquired.

  Thin layer chromatography and high pressure liquid chromatography retention data and NMR data on 20 amino acids have been acquired. Multivariate principal component analysis has been used to reduce these surrogate measures to a set of three derived principal properties which parameterize hydrophobicity, electrostatic charge/polarity and side chain bulk of the twenty amino acids.

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1996 Planned Activities:

- We will continue to examine molecular modeling software minimization procedures to evaluate the energy consistency of computer generated structures.

- We will construct computer generated structures for the 75 T4 lysozymes for which "true" crystal structures have been obtained. Computer generated structures will be compared with experimental structures to validate the methodology for generating an augmented model database.

- We will augment the 75 known T4 lysozyme structures with additional computer generated structures for selected variants from among the additional 100 which have been produced in the laboratory but not subjected to x-ray crystallographic determination.

- The T4 database (measured activities and 3-D structures) will be separated into two data sets, a model construction set and a prediction set. The construction data set will be used to develop a projection to latent structures (PLS) model to predict T4 lysozyme activity/stability. The prediction set will then be used to assess the model prediction accuracy.

- We will explore alternate methods for explicitly incorporating 3-D structural information into the PLS model.

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Structure-Activity Relationships

The high cost of synthesis and/or testing put a premium on predictive ability in the synthetic drug industry. As a result a growing repertoire of computational tools has been brought to bear on the development of Quantitative Structure-Activity Relationships (**QSAR**). Ignorance of the process relating molecular structure to biological activity, or the complexity of those processes, precludes the direct computation of those relationships and one must rely upon an empirical model to develop predictive capability. Application of QSAR methods to macromolecules is in its infancy, but the underlying assumptions and problems of prediction for enzymes suggest that the application of QSAR tools from the drug industry might be advantageous.

The primary assumptions in the QSAR paradigm are that the activity of interest must be lawfully related to the molecular structure; and, that the relevant lawful relationship has been explored by a number of experimental modifications which are then approximated by empirically established QSAR's.

Formal Declaration of the Problem

> E is a variant of a *standard* (wild-type) enzyme E_{WT} and is defined by replacement of residues in the primary sequence of E_{WT} by alternative amino acids.
>
> There is a set **E** of so-called congeneric polypeptides in existence whose activities have been measured and which may all be regarded as related to E_{WT} by different amino acid substitutions.
>
> The congeneric series of polypeptides $\mathbf{E}$ is called the construction set for the prediction of y.

Preliminary studies performed at NREL provide reasonable scientific grounds that it may be possible to find an empirical relationship between the structure of the polypeptides in $\mathbf{E}$ and their measured values of some property y (such as activity, thermal stability, etc) that would then provide a statistical prediction of the as yet unmeasured value of y for E.

Data Requirements for QSAR Development

QSAR development requires numerical descriptors of chemical structure and fundamental properties. Often the only known structural information for the enzymes is the crystallographic structure (3-D) of the wild-type enzyme, E_{WT}, and the primary amino acid sequence (2-D) for all of the congeneric polypeptides in the construction set $\mathbf{E}$. In order to make the connection between the structural location of an amino acid and its effect on the tertiary structure we have identified physicochemical descriptors that embody the individual differences among the twenty coded amino acids and reflect the non-covalent forces that drive the structural arrangement.

Certain generalizations regarding the structural patterns in globular proteins provide an indication about which forces might be important for inclusion in an empirical model. The tight packing of backbone and side-chain atoms, to densities comparable to crystalline amino acid densities, suggests that measures of molecular volume and densities will be important. The well-known contribution of intramolecular hydrogen bonding to tertiary structure indicates that measures of electrostatic interactions and polarizability will also be important. In addition, the majority of hydrophobic side-chains are buried within the protein interior and the majority of hydrophilic charged side-chains are located at the surface in contact with water. This suggests that measures of electrostatic interactions and hydrophobicity for individual amino acids will play a significant role in the development of a QSAR model.

Since there is no direct measure of features like hydrophylicity or hydrophobicity it is valuable to find suitable surrogate measures of these properties. A significant volume of literature describes the search for surrogate measures of these properties. One effective approach to parameterizing hydrophobicity relies upon the method of analogy from simple chromatographic systems. Thin layer chromatography and high pressure liquid chromatography retention data for free and derivitized amino acids in various solvent systems have been used to derive a multivariate scale than may be transferred to the protein system. In addition, NMR spectroscopy has been exploited to provide surrogate measures of intramolecular interactions. These data consist of $\propto$-proton and $\propto$-carbon chemical shifts. Multivariate principal component analysis has been used to reduce these surrogate measures to a set of three derived principal properties which parameterize hydrophobicity, electrostatic charge/polarity and side chain bulk of the twenty amino acids.

Computational Algorithms for QSAR Development

Several computational techniques have been used to develop QSAR's for polypeptides including neural nets, multiple regression analysis and partial least squares. The most advanced approach developed has been based upon partial least squares projections to latent structures, PLS, is a multivariate data analytic method that relates the systematic information in a matrix **X** to the information in a matrix **Y** with the purpose of predicting **Y** from **X**. PLS simultaneously calculates multivariate projections of the predictor variables (**X**) and the dependent variables (**Y**), so that the projection scores of the two data blocks are maximally correlated. In this way, a quantitative expression of the relationship between the two matrices is obtained.

