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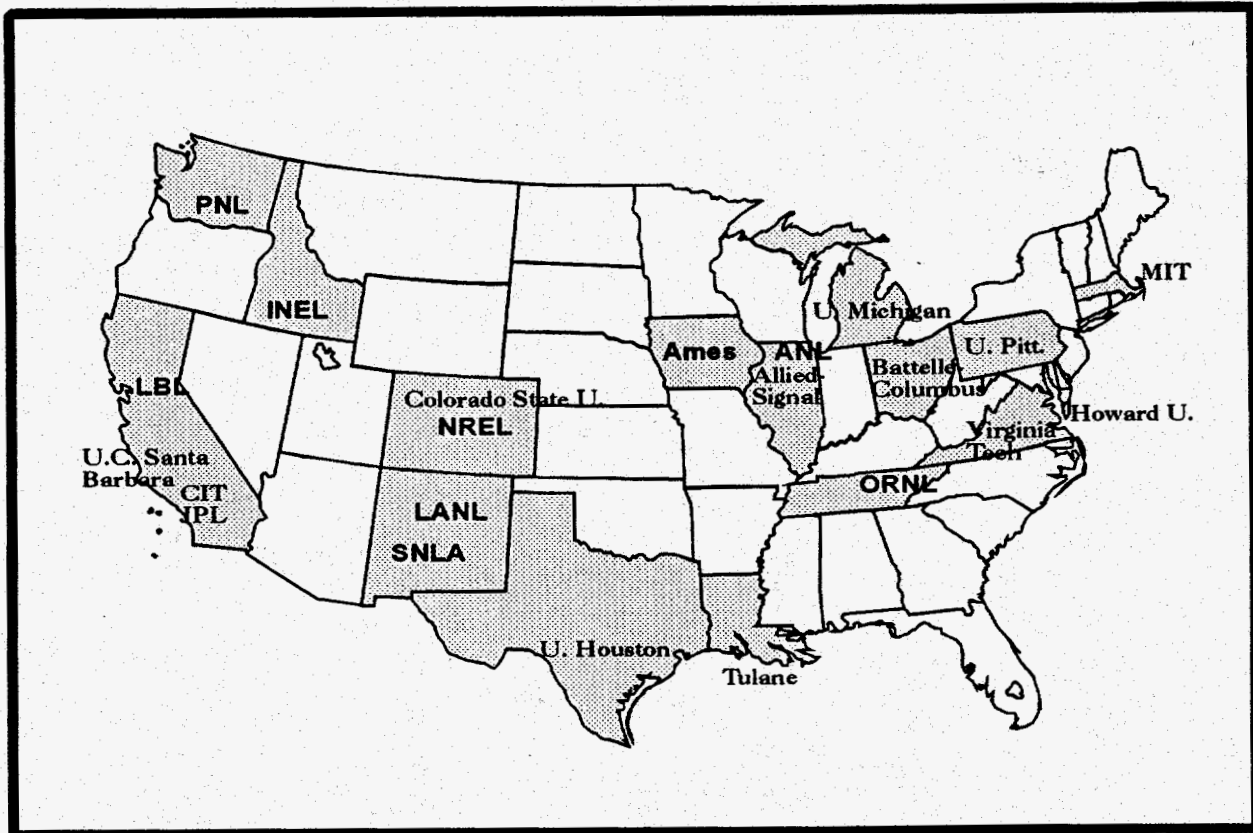
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# BCTR

Biological and Chemical Technologies Research

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## 1994 Annual Summary Report February 1995



United States Department of Energy  
Office of Energy Efficiency and Renewable Energy  
Office of Industrial Technologies  
Biological and Chemical Technologies Research

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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 that restrict the design of new chemical catalysts.

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## ABSTRACT

The annual summary report presents the fiscal year (FY) 1994 research activities and accomplishments for the United States Department of Energy (DOE) Biological and Chemical Technologies Research (BCTR) Program of the Advanced Industrial Concepts Division (AICD). This AICD program resides within the Office of Industrial Technologies (OIT) of the Office of Energy Efficiency and Renewable Energy (EE). Although the OIT was reorganized in 1991 and AICD no longer exists, this document reports on efforts conducted under the former structure. The annual summary report for 1994 (ASR 94) 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 1994; detailed descriptions of individual projects; a listing of program output, including a bibliography of published work; patents, and awards arising from work supported by BCTR.

## FOREWORD

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## 1.0 INTRODUCTION

This document contains a summary of the projects undertaken by the Biological and Chemical Technologies Research (BCTR) Program in 1994. The BCTR program is a program within the U.S. Department of Energy's (DOE) Advanced Industrial Concepts (AICD) Division that is in the Office of Industrial Technologies (OIT). In 1995 organizational changes occurred in the OIT that eliminated the AICD. Since the work described in this report was undertaken under the old organizational structure, the descriptions of the work will remain as they have in the past. In future editions of this report the new structure will be described more fully.

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 promote the introduction of these technological advances in the chemicals, petroleum refining, pulp and paper, other renewable, and emerging biotechnological industries.

Together, these advanced technologies foster U.S. competitiveness through new product and market expansion, cost-effective and environmentally safe processes, reliable domestic energy and feedstock supplies, and lower dependence on foreign energy sources. The use of renewables for fuels, chemicals, and electric power also supports the Energy Policy Act of 1992. The BCTR program serves a dual role in meeting the needs of industry and other DOE programs and channeling technology into these two areas.

The overall goal of the BCTR program is to stimulate and nurture the development, testing, and commercial deployment of advanced chemical and biological technologies. The achievement of this goal is gauged by the primary energy use that could potentially be impacted by the successful commercial deployment of innovations sponsored by BCTR and that is estimated to be 2.4 to 3.5 eJ in savings for petroleum displacement by 2030.

Toward this goal, the program objectives are to:

1. Pursue the development of technologies aimed at overcoming the limitations of the use of indigenous resources in the chemicals industry.
2. Facilitate the introduction of biotechnology into the chemicals industry.
3. Investigate innovative chemical and biological process routes that offer the potential to reduce energy use and mitigate environmental impact within the chemicals and petroleum refining industries.
4. Communicate the results of the program research efforts to OIT end-use programs or the industrial sector for continued development towards commercial deployment.

Meeting program goals and objectives involves two strategies and is based on the energy supply/demand issues of process operations.