We have attempted to demonstrate the PLS technique to predicting enzyme activity (subtilisin E) and stability (free energy of the denatured state of staphylococcus nuclease) from primary sequence data and surrogate measures of three principal amino acid properties. The y-matrix in these PLS models consists of the measured activity/stability for each of the mutants. The nature of the x-matrix is a bit more complex and will be described in the next section; but it consists of the principal properties assigned to each of the amino acids in the known sequence for the mutants. If the x-matrix adequately reflects the salient structural and physicochemical properties that determine enzyme activity the PLS technique extracts the latent structure residing in the surrogate measures and constructs a model that describes the variation in the (activity) y-matrix. The resulting model may then be used to predict the activity of new postulated mutants from the proposed primary sequence. Thus, a predictive model provides a method by which alternative experimental efforts can be evaluated prior to resource expenditure.

Explicit Incorporation of 3-Dimensional Information

As indicated earlier, activity of biomolecules is strongly dependent of specific 3-dimensional characteristics. The modeling x-matrix is constructed to include the residue-specific forces; however, the key to imparting the modeling matrix with 3-dimensional relevance is to adapt the one-dimensional autocorrelation function to this use. The nearest neighbors for each residue in 3-dimensional space may be computed from the crystallographic structure. The 3-dimensional autocorrelation function can then be calculated for each protein in the model construction set. The autocorrelation function assigns a unique pattern to every protein. While the pattern is independent of alignment, it fully represents the dependence upon adjacencies with other residues along the sequence. By constructing the x-matrix for PLS modeling from autocorrelation functions one may strengthen the model's structural inferences because the matrix is imbued with the sequence relevant information missing from other representations.

In the two examples that have been examined in our early exploratory work the only 3-dimensional data available is the crystallographic data for the wild-type enzyme. Thus, only the first row of the modeling matrix has any 3-D information. Resources to obtain crystallographic data for the remaining variants (rows of the construction set) are not likely to be available. Thus, an alternate strategy for completing the 3-D data in the construction set relies upon the availability of molecular modeling. We are examining the application of modern molecular modeling software to estimate the 3-D coordinates of variants for which measured data do not exist. We will examine whether it is possible to use the known wild-type structure as the basis for subsequent structural relaxation and free energy minimization. A separate computation will be performed for each amino acid substitution represented in the array of experimental variants. The computed 3-D coordinates will be used to approximate a 3-D relevant information for the PLS modeling matrix.

Demonstration Model System

While preliminary feasibility tests of the modeling concept have been performed on two systems, staphylococcus nuclease and subtilisin E, these enzymes have limited data for testing and validating the 3-dimensional structural component of the modeling. Therefore, T4 lysozyme has been chosen as a validation system for modeling. The Brookhaven National Laboratory (BNL) Protein Data Base (PDB) currently contains primary sequence data for 176 lysozyme variants. The extraordinary body of work, assembled principally by Brian Matthews, has completed crystallographic structure for 75 of these mutants. In addition, enzyme activities and/or thermal stabilities on all of the mutants have been published by Matthew. The existence of both sequence data and measured activities/stabilities permit construction of large x and y-matrices from which to construct a model calibration set. In addition, the existence of more than 75 crystallographic structures will permit validation of the molecular modeling. for evaluation and development of protein QSAR models.

As indicated earlier, 3-dimensional information is likely to be key to the success of QSAR models. Since crystallographic data are not always available at successive steps in mutagenesis experiments, an alternate method of estimating the 3-dimensional structural changes induced by amino acid replacement is desirable. In this work we hope to demonstrate that the wild-type crystallographic coordinates can be used as the starting-point for molecular modeling of mutants. Appropriate amino acid substitutions to the wild-type will be made in the molecular modeling system. The structure will then be relaxed and free energy minimization will be performed. The minimum energy coordinates will then be used as estimates for the mutant's 3-dimensional structure. The advantage of *T4 lysozyme* as a model system is that 75 mutants have experimental crystallographic structures that can be used to test the validity of the modeling derived coordinates. If the modeling force fields are not adequate to this task, it may also be possible to use the known crystallographic structures to parameterize minimization force fields.

Prediction and How the Model Will be Used

The importance of predictive capacity was emphasized in the initial formulation of the problem. While the method of random mutagenesis is useful for exploratory work and for developing a modeling construction set, it is clear that **directed** mutagenesis will be necessary to design a new variant that maximizes the desired enzyme properties. Furthermore, the number of alternatives for directed mutagenesis for 20 amino acids, for even the shortest polypeptides, will be large. Thus, it will be important to have a rapid, inexpensive method for the selection of worthy laboratory experiments. In a strict statistical sense any predictive model must be confined to the domain covered by the data in the construction set. Predictions about variants with characteristics outside of the model domain constitute "extrapolations". In order to avoid extrapolation we propose to use molecular modeling to "synthesize" a larger range of variants to augment the construction set for the predictive model. Each of the synthetic variants in the broadened model domain becomes a candidate for actual laboratory synthesis, but the model provides a rational basis for selecting viable options for actual laboratory work.