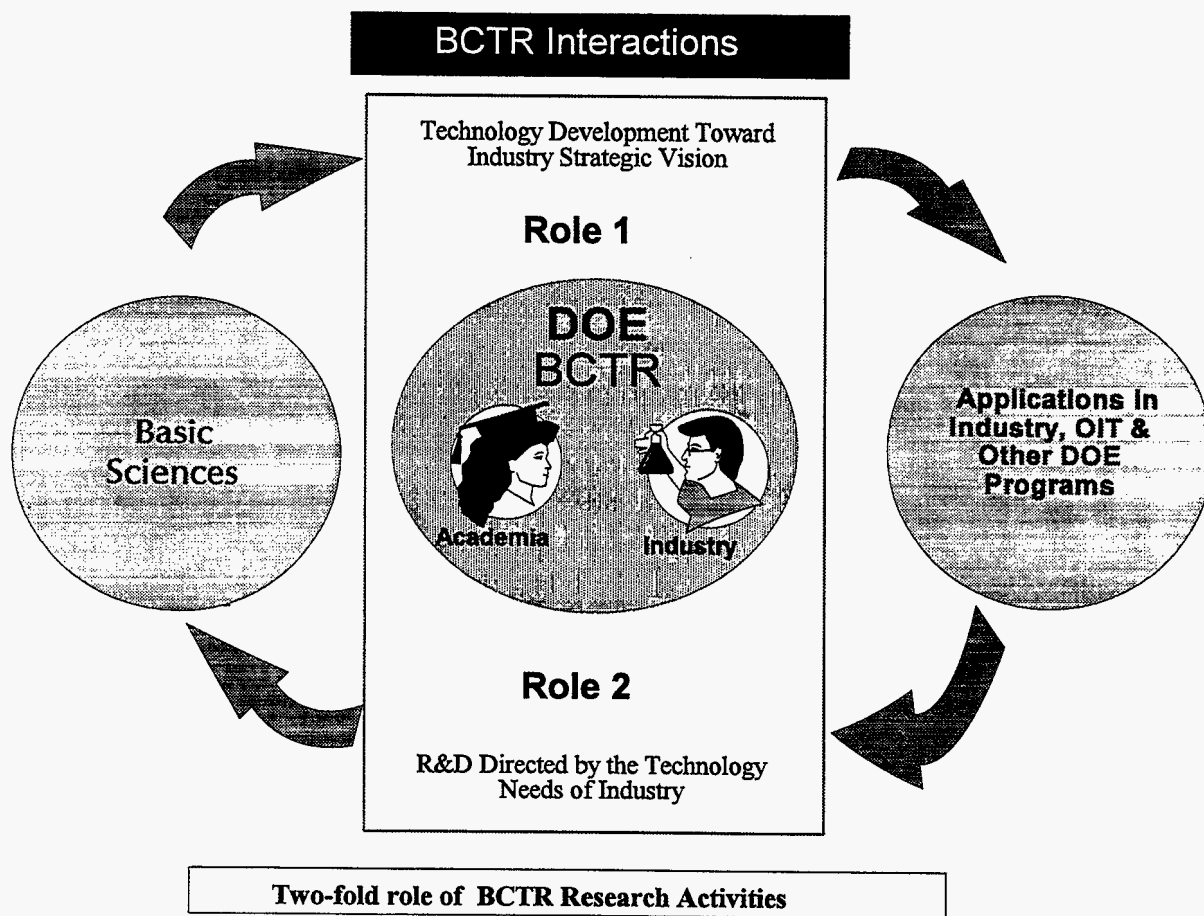


Exhibit 1.1

- ▶ Strategy 1 - **Boost 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 and activating technologies that reduce energy demands for chemicals and materials processing.

The program pursues two basic approaches in implementing its program objectives. First, it builds upon the findings of basic science to enhance technology applications within industry and end-use programs. Second, it applies technology developed by BCTR in response to the research and development (R&D) needs of industry and other OIT programs. These approaches are displayed in Exhibit 1.1.



These two approaches are embodied in a program structure containing elements that address the industry needs and vision of the future (see section 2.0). The five elements are described below including programmatic objectives and goals.

Computer Aided Catalyst Design (CACD) **Objective:** Create tools to aid in the design of chemical and biological catalysts for chemicals and materials production. **Sub-activities:** Investigate chemical CACD and biological CACD. **Goals:** Have chemical and biological CACD sub-model applications by 1996 and model applications by 2008.

Advanced Bioprocess Systems (ABS) **Objective:** Develop and integrate biological processes and schemes into chemicals and allied products industries. **Sub-activities:** Bioprocessing for commodity chemicals and integrating bioprocesses for chemicals processing. **Goals:** Have a novel aqueous bioreactor system in commercial operation by 1996; other aqueous and organic-phase bioprocesses in operation by 2005.

Feedstocks/Process Interaction (FPI) **Objective:** Develop base technology for biological or chemical approaches to expand renewable and recoverable materials use in conventional process operations. **Sub-activities:** Greater use of renewables / Lignocellulosics and Recoverables / Inorganics. **Goal:** Commercially use renewables, including CO<sub>2</sub>, as feedstocks for chemicals production by 2015.

Novel Process Development (NPD) **Objective:** Exploratory screening of novel biological and chemical processes and syntheses developed with support from Basic Energy Sciences, National Science Foundation, and industry for the evaluation of energy efficiency, productivity, and environmental soundness in industrial process operations. **Goal:** Transfer three novel methods to Energy, Efficiency and Renewable Energy (EE) end-users and industry by 2015 for continued engineering development.

Process Control and Systems Analysis (PCSA) **Objective:** Develop process models and simulation capabilities that researchers can use to guide the engineering development of industry process systems. **Goals:** Have industrial applications of a process simulation model by 1997; a process control(s) for advanced systems by 2010.

BCTR projects are specifically designed to evaluate high risk opportunities for industrial applications that lead to investment grade technology. BCTR projects stop short of developing technology that provides immediate commercial deployment by industry. Also, BCTR projects are not the high risk, broad reward efforts typically found in Basic Energy Sciences.

The program has a number of significant achievements through 13-plus years of exploratory research and development which include:

- **Computer Aided Design of Catalysts**

Monsanto, General Motors and DuPont are using catalyst design tools developed at Pacific Northwest Laboratories and Ames to characterize the surface properties of catalysts and for determining the chemical composition of multimetallic automobile catalysts.

Catalytic antibody technology, partly developed under BCTR support, is being employed at Affymax, a small biotechnology firm specializing in pharmaceutical and specialty chemical applications, to investigate chemical catalysis opportunities.

- **Advanced Bioprocessing Systems**

The DOE Alcohol Fuels and Alternative Feedstocks programs are employing bioreactor systems developed at Oak Ridge National Laboratory that provide continuous operation with high productivities and yields in process scale-up for ethanol and succinic acid production, respectively.

AlliedSignal Corporation is employing enzyme technology developed at AlliedSignal and MIT for the production of unique chemicals using immobilized enzyme systems operating in organic solvents.

- **Novel Processing Systems Development**

A novel bioprocessing system that extracts phosphate from ores, developed at the Idaho National Engineering Laboratory, is being scaled-up to pilot-plant operation in collaboration with J.R. Simplot, Inc.

Advanced, low-energy complexation/separation technologies for isolating dilute organic acids produced by fermentation developed at Lawrence Berkeley Laboratory are being employed in the commercial production of polymer precursors by a major agriprocessor.

The ability to improve aerobic fermentations for more efficient productivity and energy use by engineering microorganisms has been demonstrated and is being commercialized by a spin-off small business, Exogene, that started as a direct result of BCTR funding. (Exogene ceased operations in 1994)

- **Industry/Academic/Government Research Center**

A state-of-the-art, hands-on research center that promotes and facilitates government-industry-university interaction and collaboration on computer-aided catalyst design was established at the California Institute of Technology in 1991 with DOE, the Department of Defense (DOD), and industry funding. It currently operates with 8-10 industrial partners who are able to evaluate and develop modeling tools for the study of their own catalysis and biocatalysis problems.

The **economic benefits** from BCTR supported projects has most directly been evidenced by the formation of small businesses. The following is a list of the six small businesses that have begun partly or wholly as a result of DOE BCTR funding. Almost all continue to operate today, employ over 400 individuals, and return over \$60 million to the economy in salaries and benefits.

**Molecular Simulations, Inc.** — Integrated modeling software company

**Exogene** — R&D in chemicals and pharmaceuticals

**Affymax** — Biotechnology and pharmaceuticals R&D

**Alkermes, Inc.** — Value-added products for pharmaceutical and food industries

**Optifood Ingredients, Inc.** — R&D in the food processing industry, and

**Schrodinger, Inc.** — Simulation tools for molecular modeling.

### **Industrial Involvement and Impacts**

Industrial cost-share, industrial interest, participants and program output is found in Section 5. In FY94, industry cost-shared \$ 1677 K with program participants. Non-contractual and leveraged cost-share amounted to another \$ 3042 K. The total BCTR budget for FY94 was about \$ 4.7 M which means that direct cost-share, program wide was 36% and if the non-contractual or leveraged cost-share is included, the industrial contribution, program-wide was 98%. There were 29 companies which were involved as project partners and 18 more which expressed potential future partnerships with program participants. Four potential partners from FY93 became cost-sharing partners in FY94 (Eli Lilly, Catalytica, DuPont Chemical, and AMOCO Chemicals). Although three cost-sharing industrial partners did not continue their participation with projects, five more signed on as new partnerships. Six offices within DOE or other federal agencies were involved in cost-sharing or collaborative efforts with BCTR projects. There was one association with the Norwegian agricultural community and with the private non-profit research organization, Michigan Biotechnology Institute. Program support to participants resulted in the employment of over 58 full-time equivalents. Industry personnel who participated in working towards project goals and objectives amounted to nearly 10 full-time equivalents.

### **Program output - Publications, Patents and Awards**

Program output measured in papers, awards, and patents is also noted in section 5. In FY94, program participants produced 72 peer reviewed publications, had two patents awarded, and filed 4 additional patents and licensed BCTR related technology to one company. Also, several technical reports and conference proceedings resulted from BCTR supported work.

## **2.0 BIOLOGICAL AND CHEMICAL TECHNOLOGIES RESEARCH PROGRAM**

### **2.1 Introduction**

The Department of Energy has five core businesses of which the BCTR program directly supports two: supporting industrial competitiveness via science and technology, and promoting diverse energy supply and demand through technology development and deployment. The BCTR program mission, goals, objectives, and strategies support DOE core business priorities by managing science and technology for results through the interaction and use of DOE's organizational systems.

### **2.2 Program Description**

The Advanced Industrial Concepts Division (AICD) Biological and Chemical Technologies Research (BCTR) Program evolved from the Energy Conversion and Utilization Technologies (ECUT) projects/programs in the Chemical Processes, Catalysis and Biocatalysis areas which originated in 1981. In 1983 only the biocatalysis component was continued as the biocatalysis research activity. Since 1984 the BCTR Program (and its predecessors) has been funded through a budget line item. In 1990, with an increased interest in novel chemical processing, the program was renamed Catalysis/Biocatalysis. In April of 1990, a reorganization of the Office of Conservation and Renewable Energy moved this program to the AICD under the OIT and the Office of Industrial Processes (OIP). The program is now titled Biological and Chemical Technologies Research and is in the Energy Efficiency and Renewable Energy Office. The operational model for the BCTR program is depicted in Exhibit 2.1

This operational model allows BCTR to evaluate the industrial applicability of fundamental findings, and to respond to DOE end-use programs and industry needs that can be filled with existing technology previously developed by BCTR. Program technology development stops short of large-scale pilot plant operations, but is intended to demonstrate the applicability of technologies for industrial uses. Often, some fundamental research is required in the course of demonstrating proofs of concept, but the general scope of BCTR program work is not fundamental research.

### **2.3 Industry Drivers and Vision and BCTR Response**

The chemical, by-products, and petroleum industries will continue to influence U.S. energy, economy, and environmental matters in the 21st Century. The renewables industry is expected to increase its role in the U.S. economy, initially as a response to environmental requirements, but ultimately as a reliable feedstock serving a diverse energy market. Exhibit 2.1 displays the current drivers of these three industries and the vision they are forming for the future.

Industry	Technology Drivers	Technology Visions
<b>Petroleum Refining</b>	<b>Emissions regulations and changing product specifications</b> <b>Lower quality crude oils containing higher impurities</b> <b>Natural gas use</b>	<b>Become environmentally benign</b> <b>Enlarge heavy crude conversion technologies</b> <b>Use syngas and convert natural gas to liquid products</b>
<b>Chemicals</b>	<b>Emission regulations and reducing wastes</b> <b>New products for market expansions</b> <b>Produce commodities near source</b> <b>Maintain competitiveness with current products</b>	<b>No waste processes or minimized wastes from processes</b> <b>Environmentally friendly biotechnological products</b> <b>High value-added products through bioprocessing</b> <b>Processes that offer feedstock flexibility</b>
<b>Renewables</b>	<b>Effective land use - eliminate farm subsidies</b>  <b>Higher profit margins</b>  <b>Stabilize rural America</b>	<b>Use the 40-60 M acres of set-aside land for productive farming</b> <b>Increase level of biobased products in the market</b> <b>Site biobased products near sources</b> <b>Employ novel biorefinery/bioprocessing concepts</b>

**Exhibit 2.1** Industry technology drivers and vision for three related industries

**Economic and market competitiveness:** The following are examples of how economics and market forces are changing the face of these three industries.

- The value of products derived from catalysts is about 17% of the U.S. gross national product (GNP), but the development of catalysts is highly empirical and expensive. Catalyst R&D is losing favor in the U.S. while growing in importance in Europe.
- Although the U.S. chemical processing industry has an enviable record in world trade, exports of commodity chemicals is decreasing as Asian nations and other third world countries are building their own capacity. This moves jobs off U.S. shores. Forces affecting the market include dwindling oil supply, poor quality feedstocks, exchange rate shifts, political upheavals, and environmental controls. Competitiveness for United States industries will require that production facilities be flexible in feedstock use and product swing.
- The North American Free Trade Agreement will solidify the strong export market to Canada and Mexico and help maintain commodity chemicals production. Overall, the exports of large volume and large dollar chemical categories, organics and synthetic resins, are showing slow growth, while low-volume, low-dollar chemical categories are showing larger growth. This trend is expected to continue as other nations build their own capacity, especially in Asia.

**Energy:** These industries are major energy users. Improved process efficiency will become important as energy costs rise. Using alternative feedstocks lowers the need for petroleum imports and may be less energy demanding.

**Environment:** Environmental laws and more stringent solid, liquid, and air emission controls mandate industry to reduce wastes, use dilute feedstock streams and combustion gases, and to develop novel processes to effect zero-discharge processes. Capital investments for environmental compliance or divestiture of processes will continue into the next decade. This will be followed by a life extension program to maintain the capital investments and the search for clean processes. Ultimately, new processes will be required to meet public demands for environmentally clean and energy efficient processes.

The research activities within the BCTR program apply across much of the industrial sector. However, the focus of the program is toward the chemical and allied products (SIC 28) and petroleum refining (SIC 29) industries. Exhibit 2.1 gives the broad vision needs of the three constituent industries that the BCTR program seeks to support. In Exhibit 2.2 additional items are added that show the technical areas that the BCTR program addresses and that meet the needs of the industry vision. These are noted in bold type.

Industry	Industry Technology Visions	BCTR Technology Meets Industry Vision
Petroleum Refining	<b>Become environmentally benign</b> <b>Enlarge heavy crude conversion technologies</b> <b>Use syngas and convert natural gas to liquid products</b>	<b>Highly specific catalysts</b> <b>More robust catalysts</b> <b>New catalysts and processes</b>
Chemicals	<b>No waste processes or minimized wastes from processes</b> <b>Environmentally friendly biotech products</b> <b>Processes that offer feedstock flexibility</b>	<b>High specificity catalysts</b> <b>Value-added products through bioprocessing</b> <b>Integration of bioprocessing into typical chemical processes</b>
Renewables	<b>Use the 40-60 M acres of set-aside land for productive farming</b> <b>Increase level of biobased products in the market</b> <b>Site biobased products near sources</b> <b>Employ novel biorefinery/bioprocessing concepts</b>	<b>Helping alternative feedstocks become part of agricultural product slate</b> <b>Develop novel processes that employ biomass</b>  <b>Biomass refineries or Renewable Energy Parks</b> <b>R&amp;D in chemical and biological processes</b>

**Exhibit 2.2** BCTR response to industry technology drivers and vision for three related industries

The program structure described in section 1.0 of this report is designed to organize the efforts of the BCTR program in response to the needs of industry. The following displays the structure in diagram form including the subactivities, where applicable.

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**PROGRAM ELEMENTS**

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<b>Computer-Aided Catalyst Design</b>	<b>Advanced Bioprocess Systems</b>	<b>Feedstock/Process Interactions</b>
Chemical CACD	Bioprocessing for commodity chemicals	Renewables/Lignocellulosics
Biological CACD	Chemical industry integration	Recoverables and Inorganics
<b>Novel Process Development</b>	<b>Process Control/Systems Analysis</b>	

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## 2.4 BCTR projects in FY94

Listed below are the specific projects undertaken in 1994 that support each of the program elements. Acronyms for institutional sites are as follows. Lawrence Berkeley Laboratory (LBL); Los Alamos National Laboratory (LANL); Jet Propulsion Laboratory (JPL); California Institute of Technology (CIT); Sandia National Laboratory Albuquerque (SNLA); Oak Ridge National Laboratory (ORNL); Idaho National Engineering Laboratory (INEL); Massachusetts Institute of Technology (MIT); National Renewable Energy Laboratory (NREL); Pacific Northwest Laboratory (PNL).