It is interesting to note that after the experimentally based model has been constructed and if augmentation through molecular modeling is sufficiently robust, any polypeptide sequence could be "evaluated" for its activity toward the reaction represented in the y-matrix. Thus, smaller subunits (active sites) or even wholly synthetic polypeptides could be evaluated.

20.	**Project Title**	**Biochemical Engineering Simulation Technology**
	Principal Investigators	**Robert J. Wooley**
	Project Site	**National Renewable Energy Laboratory**

Description:

Decomposition of cellulose to glucose is one of the primary reactions necessary to utilize biomass as a feedstock for various fuels and chemicals. Cellulose is a natural homopolymer of glucose, so the reaction of cellulose to glucose is a depolymerization or a reverse polymerization reaction.

Aspen Technology, Inc. (Aspen Tech.) has developed the capability in their ASPEN+ process simulation software to model various polymerization reactions, including those using condensation mechanisms of interest here. Aspen Tech. uses a novel approach to modeling of polymerizations by parameterizing only the reactions of various repeat and end groups. This simplifies the user input considerably. Using this approach various properties of the polymer can be tracked throughout a batch reaction. These properties include, degree of polymerization, weight average molecular weight, number average molecular weight and others. By knowing these properties of the polymer and the molecular structure of the repeat units other properties such as density and heat capacity can be predicted. If cellulose depolymerization can be modeled in this fashion it would prove to be a simple technique that could also provide physical properties as the reaction progresses.

To prove the concept of depolymerization using this model, a well characterized system, polymerization of hexamethylenediamine and adipic acid to form nylon 66 was calculated in reverse.

The cellulose system was then modeled in a fashion similar to the nylon depolymerization. To fit parameters necessary in the model, literature data was fit to the ASPEN+/PP model. Results of this regression and the simulation of depolymerization of cellulose will be given.

Annual Technical Summary Report:

This project has been completed, and this final report is submitted at the 1995 Annual Summary Report.

Introduction

One method to utilize biomass as a feedstock is to convert the carbohydrate portion to sugars. This carbohydrate portion is made up of high molecular weight polysaccharides, cellulose and hemicellulose.[1] Cellulose is the main component, making up sometimes over 50% of wood. Hemicellulose is also important as it can comprise 10-40%[2]. Cellulose is a polymer of glucose connected by β-1,4-glycosidic linkages. This linkage, results in the "chair" configuration of a 6 member ring, exposing the chain to hydrogen bonding with other chains allowing the cellulose to form highly crystalline polymers.

Aspen Technology, Inc., developers of the advanced chemical process simulation package, ASPEN+ have also developed an add-on package for the modeling of polymer reactions (Polymers Plus). Aspen Tech.[3] use a relatively simple approach of modeling repeat units and end units as reacting species and require kinetic rate parameters to describe only reactions of these units. From this, the simulator is able to track the progress of the polymerization. The simulator was developed to model polymerizations, i.e., the growth of polymers. However, the reactions will go in reverse with the proper conditions and there is no reason that this simulator will not model the reverse reaction as well.

As a first example, the well described reaction of adipic acid and hexamethylenediamine to form nylon 66 was modeled as a depolymerization, using kinetic rate parameters developed for the forward polymerization. Then a simple model was outlined for cellulose depolymerization. To obtain the kinetic rate parameters necessary for the model, ASPEN+ has the capability to fit experimental composition versus time data to the rate expression in a batch reaction.

ASPEN+/Polymers Plus Simulation Approach

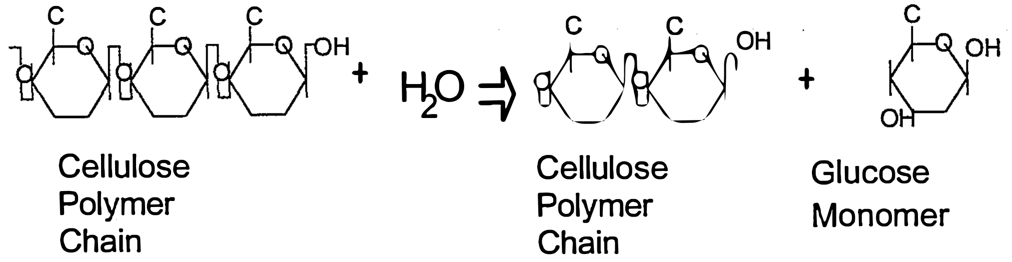
ASPEN+/Polymers Plus (ASPEN+/PP) has the built in capability to simulate polymerization reactions by several mechanisms. The mechanism of interest here for the cellulose and nylon systems is condensation. The polymerization of 1,6-hexamethylenediamine with adipic acid is given as:

$$NH_2(CH_2)_6H_2N + HOOC(CH_2)_4COOH \rightarrow NH_2(CH_2)_6HN\text{-}CO(CH_2)_4COOH + H_2O$$

where water is a by-product of the reaction, thus the classification, condensation. Depolymerization of nylon would be shown as:

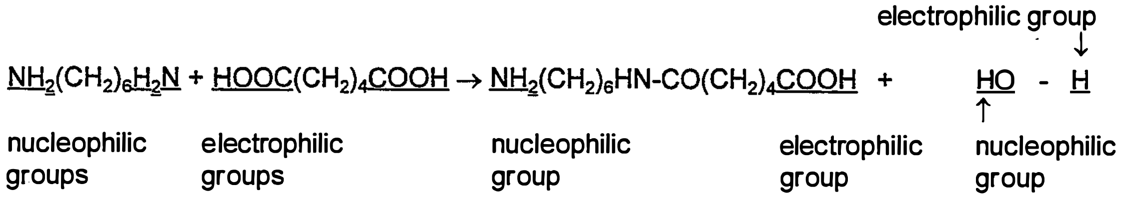
$$\text{—}NH\,(CH_2)_6HN\text{-}CO(CH_2)_4CO\text{—} \quad + H_2O \rightarrow \quad \text{—}NH(CH_2)_6H_2N \quad + \quad HOOC(CH_2)_4CO\text{—}$$

and the depolymerization of cellulose:



The mechanism used for condensation polymerization is step-growth. In these types of reactions, two monomers or a polymer chain and monomer or two polymer chains react. In these reactions one molecule contains a electrophilic functional group which reacts with a necleophilic group in the other molecule. Electrophilic groups are electron weak groups, such as aldehydes (-CHO) or acids (-COOH) and nucleophilic groups are electron-strong, such as alcohols (-OH). In the nylon reaction adipic acid contains two electrophilic groups (-COOH) and the hexamethylenediamine contains two nucleophilic groups ($-NH_2$).

In the case of condensation reactions, a nucleophilic group from one reactant exchanges with a nucleophilic group on the other reactant creating two new molecules. In the reactions of interest here the bi-product molecule generated is water, hence the name condensation reaction.



$$\underline{NH_2}(CH_2)_6\underline{H_2N} + \underline{HOOC}(CH_2)_4\underline{COOH} \rightarrow \underline{NH_2}(CH_2)_6HN\text{-}CO(CH_2)_4\underline{COOH} + \underline{HO} - \underline{H}$$

The ASPEN+/PP can also accommodate branching and end-blocking. Essentially, a trifunctional reactant will generate cross-linking or branching and a monofunction monomer will block the polymer to further reaction. It will become clear that either of these cases can be handled when the monomers are characterized.

Oligomers are identified to the system by the number of repeat units in each, the concentrations of these species will then be tracked. Only oligomers important to the process need to be included as tracking these components add to the calculation time.

Physical properties of the polymer and any oligomers are modeled based on the properties of repeat units and the degree of polymerization. By knowing the structure of the repeat units, a prediction method, such as that developed by van Krevelen[4] can be used to calculate the density and heat capacity of the polymer solution through out the reaction.

To characterize the reaction system, ASPEN+/PP requires that each monomer, repeat unit and end unit be characterized as to its reacting functional group. Once these reactions are identified the ASPEN+ system will develop a set of unique reactions describing all the possible interactions. The user can specify kinetic parameters using a power law expression such as:

$$\text{rate} = [\text{Nucl}][\text{Elec}][\text{Cat}][\text{Prob}]A_{ij}e^{\frac{-E_{ij}}{RT}}T^{b_{ij}}$$

where:

[Nucl]	Concentration of nucleophilic species
[Elec]	Concentration of electrophilic species
[Cat]	Concentration of catalyst
A_{ij}	Pre-exponential factor
E_{ij}	Activation Energy
b_{ij}	Temperature Exponent

The user can specify rate constants to the specific group (electrophilic or nucleophilic) or species (electrophilic or nucleophilic reactant). The system will then apply the rate constants to the reactions generated according to a preference. In general, the more that is specified about a rate parameter, the fewer reaction it will be applied to. If both types of groups and species are all specified the rate parameters will only be applied to one reaction, if only a group is specified the rate parameter will be applied to all reaction involving that group.

As an example the nylon system was characterized as follows:

Reacting Groups

Name	**Type of Group**
TNH_2	Electrophilic Leaving Group
TCOOH	Nucleophilic Leaving Group
BADA	Electrophilic Repeat Unit
BHMA	Nucleophilic Repeat Unit

Species

Species	**Species Descriptio**	**Number & Reacting Group**
HMD	Monomer	2-TNH_2, 1-BHMD,
ADIPA	Monomer	2-TCOOH, 1-BADA
T-HMD	End Unit	1-TNH_2, 1-BHMD
T-ADA	End Unit	1-TCOOH, 1-BADA
B-HMD	Repeat Unit	1-BHMD
B-ADA	Repeat Unit	1-BADA

Knowing which groups are electrophilic and nucleophillic, ASPEN+/PP is able to determined the following set of unique reactions:

1. HMD + ADIPA → H_2O + T-HMD + T-ADA
2. HMD + T-ADA → H_2O + T-HMD + B-ADA
3. H_2O + T-ADA + T-HMD → ADIPA + HMD
4. H_2O + T-ADA + B-HMD → ADIPA + T-HMD
5. H_2O + B-ADA + T-HMD → T-ADA + HMD
6. H_2O + B-ADA + B-HMD → T-ADA + T-HMD
7. T-HMD + ADIPA → H_2O + B-HMD + T-ADA
8. T-HMD + T-ADA → H_2O + B-HMD + B-ADA

The rates were specified for the following:

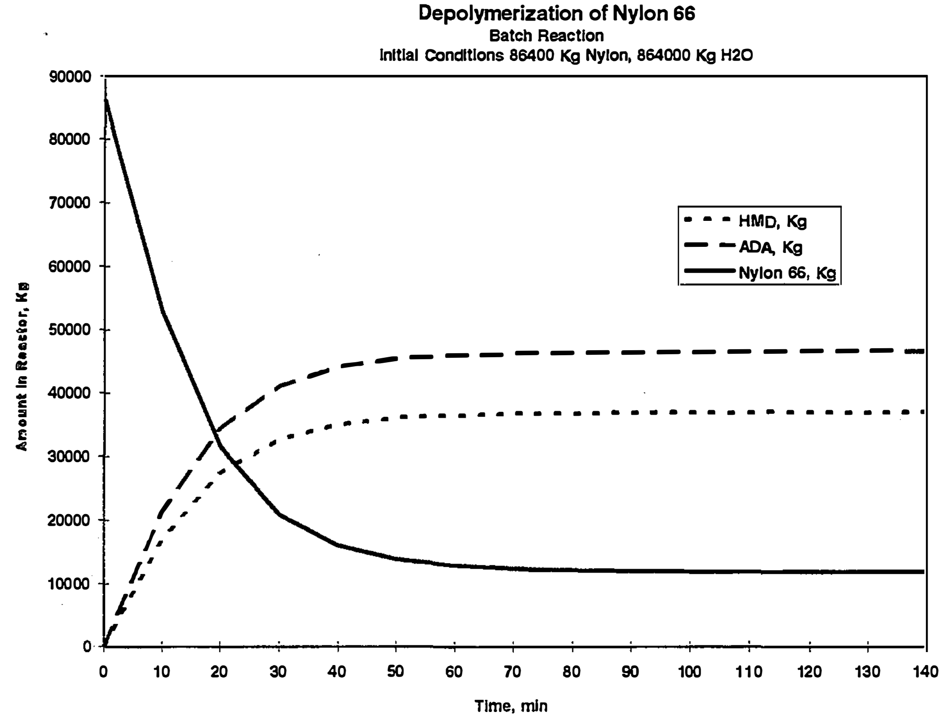
Kinetics 1	Kinetics 2
[Nucl. group] = TNH_2	[Nucl. group] = H_2O
[Elec. group] = TCOOH	

$A_{ij} = 7\ \text{sec}^{-1}$
$E_{ij} = 2.5\text{E}7$ J/Kmol

$A_{ij} = 0.014\ \text{sec}^{-1}$
$E_{ij} = 2.5\text{E}7$ J/Kmol

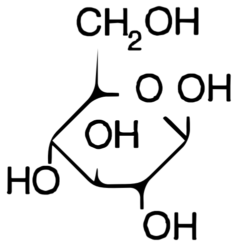
Kinetics 1 were applied to reactions 1, 2, 7 and 8 and Kinetics 2 were applied to reactions 3, 4, 5 and 6.

The result for batch depolymerization calculated using these kinetics are shown as in the following graph. Details of the input file and resulting report file from ASPEN+/PP are given in Appendix A.



Depolymerization of Cellulose

The cellulose system was model in a fashion very similar to the nylon. The primary difference is cellulose is a homopolymer with only one repeat unit, glucose. Upon examination of the glucose molecule, it is easily seen that one of the reacting ends is electophilic (hydroxy group) and the other nucleophilic (acid like group).



Glucose
Monomer

The reacting groups of the cellulose depolymerization were characterized as follows:

Reacting Groups

Name	Type of Group
COOH	Electrophilic Leaving Group
COH	Nucleophilic Leaving Group
GLUC	Mixed (Electrophilic and Nucleophillic) Repeat Unit

Species

Species	Species Description	Number & Reacting Group
GLUCOSE	Monomer	1-COOH, 1-COH, 1-GLUC
E-OH	End Unit	1-COH, 1-GLUC
E-COOH	End Unit	1-COOH, 1-GLUC
R-GLUCOSE	Repeat Unit	1-GLUC

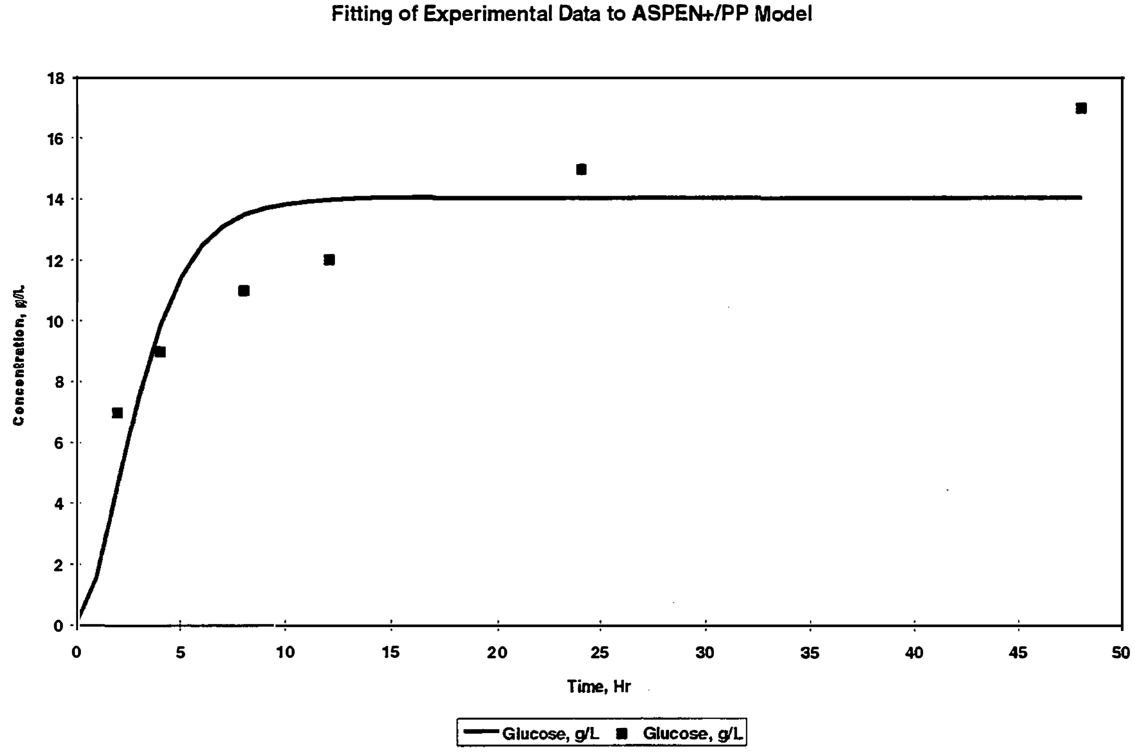
Given these reacting species the system will generate the following set of unique reactions:

1. GLUCOSE + GLUCOSE → H_2O + E-OH + E-COOH
2. GLUCOSE + E-OH → H_2O + E-OH + R-GLUCOS
3. E-COOH + GLUCOSE → H_2O + R-GLUCOS + E-COOH
4. E-COOH + E-OH → H_2O + R-GLUCOS + R-GLUCOS
5. H_2O + E-COOH + E-OH → GLUCOSE + GLUCOSE
6. H_2O + E-COOH + R-GLUCOS → GLUCOSE + E-COOH
7. H_22O + R-GLUCOS + E-OH → E-OH + GLUCOSE
8. H_2O + R-GLUCOS + R-GLUCOS → E-OH + E-COOH

Using data from the literature[5] kinetic rate parameters were fit for two different types of reactions, one involving the COOH electrophilic group with the OH neucleophilic group and one involving water. The results of fitting that data are given in the following table and graph.

Table 1
Decomposition of Cellulose to Glucose

Time	Glucose g/L	
hr	ASPEN+/PP	Experimental[5]
0	0	
2	4.4721	7
4	9.8407	9
8	13.491	11
12	13.977	12
24	14.051	15
48	14.054	17



The calculated rate parameters were, $A_{ij} = 0.444$ sec^{-1} (reactions 1, 2, 3 and 4) and $A_{ij} = 177$ sec^{-1} (reactions 5, 6, 7 and 8). The results are not very good. The expected problem is that the power-law kinetics used in this model are not sufficient. Several considerations, such as competitive inhibition of the enzymes from both the glucose and cellobiose and non-homogeneous solution. In addition enzyme reactions are generally considered to follow Michaelis-Menten[6] type of kinetics. This type of kinetics rate expression starts out first order with respect to the substrate (this is at high substrate concentration) and then approach zero order as the reaction continues and substrate is consumed. The data used here tends to continue reacting at a fair rate even after the substrate is nearly consumed, thus supporting this type of kinetics.