### Computer-Aided Catalysis Design (CACD)

#### Chemical CACD

Periodic *Ab initio* Hartree-Fock theory/site isolation in selective alkane oxidations (PNL)

Design characteristics and testing of multimetallic catalysts for pollution control (Ames)

Theoretical studies of hydrocarbon catalysis on zeolites (LANL)

Theory-assisted design of metal and zeolite catalysts (LBL)

#### Biological CACD

Genetic engineering of methanogenic bacteria for industrial/chemical applications (JPL)

Chemistry, immunology, and modeling as tools for the rational design of enzymes (LBL)

Theory of biocatalysis-electron transfer reactions (University of Pittsburgh)

Predictive models and effects of structure on catalytic properties and materials and molecular simulation center (CIT)

### Mimetics

Computer aided molecular design of biomimetic CO<sub>2</sub> activation catalysts (*SNLA*)

## **Advanced Bioprocessing Systems**

### Bioprocessing for Commodity Chemicals

Engineering enzymes for stability and activity in organic solvents (*CIT*)

Bioprocess engineering: Immobilized cell systems for continuous, efficient biocatalyzed processes (*ORNL*)

### Integration into Chemicals Industry

Designing and improving enzymes for use in organic solvents (*MIT*)

Immobilized enzymes in organic solvents (*AlliedSignal*)

Biological separation of phosphate from ores (*INEL*)

## **Feedstock/Process Interactions**

The carbon dioxide bioreactor: Conversion of industrial waste carbon dioxide into polyhydroxybutyrate (PHB) (*JPL*)

Photobiological conversion of syngas into microbial polyester (*NREL*)

CO<sub>2</sub> photoreceptor research (*ORNL*)

Levogucosan polymers (*NREL*)

Clean fractionation process (*NREL*)

## **Novel Process Development**

Overproduction and enhanced secretion of enzymes (polyphenol oxidase)  
(*Howard University*)

Separations by reversible chemical association (*LBL*)

Electroreduction of lipids (*Tulane*)



Engineering analysis of electrochemical conversion of methane to C<sub>2</sub> hydrocarbons and synthesis gas (*Tufts University*)

**Process Control/Systems Analysis**

Bioengineering Simulation Technology (BEST) development (*NREL*)

A Biological and Chemical Process Integration and Assessment Computer Model: (BCP1) (*JPL*)

BCTR program planning and support (*PNL*)

Brief Characterization of the Top 50 U. S. Commodity Chemicals (*PNL and JPL*)

Impact of Catalysis on the Production of the Top 50 U.S. Commodity Chemicals (*PNL*)

Brief Characterization of the Top 50 U.S. Commodity Chemicals (*PNL and Battelle Columbus*)

### 3.0 SELECTED HIGHLIGHTS FOR FY 1994

#### 3.1 PACIFIC NORTHWEST LABORATORY AND JET PROPULSION LABORATORY

*Significant assessment completed by PNL and JPL in 1994.*

The report, "Brief Characterization of the Top 50 U. S. Commodity Chemicals" compiles data about the 50 commodity chemicals produced in the largest amounts in the United States. The report contains information on volumes, energy, intensity, estimated energy consumption, price, revenue, capital intensity, and environmental considerations for each chemical. This assessment will assist in determining priorities for research areas that should result in maximum improvements in energy efficiency and in greater use of renewable energy resources for the industrial production of chemicals.

#### 3.2 PACIFIC NORTHWEST LABORATORY

*Impact of catalysis on chemicals production analyzed*

A project to investigate the impact of catalysis on energy consumption was completed and published as a PNL report, "Impact of Catalysis on the Production of the Top 50 U.S. Commodity Chemicals". The maximum feedstock and process energy savings during the production of the top 50 commodity chemicals is 0.47 quads/year. The energy savings resulting from process yield improvements ranging from 1% to 25% is also quantified.

#### 3.3 BATTELLE COLUMBUS AND PACIFIC NORTHWEST LABORATORY

*Project opportunities based on top 50 chemicals outlined*

By using the reports, "Brief Characterization of the Top 50 U.S. Commodity Chemicals" and "Impact of Catalysis on the Production of the Top 50 U.S. Commodity Chemicals," another significant report was published that identified the opportunities for non-incremental changes in process technology and the alternatives for more environmentally benign processes. This report is titled "Initiatives for Top 50 Commodity Chemicals" and will be used to guide program initiatives.

#### 3.4 TULANE UNIVERSITY

*BCTR technology to transfer to USDA and industry*

Work at Tulane University supported by the BCTR program has demonstrated the technical and economic feasibility of a process to electrochemically hydrogenate edible oils. Industry and the United States Department of Agriculture will pursue the commercialization of this technology. Work in the past year has provided three significant advances that lead to the transfer of this technology. A semi-empirical porous electrode Raney nickel flow reactor model has been developed that can predict the dependence of soybean oil hydrogenation current efficiency on applied current and

electrolyte oil content. Also a porous electrode reactor FORTRAN code has been linked to a commercially available computer design software package (PRO/II™ by Simulation Sciences, Inc.) for heat and mass balance calculations on an entire soybean oil electro-hydrogenation plant. An equipment size and cost analysis of an oil electro-hydrogenation plant (6.5 MM lbs./yr capacity) was performed, based on a tentative plant flowsheet, the porous electrode reactor model, and the results of the PRO/II heat and mass balance calculations.

### 3.5 OAK RIDGE NATIONAL LABORATORY

#### *Fluidized bed technology shown cost effective and efficient in larger scale*

Three significant findings highlight work at ORNL in 1994. In a joint effort with the Office of Transportation Technologies, the 2" fluidized bed reactor developed under BCTR support was scaled to 4" to meet the needs of the Biofuels Systems Division. The shake-down experiments show 2-3 fold increased productivity and raised glucose conversion efficiencies to 99% from 94-97%. Extended testing will now be undertaken.

An economic analysis using of fluidized bed technology for ethanol production over fed-batch systems by Fluor-Daniel indicated a potential of 6¢/gal savings in processing costs.

Continuous and simultaneous fermentation and purification lactic acid was demonstrated in a biparticle fluidized bed reactor. Results for short runs included a 12 fold improvement in productivity in similar reactors without *in situ* product extraction, an 8-fold increase in product concentration, and a 1,734 fold enhancement in separating the product from substrate by continuous regeneration of the sorbent particles.

### 3.6 IDAHO NATIONAL ENGINEERING LABORATORY

#### *New encapsulation process may permit direct application of technology*

INEL has developed a "microencapsulation" process wherein microbes are coencapsulated with phosphate ore within 2mm beads. These microfactories are capable of solubilizing phosphate and providing *in situ* release of phosphate at the site of most need rather than the indiscriminate application of phosphate fertilizer. This approach would avoid the problems of large-scale bioreactor processing found in the earlier developmental efforts at INEL.

### 3.7 HOWARD UNIVERSITY

#### *Enzyme process for biological pulping demonstrated at lab scale*

Research, at the laboratory scale, has demonstrated the ability for high-volume production of enzyme biocatalysts important in processing the intractable lignin portions of lignocellulosics. The production of sufficient quantities of enzymes suitable for pulping wood has been a stumbling block in the

development of low-polluting, pulping bioprocesses. These results provide an important milestone in the quest to develop bioprocesses for pulping wood.

### 3.8 LAWRENCE BERKELEY LABORATORY

*Energy efficient production of de-icer demonstrated; new biocatalytic capabilities demonstrated for chemicals production; and simulations of workhorse chemical catalysts verified for use*

Laboratory experiments have established a viable approach to making calcium magnesium acetate (an environmentally benign road de-icer) from dilute acetic acid without recovering and concentrating the acetic acid.

Antibodies were used to conduct site specific hydrolysis of oligosaccharides, to mediate chemically interesting oxygenation reactions, and to elicit stereospecific reactions with oximes and esters. These were conducted in partnership with a spin-off company, AFFYMAX, which has been instrumental in developing unique synthesis routes for chemicals, polymers, and medical products.

The ability to simulate, with good quantitative accuracy, the interaction of Brönsted acid sites and adsorbents in zeolites has been demonstrated. This will permit better characterization of zeolite catalyst systems. In addition, good agreement between experiment and simulation was achieved for the occupancy and diffusion of long chain molecules within the zeolite catalyst structure. This will permit more accurate simulations of the cracking and separation of such molecules and simulations will aid in developing of crude oil cracker processes.

### 3.9 PACIFIC NORTHWEST LABORATORY

*Use of massively parallel computers for molecular modeling shown feasible*

The ability to use massively parallel computer architectures for molecular modeling required that simulation of the properties of crystalline materials be demonstrated in successively more complex systems. In 1994, the ability to use such architectures exceeded the phase I milestones for implementing this technology. Additionally, work was completed on the development and demonstration phase for the periodic *Hartree-Fock* theory. This now mature technology is being applied to many first-of-a-kind simulations with zeolite and metal oxide catalyst systems thought to be beyond the scope of *ab initio* methods.

### 3.10 AMES LABORATORY

*Structure of small metal clusters defined and software readied for release*

An outstanding question about application of the entire range of methods in DeCal has been the compact shape of metal clusters. Small metal clusters have been shown to have compact shapes. The above accomplishment validated the DeCal methodology. In addition, a "seamless integration"

of CEM, MD/MC-CEM and BOS codes has been produced. This effort is the first step in producing a user-friendly software package. Further steps will be accomplished in FY 1995 and FY1996, leading to software release in September 1996.