To override the simple power-law kinetics the complete mechanism and kinetic rate parameters need to be specified. The ASPEN+/PP system allows for that approach to modeling, but little is gained over any general kinetic modeling system. A more rigorous mechanism is given by Fan and Lee[5] involving the general formation of an enzyme-substrate complex followed by reaction to the dimer cellobiose. The enzyme used to convert crystalline cellulose to cellobiose is really a pair of enzymes, β-1,4-glucan cellobiohydrolase and endo-β-1,4-glucanase. A third enzyme, β-glucosidase, is then used to react the cellobiose (dimer) to glucose. The mechanism given by Fan and Lee[5] can be converted into a mechanism for ASPEN+/PP as follows:

Decomposition of the cellulose to cellobiose using the enzymes, β-1,4-glucan cellobiohydrolase and endo-β-1,4-glucanase is given in the following set of reactions,

$$E_A + S \leftrightarrow E_AS \tag{1}$$

$$E_AS + P_2 \leftrightarrow E_ASP_2 \tag{2}$$

$$E_AS + P_1 \leftrightarrow E_ASP_1 \tag{3}$$

$$E_AS \rightarrow E_A + P_2 \tag{4}$$

Reaction of the cellobiose to glucose using the enzyme, β-glucosidase

$$P_2 + E_\beta \leftrightarrow P_2E_\beta \rightarrow E_\beta + P_1 \tag{5}$$

This is also inhibited by glucose

$$P_1 + E_\beta \leftrightarrow P_1E_\beta \tag{6}$$

Where

P_1	Glucose
P_2	Cellobiose
E_A	Enzymes, β-1,4-glucan cellobiohydrolase and endo-β-1,4-glucanase
S	Cellulose
E_β	Enzyme, β-glucosidase

This mechanism can be converted using various polymer segments to a set of 12 reactions compatible with the ASPEN+/PP system. This set of reactions would need to be fit by hand or by regressing some parameters while keeping other held fixed. The advantage to the ASPEN+/PP system seems to be lost when you must go to such a complex mechanism. It would be easier to use the exact mechanism, without segments and repeat units, as outlined by the original authors[5] in a user specified kinetics routine to ASPEN+.

If you define the following in ASPEN+/PP

Group	**Group Definition**
SEG-S	Cellulose Repeat Segment
SEG-ES	Cellulose Repeat Segment with Enzyme A attached
SEG-ESD	Cellulose Repeat Segment with Enzyme A and Cellobiose Attached
SEG-ESG	Cellulose Repeat Segment with Enzyme A and Glucose Attached
DE	Cellobiose Attached to β Enzyme
GE	Glucose Attached to β Enzyme

The following reactions in ASPEN+/PP correspond to the above mechanism of Fan and Lee[5].

E_A + SEG-S → SEG-ES (1a)
SEG-ES → E_A + SEG-S (1b)

SEG-ES + P_2 → SEG-ESD (2a)
SEG-ESD → SEG-ES + P_2 (2b)

SEG-ES + P_1 → SEG-ESG (3a)
SEG-ESG → SEG-ES + P_1 (3b)

SEG-ES + SEG-S + H_2O → E_A + P_2 (4)

P_2 + E_β → DE (5a)
DE → P_2 + E_β (5b)
DE → H_2O → P_1 + E_β (5c)

E_β + P_1 → GE (6a)
GE → E_β + P_1 (6b)

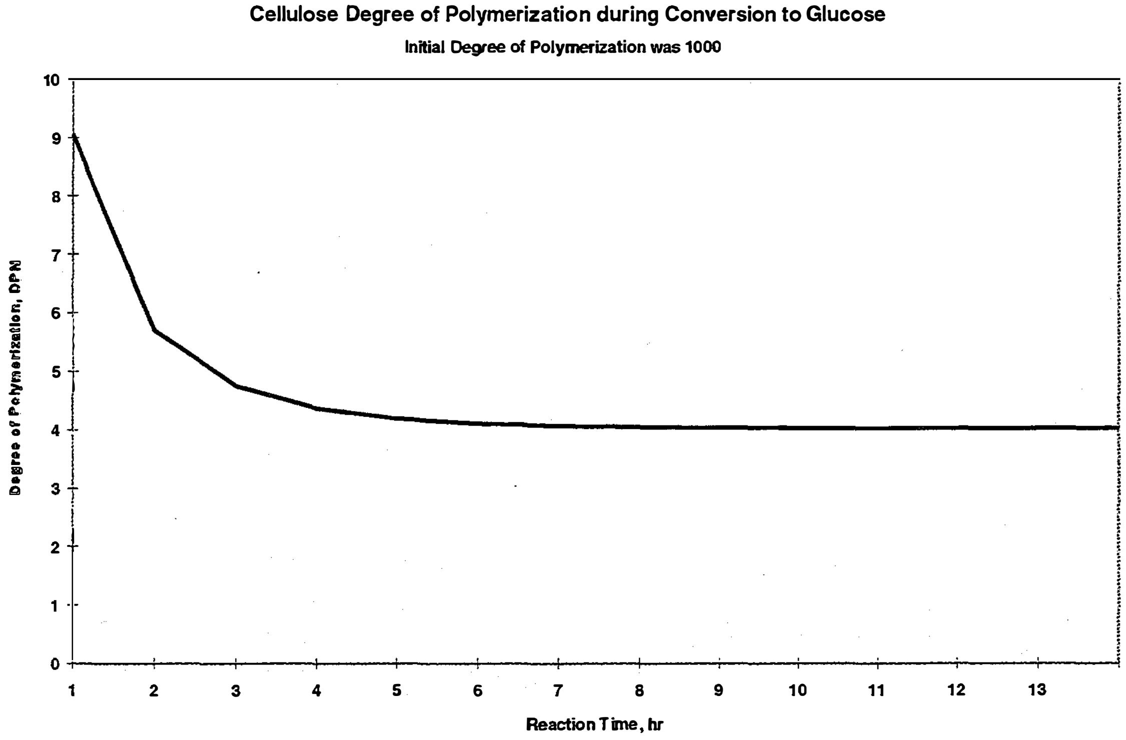
Acid Hydrolysis

Besides using enzymes to break down cellulose, strong acids, such as HCl or H_2SO_4 can be used. These appear to follow power law kinetics better, and it is possible that this modeling scheme might work for these systems. However, there is a side reaction, the formation of hydroxymethylfufural (HMF) from glucose in the presence of acids. This degradation is seen in all experimental data. To properly model that system an additional reaction, that of glucose to HMF, needs to be included in the model. ASPEN+/PP is suppose to be able to handle this, but the second decomposition reaction was ignored by the calculations, therefore regression of the depolymerization and decomposition rate parameters proved unsuccessful.

Physical Properties

There were two primary reasons for modeling the cellulose depolymerization using ASPEN+/PP. They were, ease of use, and prediction of physical properties. The prediction of physical properties is made possible by keeping track of various characteristics of the polymer, most notably, the degree of polymerization, and knowing the repeat unit structure. Knowing this information the predictive methods of van-Krevelen perform very well in predicting the properties of the polymer solution. In addition, vapor liquid equilibrium can also be model in this fashion, if necessary.

For the cellulose system the degree of polymerization is calculated according to the kinetic model derived, see graph below. However, until the polymer has been reduced to a dimer or trimer, the cellulose is insoluble. Therefore, the density and heat capacity of the solution are more determined by that of a slurry than that of a polymer solution. If the kinetic model had been successful, then the concentrations of dimer in the solution as well as glucose would have been known and the properties of the solution predictable. However, the overall properties of the slurry would have concentrations of dimer in the solution as well as glucose would have been known and the properties of the solution predictable. However, the overall properties of the slurry would have been greatly influenced by the solid present and the model has no way of handling that. In addition to better calculate agitator power requirements, the solution viscosity is also required. Therefore, it was hoped that a correlation of degree of polymerization and viscosity would be available. This was not possible either because of the influence of the solid.



The result was that this method of modeling cellulose depolymerizations was of no help in being able to predict the physical properties (density, heat capacity and viscosity) of the solution necessary for detailed reactor design.

Conclusions

The power law kinetic model is not sufficient to describe the enzymatic kinetics of cellulose depolymerization. This is the only expression available within the frame-work of the ASPEN+/PP model. Depolymerization is generally known to follow the Michaelis-Menten type kinetics. Michaelis-Menten systems are characterized by first order at high substrate concentrations (beginning of reaction) and zero order at lower substrate concentrations (reaction continues at low substrate concentration). The ASPEN+/PP system can be used with a user specified mechanism, but the benefits of the ASPEN+/PP easy to use system would be lost and there would be no advantage of ASPEN+/PP over standard kinetics modeling systems.

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6.0 Program Output

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6.2 Patent Awards and Filings

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Filed

Idaho National Engineering Laboratory

'Biomediated Continuous Release Phosphate Fertilizer'

'Genetic and Biochemical Pathway For Bacterial Solubilization Of Rock Phosphate'

California Institute of Technology

'Improved para-Nitrobenzyl Esterase Enzymes by Directed Evolution'

6.3 Significant Achievements

Dr. Klibanov	Elected to the National Academy of Sciences in April 1995, for his work on enzymatic catalysis in organic solvents.
Tony Redondo	Made group leader of the Theoretical Chemistry Group at Los Alamos National Laboratory
Gene Petersen	Midwest Research Institute Presidents Award for Excellence
Peter Schultz	Selected as a Howard Hughes Medical Investigator
	Received the Wolf Prize in Chemistry for Converting Antibodies into Enzymes
	Received an honorary degree - Doctor of Sciences from Uppsala University in Sweden
	Elected to the National Academy of Sciences, the youngest member elected.
David Beratan	Awarded tenure by the University of Pittsburgh
	Continues to hold the National Science Foundation National Young Investigator Award
John Shelnutt	Sandia Award of Excellence for Molecular Design
Eli Greenbaum	Selected Editor in Chief of the AIP (American Institute of Physics) - International Theory in Basic and Applied Biological physics
Tim Scott	Named the Director of the Bioprocessing Research and Development Center at Oak Ridge National Laboratory
	1995 Federal Laboratory Consortium Award for Excellence in Technology Transfer presented by Hazel O'Leary
Francis Arnold	Invited speaker at the Chancellor's Lecture Series Honoring Distinguished Women Scholars at the Department of Chemistry, University of Pittsburgh
Brian Davison	Recently named co-chair and co-editor of the Symposium of Biotechnology for Fuels and Chemicals