### 3.11 MASSACHUSETTS INSTITUTE OF TECHNOLOGY

#### *Characterization of enzymes in organic solvents further defined*

MIT researchers have demonstrated that the marked solvent dependence of substrate specificity of the protease subtilisin Carlsberg can be quantitatively explained on the basis of thermodynamic parameters, such as activity coefficients of the substrates in different solvents which we calculate using the UNIFAC computer algorithm. This achievement is a significant step toward controlling substrate specificity of enzymes at will, thus providing heretofore unavailable means of optimization of enzymatic processes. In addition, the researchers have found that enantioselectivity in organic solvents of subtilisin Carlsberg covalently attached to macroporous polymeric supports may be controlled by the support's aquaphilicity. Furthermore, the catalytic activity of immobilized subtilisin in organic solvents is strongly affected by the enzyme pretreatment. These findings suggest additional approaches to enhancing efficiency of enzyme-catalyzed processes.

### 3.12 NATIONAL RENEWABLE ENERGY LABORATORY AND OAK RIDGE NATIONAL LABORATORY

#### *New techniques to convert biomass and carbon dioxide into chemicals demonstrated*

Four small projects at these two laboratories produced laboratory scale results that could prove useful in processing and fractionating biomass to produce of chemical intermediates.

A new class of polyurethanes has been synthesized from *cis*-1,3-dioxolane-2,4-dimethanol derived from the pyrolysis of wood, using methylenedi-*p*-phenyl diisocyanate (MDI) and hexamethylene diisocyanate (HDI). Although the study of the thermal properties of these specific new polyurethanes using Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) indicated poor performance, the production of such starting materials is potentially useful for production of new polymers.

A review of the literature on the chemical use of CO<sub>2</sub> as a chemical intermediate pointed out the need for identifying and developing new catalysts if CO<sub>2</sub> use is to be implemented. One area to begin searching for new catalysts is syngas conversion technology. These catalysts, when modified, may work for CO<sub>2</sub> conversion as well. A review paper on CO<sub>2</sub> use was completed, submitted and accepted for publication in Chemistry and Industry.

A new single-light photosynthetic pathway was discovered for the conversion of atmospheric CO<sub>2</sub> to value-added chemicals in the genetically engineered mutant B4 of *Chlamydomonas reinhardtii*. From an applied and industrial perspective, these results are important because they imply a potentially greater efficiency in the conversion of CO<sub>2</sub> to value-added chemical compounds.

Correlations were made in organosolv pulping processes between the solvent severity and the increase in lignin and xylan removal from treated wood. Also, novel products formed from the degradation of glucose and xylose were observed under organosolv and clean fractionation conditions. These previously unreported products only appear in the presence of organic solvent. The chemical structure of the products has not been positively identified.

### 3.13 CONFERENCES AND REVIEW MEETINGS

*BCTR program continues to build relationships among the professional community*

The BCTR program provided support to four significant symposia and conferences: The Sixteenth Symposium on Biotechnology for Fuels and Chemicals; the Biomass of Americas Conference; the International CO<sub>2</sub> Utilization Conference; and the Biobased Products Expo'94.

In 1994, the first combined meeting of all principal investigators was held in Lakewood, Colorado, along with a meeting of the BCTR industrial review panel which also reviewed all the projects.

## 4.0 FY 1994 TECHNICAL PROJECT DESCRIPTIONS

The following includes detailed descriptions of the FY 1994 projects. The projects have been categorized according to the five program elements discussed in Section 2.5. Each project summary contains a brief description of the effort including the names of the investigators and the site of the research, a summary of the major 1994 accomplishments, a description (where applicable) of the anticipated 1995 efforts. A more in-depth discussion of 1994 research results is also included.

### 4.1 Computer-Aided Catalyst Design - Chemical

1. **PROJECT TITLE:** Heterogeneous Catalysis: Chemistry and Physics of Zeolites and Metal Oxides
- PRINCIPAL INVESTIGATORS:** A. C. 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.

#### 1994 ACCOMPLISHMENTS:

Exceeded the Phase I milestones for a periodic Gaussian basis density functional theory which targets massively parallel computer architectures. The goal of this project is to generate a low order scaling quantum mechanical theory which is efficiently implemented on MPP architectures capable of accurately treating molecules, external surfaces and bulk crystalline materials (0-,2- and 3-dimensionally periodic objects). The current versions of the code, NWDFT, include molecular energies and first derivatives which demonstrate 90-95% efficiency on shared, distributed/shared and distributed memory machines on tested cases to 250 processors. The current scaling with the size of

system is of the order  $O(N^{2.4})$ . Presently, NWDFIT, also contains a first generation object capable of evaluating the ground state energy and density of 3-dimensionally periodic (bulk crystalline) materials.

Obtained funding for CRADA with Catalytica Inc. of Mountain View, CA. Catalytica was originally an industrial partner of ours which was interested in applying advanced computational methods to general problems associated with liquid and solid acid catalysts. As we became more involved with that company it eventually became clear to both parties that a more focused effort on perhaps less generic problems would be of benefit to them. A CRADA proposal was written and has now been funded. The total value of the project of over a 3 year period of time will be 480K.

Two new industrial partners for FY94. Amoco Chemical and DuPont have joined as industrial partners during this past fiscal year. Work with both partners involves various aspects of oxidation catalysis and the use of the soon to be release Gaussian basis density functional program, NWDFIT. The gradient codes within this program will find widespread application to problems associated with structural optimizations of bulk and surfaces as well as in the identification of reaction paths and the associated details of the reactants, intermediates and products involved in chemical reactions.

Completed development goals and demonstration phase for periodic Hartree-Fock theory. During FY94 we have completed the development, validation and testing phase of periodic Hartree-Fock theory. This is now viewed as a mature technology which is experiencing widespread use in the field. Using this approach we have performed many first of a kind applications in zeolite and metal oxide catalysis previously felt to be beyond the scope of ab initio methods. No further development activities are planned for this technology.

#### **1995 PLANNED ACTIVITIES:**

Our FY95 research plans are composed of two primary components. First we intended to use today's state-of-the-art solid state methods (eg, periodic Hartree-Fock theory ) to assist our industrial partners in achieving solutions to their "in house" catalyst problems. This type of activity is a critical component of a successful program of interaction with industry. It serves the purpose of providing our partners with the necessary familiarity with high level quantum mechanical methods to utilize the technology independently. As such, it is a necessary and important step in any technology transfer activity. Second, we will focus essentially all of our development effort on producing the next generation of quantum mechanical software tools which, even at this early stage, exceed the capabilities of the current "state-of-the-art" tools. This new technology, based upon density functional theory, is the result of several years of mathematical research which has allowed us to reformulate various aspects of the theory such that it achieves a very low order scaling as a function of system size. An additional aspect of this work, is to implement the resulting formal equations efficiently on massively parallel computer architectures. Taken in combination, we can experience approximately a 1000-fold increase in computational capabilities over current theoretical tools without a sacrifice in accuracy. Recently we have completed a proper molecular implementation of our approach and have proven that our new ideas are capable of this significant jump over the capabilities of current methods. This degree of increase is absolutely necessary if we are to move beyond descriptions of the physical state of the catalyst to the point where we provide industry with tools to investigate chemical reactions directly at the atomic level. In the context of this development activity (and within modern theoretical frameworks) this can be done by careful investigations of the



reaction paths which yield the necessary information to begin to predict and understand the overall kinetics and thermodynamics of the catalytic process on time scales important to a chemical engineer. Briefly, our milestones for industrial involvement and NWDFT development are as follows:

**Molecular Module of NWDFT:** One of our primary goals for the molecular module during FY95 will be to distribute it to other principles in the CACD program set (A. Bell, J. Shelnut and J. Hay have requested the code) and to our or other CACD program members industrial partners. We intend to make this distribution early in the first quarter CY95. Additionally, we will focus our attention on some (perhaps all) of the following three technical refinements to the existing modules a) multipole expansions will be used to evaluate components of the Coulomb contribution to the Hamiltonian b) fully numerical evaluations of all components of the Hamiltonian (with the exception of the kinetic energy) will be investigated c) replacements for explicit matrix diagonalization (conjugate gradient techniques, etc.) will be implemented and tested. All three of these modifications have the potential to further reduce either the formal or effective scaling of the molecular code. This translates directly into lower cost and faster response time for theoretical industrial scientists working with experimental colleges. Molecular reaction path following should be completed some time in the second quarter.

**Three- and 2-dimensionally periodic NWDFT modules:** The completion of a stable, efficient 3- and 2-dimensionally periodic modules capable of treating crystalline materials and their external surfaces is the primary goal of FY95. This is, and will be, a code which fully exploits massively parallel computer architectures and evaluates the ground state energy and density of this type of matter. In addition to the ground state energy and charge density we will also provide simple properties (e.g., band structure, density and projected density of states and population analysis) in the first release of this code to our partners. An implementation which scales as  $O(N^3)$  for both 3- and 2-dimensions and which operates efficiently on shared, distributed shared or distributed memory architectures up to 250-512 processors will be judged to be acceptable for this fiscal year. FY96 milestones focus upon delivering atomic forces and algorithms designed to follow reaction paths for solid and solid surfaces.

We also intend to continue to employ today's state-of-the-art methods (e.g., periodic Hartree-Fock theory) to assist our industrial partners in solving problems involving solid state systems. As stated above, this is essentially a requirement for a successful technology transfer with such advanced software. As in the past, the industrial partners specify the problem set and we help them to work through applications of the theoretical tools to problems of relevance to them. This type of activity is central to our deployment strategy. Industrial partners are often selected on their ability to properly utilize these tools and to subsequently demonstrate leadership to the rest of the industry in terms of its application.

## **ANNUAL TECHNICAL SUMMARY REPORT:**

### **Heterogeneous Catalysis: Chemistry and Physics of Zeolites and Metal Oxides**

The role of catalysts 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. The use of catalysts to facilitate the chemical transformation of raw materials into economically important compounds has

long been a crucial component of the energy posture of the United States. Used in an analogous manner, catalysts can also be employed in the environmental restoration activities currently taking place throughout U.S. Department of Energy facilities. A key component of the solid state theory effort is to gain a fundamental understanding of the atomic level behavior of solid catalysts. The work outlined below demonstrates the application of several advanced theoretical methods to metal oxide and zeolite catalysts.

Our FY94 research activities have focused upon both the application of current quantum mechanical tools to selected catalyst systems and the development of next generation methods designed to take advantage of massively parallel computer architectures. In this fiscal year, as well as in previous years we have applied ab initio periodic Hartree-Fock (PHF) theory to a wide variety of complex heterogeneous systems. The purpose of this activity was to both gain additional scientific understanding of these systems and to assess the strengths and weaknesses of this method in treating problems important to catalysis. Briefly, the application of PHF theory to problems in this field has resulted in several first of a kind studies and the approach is commonly used in the field. However, we find that the cost of such calculations and the amount of real time required to achieve a solution remains high when addressing realistic models of many common catalysts. We find, for example, that one can quite readily evaluate observable quantities associated with the physical properties of ground state of the system at reasonable cost even for very large systems but the cost of gathering the correct type and volume required to understand a chemical change in the system is often prohibitively expensive. As catalysis, in naive sense, is primarily concerned with kinetics, which within modern theoretical frameworks is extracted by understanding the reaction path for a given chemical reaction a theoretical tool powerful enough to yield this information directly would be of considerable value. Having realized this during late CY92 we began to consider alternative ab initio methods and to monitor the progress of researchers developing high performance computational chemistry codes for massively parallel computer architectures. By the close of FY93 we had conclude that a next generation solid state theory based upon Gaussian basis Density functional theory implemented from its inception on massively parallel computer architectures (defined for our purposes as a machine possessing over 100 three hundred megaflop processors) could provide the factor of 1000 required over current technology to monitor/predict chemical changes in solid/gas and perhaps simple solid/liquid interfaces. During FY94 we have completed the initial implementation phases of a new massively parallel density functional program, NWDFT. At present this package is capable of evaluating total molecular energies and forces and contains a prototype 3-dimensionally periodic solid state module which converges the ground state energy and density. It is important to realize that writing such a package is a massive undertaking the burden of which is shared between researchers in this program, and the High Performacs Computational Chemistry group of the Environmental and Molecular Sciences Laboratory at Pacific Northwest Laboratory.

In what follows we will first discuss recent results using NWDFT to study very large molecular systems and demonstrate the scaling properties and degree of parallism which exists in the current implementation. Following this discussion we will briefly report on some of the systems studied using PHF as an example of what this type of technology is currently capable of when used to study zeolites and metal oxides.

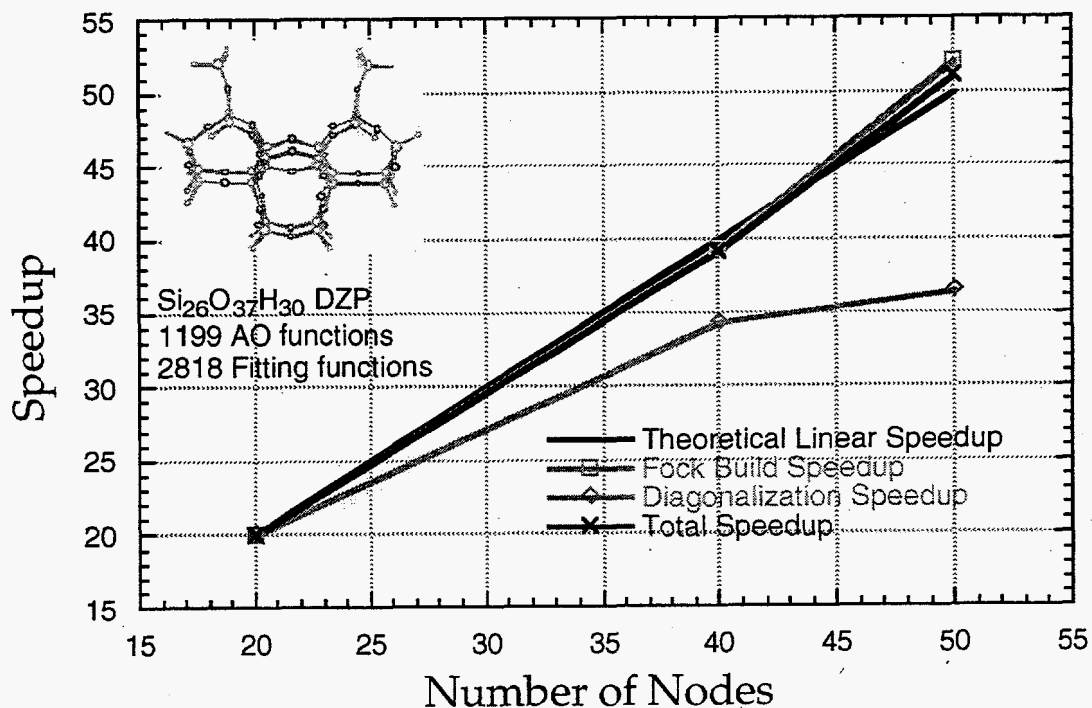
## Massively Parallel Gaussian Basis Density Functional Theory

Our work on advanced computational strategies has been motivated by our industrial partners need to understand changes occurring as reactant species undergo chemical transformations on the way to products at the internal and external surfaces of solid materials. Our approach in designing a next generation computational tool has been to first reduce the formal mathematical order of the theory to its lowest form. One of the primary scientific goals of this project is to provide a first principles solid state quantum mechanical method which can efficiently perform reaction path following for the purpose of identifying reactants, intermediates, and products as well as any associated energy barrier to the overall process. Since many aspect of our industrial partners work involves dynamical phenomena one of our primary goals centers upon the evaluation of atomic forces to drive molecular dynamics calculations, optimize structures and follow reaction paths. The principle application target is therefore a tool which will allows researchers to better understand the interaction of molecular species with the internal and external surface of materials. This level of detailed information concerning the reaction path will provide the necessary information to begin to understand the kinetic and thermodynamic properties of the process. As stated above, we have completed a first stage of the new density functional method designed to treat molecules, polymers, surfaces and bulk materials. The design goals for the first phase of the project included the development of a robust molecular dft code (including first derivatives of the energy) which demonstrate at least  $O(N^3)$  scaling that functioned efficiently on both distributed and shared memory machines. In addition, the goals also included the generation of a prototype 3-dimensionally periodic program. The current versions of NWDFT meet or exceed all milestones for the first phase of the project. What follows is a brief report on details of the package and indication of its current performance.

NWDFT, is a FORTRAN program which uses C preprocessor directives to activate code that is specific for various hardware platforms. We heavily utilize tools developed in the High Performance Computational Chemistry (HPCC) initiative, for example, the dynamic memory allocator (MA), the message passing library (TCGMSG), and the global array (GA) tools all combine to form a coherent and usable back plane. Additionally, all data greater then  $O(N)$  is stored in global arrays. The code we have developed is a MPP implementation of the Hohenberg-Kohn-Sham formalism (Hohenberg and Kohn, 1964; Kohn and Sham, 1965) of DFT. DFT is able to give results similar to those from correlated ab initio methods at substantially less cost; it assumes a charge density and successively better approximates the Hamiltonian (the exchange correlation functional). In our Gaussian basis DFT method the Hamiltonian is broken down into the same basic one-electron and two-electron components as traditional methods. The two-electron component is further reduced to a Coulomb term and an exchange-correlation term. The treatment of the Coulomb term can be accomplished in identical fashion to that used in traditional SCF methods or by first projecting the charge density into a set of auxiliary Gaussian functions and with subsequent analytical integration of the resulting Coulomb repulsion integrals form by the orbital basis and that of the auxiliary basis. In our approach we provide the means to either evaluate the exchange-correlation potential either by direct numerical integration, or by a projection of the potential into an auxiliary Gaussian basis and subsequently integrated analytically. In NWDFT, the electron density and the exchange-correlation functional can be expanded in terms of auxiliary Gaussian basis sets. We have incorporated the exchange-correlation functionals (Dirac's exchange functional (Dirac, 1930) and Vosko, Wilk, Nusair's correlation functional

(Vosko, Wilk, and Nusair, 1980)) required to obtain the Local Density Approximation (LDA) and Local Spin Density Approximation (LSD) for open shell systems. In addition, we have implemented two gradient corrected functionals for the exchange-correlation contributions; Becke's exchange functional (Becke, 1988) and Perdew's correlation functional (Perdew, 1986). The above functionals have been implemented for both closed and open shell systems. We have also incorporated several techniques to improve convergence in the iterative SCF procedure: damping, level shifting and DIIS. Of course these have all been implemented in a manner to exploit MPP technology with maximum efficiencies. In addition to tuning the current versions of the molecular code for parallel efficiency, we are carrying out systematic benchmark calculations to optimize quadrature schemes and fitting bases for accuracy and conjugate gradient techniques to avoid the explicit diagonalization of eigen systems.

In the next three figures we illustrate the current computational behavior on both shared memory and distributed memory machines as well as the overall scaling of our approach as a function of system size.



#### Actual Wall Times in Seconds

Nodes	Fock Build	Diagonalization	Total
20	7441.60	336.50	7778.10
50	2859.40	184.65	3044.05

Figure 1: The speedup obtained for calculations of Fock matrices and subsequent diagonalization of zeolite fragment Si<sub>26</sub>O<sub>37</sub>H<sub>30</sub> on up to 50 nodes of the KSR-2.

Figure 1 demonstrates the speedup of the most time consuming portions of our DFT calculations using our in-house KSR-2 parallel platform on the zeolite fragment  $\text{Si}_{26}\text{O}_{37}\text{H}_{30}$  (1700 atomic orbitals and 4000 fitting functions) on up to 50 nodes of this machine. The four time consuming steps are: fit of the charge density, calculation of the Coulomb potential, evaluation of the exchange-correlation potential, and diagonalization of the Fock matrix. We are currently obtaining an overall speedup of greater than 90% using up to 67 processors for building the Fock matrix and subsequent diagonalization. This figure specifically shows the results obtained using 50 nodes of the KSR-2 for  $\text{Si}_{26}\text{O}_{37}\text{H}_{30}$ . To the best of our knowledge, the current generation of NWDFE exceeds the capabilities of all known commercial or public domain DFT codes with respect to the size and complexity of system.

In the figure below we illustrate the scaling of the molecular version of the NWDFE for a variety of zeolite fragments. From the figure it can be clearly seen that our method is scaling as  $O(N^{2.5})$  which exceeds the initial milestones. In addition, the largest of these fragments adequately represents the state-of-the-art today using a first principles theory.

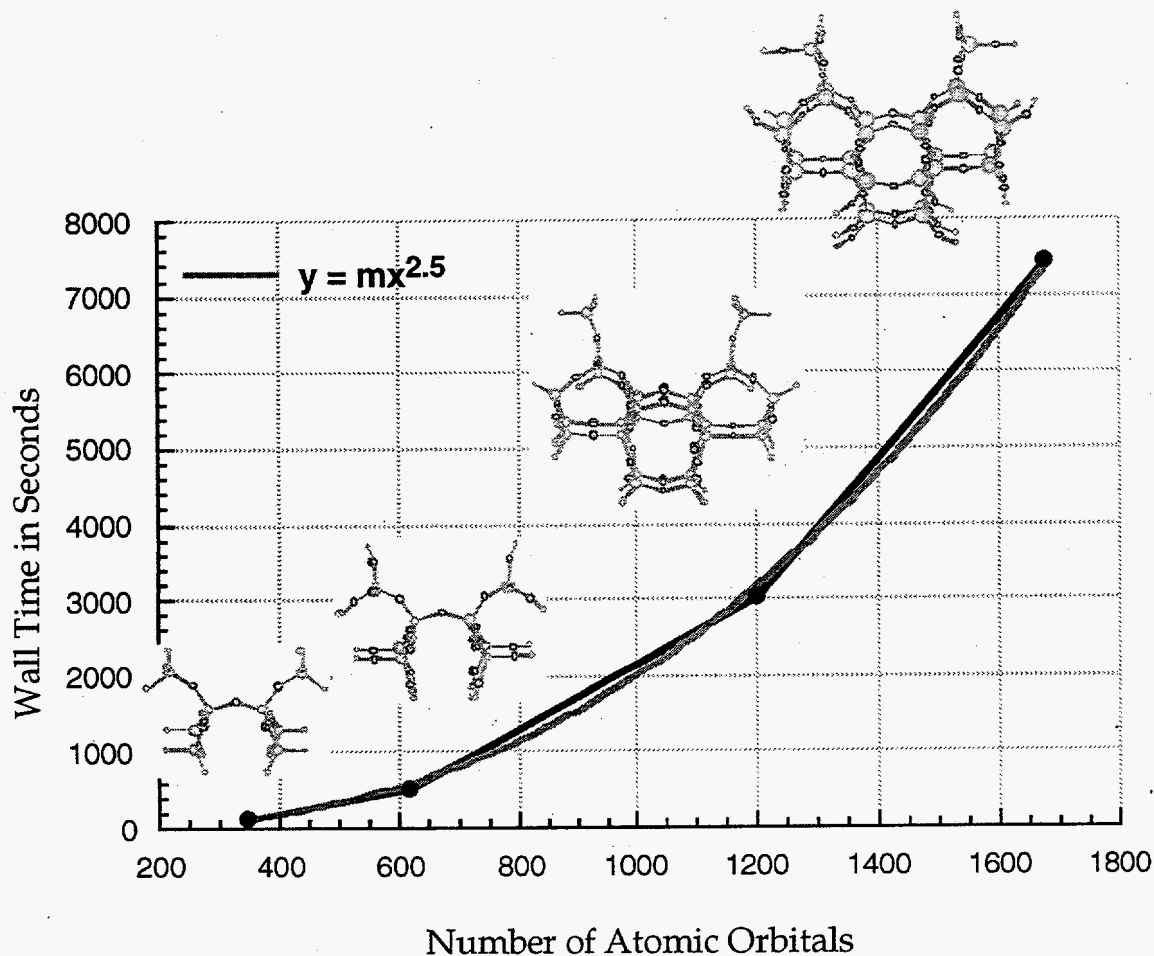
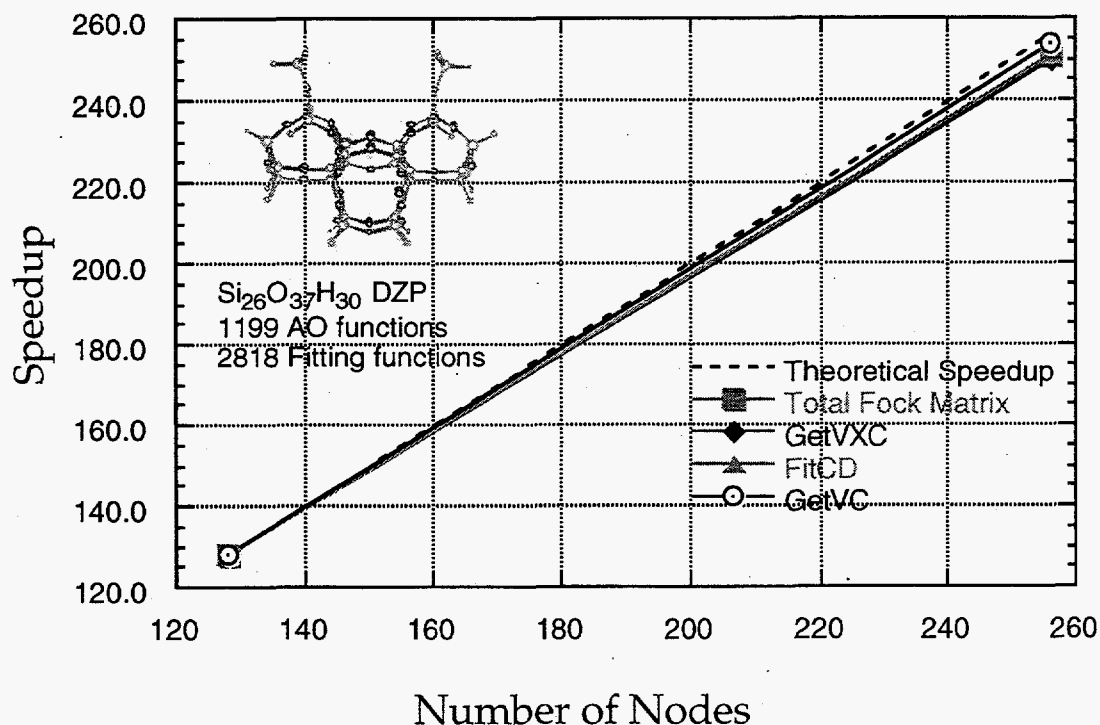


Figure 2: The overall scaling of the DFT prototype code (wall time versus number of atomic orbitals) on various zeolite fragments using 50 nodes of the KSR-2.

Intel Touchstone Delta The Intel machines (unlike the KSR-2 which has an ALLCACHE "shared memory" architecture) is truly a massively parallel distributed memory platform. In porting to the Touchstone Delta we encountered a few algorithmic flaws (with respect to data locality) which had to be corrected in order to obtain the excellent speedup as shown below for  $\text{Si}_{26}\text{O}_{37}\text{H}_{30}$  in Figure 3 which illustrates the ability of our implementation to function efficiently on distributed memory machines.



Actual Wall Times in Seconds				
Nodes	Total Fock Build	GetVXC	FitCD	GetVC
128	4171	2517	785	869
256	2123	1287	399	437

Figure 3: The speedup obtained for calculations of the Fock matrix and it's components for zeolite fragment  $\text{Si}_{26}\text{O}_{37}\text{H}_{30}$  on up to 256 nodes of the Intel Touchstone Delta.

#### Solid State Component

The following is a brief synopsis of the solid state formalism associated with the new Gaussian basis. The equations are currently implemented in a prototype code (prerelease) current activities center upon the development of a more general applicable tools which will be made available to industrial partners. The purpose of this effort is to develop a low order scaling ( $N \sim 2.5$ ) solid state and molecular method which can be used to investigate large complex systems. We achieve this

low order scaling by expanding both operators and charge densities in auxiliary Gaussian basis sets where appropriate. In previous studies we have shown that such expansions can be done without sacrificing chemical level accuracy for energy related terms. The use of an analytical representation for the charge distribution to obtain the total energy of 3- and 2-dimensionally periodic systems is unique in its formulation and is a highly effective way to obtain many ground state properties of the system of interest.

What follows below is a brief synopsis of the new solid state formulation. We use an orbital basis set of localized functions (contracted Gaussians) which form eigenfunctions obeying Bloch's theorem according to

$$\Psi_{kn}(\mathbf{r}) = \sum_i C_{kni} \sum_R e^{ik \cdot R} X_i(\mathbf{r} - \mathbf{R})$$

where  $\mathbf{R}$  is a Bravais lattice vector. The coefficients are found by iterative solution of an eigenvalue problem:

$$\sum_j H_{ij}(\mathbf{k}) c_{knj} = \epsilon_{kn} \sum_j S_{ij}(\mathbf{k}) c_{knj}$$

where  $S_{ij}(\mathbf{k})$  is an overlap matrix (the eigenvalues  $\epsilon_{kn}$  determine the band structure of the solid.) A new feature of our approach is the use of auxiliary basis functions  $h_p$  and  $G_q$  to fit the periodic electron density and exchange-correlation potential:

$$\tilde{V}_{XC}(\mathbf{r}) = \sum_R \sum_q b_q G_q(\mathbf{r} - \mathbf{R})$$

$$\tilde{\rho}(\mathbf{r}) = \sum_R \sum_p a_p h_p(\mathbf{r} - \mathbf{R})$$

In the periodic context this fitting leads to a Hamiltonian which is built from the formula

$$H_{ij}(\mathbf{k}) = \sum_R e^{ik \cdot R} [H_{ij}^{\text{kin}}(\mathbf{R}) + H_{ij}^{\text{XC}}(\mathbf{R}) + H_{ij}^{\text{coul}}(\mathbf{R})]$$

where

$$H_{ij}^{\text{XC}}(\mathbf{R}) = \sum_q b_q X_{ijq}(\mathbf{R})$$

$$H_{ij}^{\text{coul}}(\mathbf{R}) = \sum_p a_p C_{ijp}(\mathbf{R})$$

and

$$X_{ijq}(\mathbf{R}) = \int \mathbf{X}_i^*(\mathbf{r}) \mathbf{X}_j(\mathbf{r} - \mathbf{R}) \sum_{\mathbf{R}'} G_q(\mathbf{r} - \mathbf{R}') d^3 r$$

$$C_{ijp}(\mathbf{R}) = \int \mathbf{X}_i^*(\mathbf{r}_1) \mathbf{X}_j(\mathbf{r}_1 - \mathbf{R}) \frac{1}{|\mathbf{r}_1 - \mathbf{r}_2|} \sum_{\mathbf{R}'} h_p(\mathbf{r}_2 - \mathbf{R}') d^3 r_1 d^3 r_2$$

The calculation of  $X_{ijq}(\mathbf{R})$  is straightforward, but  $C_{ijp}(\mathbf{R})$  is difficult due to the long ranged nature of the Coulomb interaction. This difficulty is removed by including a portion of the nuclear charge in  $h_p$  so that  $\rho(\mathbf{r}, \tau)$  represents the total charge density, which has zero spatial average in a neutral system. Conditional convergence in the  $\mathbf{R}'$  sum in  $C_{ijp}(\mathbf{R})$  is then dealt with by expressing the long range multipole interactions in terms of the Ewald potential, which guarantees absolute convergence to the correct physical limit. The same technique solves similar convergence problems that occur in finding the charge fitting coefficients  $a_p$ .

Our general strategy for dealing with the above expressions is to construct the necessary matrix elements in direct space of the operators in the Hamiltonian by evaluating the necessary multi-center integrals (either numerically in the case of the exchange-correlation potential or analytically for the other operators). The resulting matrix representation of the Hamiltonian is then diagonalized at various points in the irreducible portion of the first Brillouin zone with the intent of obtaining a representation of the density matrix. The resulting density matrix is then reassembled in direct space and used to construct the next cycle of matrix representations. This process is continued until convergence has been achieved in the total energy and or rms deviations in the density are acceptable. Our goal current goal for this DFT software consists of calculations with 1,000 atoms, 10,000 orbital functions, and up to 30,000 auxiliary functions using a variety of functionals (including gradient corrections).

#### Periodic Hartree-Fock Study of NaCa Zeolite-A

Zeolites are among the most industrially important compounds in use today. Naturally occurring as well as synthetic zeolites have applications in a wide variety of fields, including heterogeneous catalysis, chemical separation processes, gasoline refinement, and long-term storage of nuclear waste products. The materials are characterized by open-framework structures typically composed of  $\text{SiO}_4$  and  $\text{AlO}_4$  tetrahedra in conjunction with charge compensating cations. The  $\text{TO}_4$  tetrahedra (T=Si and Al) are interconnected by sharing corners to form 1-, 2-, or 3-dimensional channels throughout the material. The resulting system of channels allows these compounds to function as molecular sieves. In addition, the high internal surface area provides a large number of sites where processes such as ion exchange, adsorption and catalysis can take place.

Zeolite A is a widely used industrial zeolite. Its ion-exchange capability has made it a popular alternative to phosphate use in detergents. The framework of zeolite A is constructed from b-cages in which the T atoms form a truncated octahedron. The b-cages are joined via double 4-rings, a cubic arrangement of eight covalently bound  $\text{TO}_4$  tetrahedra. This produces large vacant



a-cages, interconnected by 8-ring channels, running in three dimensions throughout the crystal. The effective radius of the narrowest section of the channel is approximately  $4.1\text{\AA} \times 4.1\text{\AA}$ <sup>(21)</sup>. The typical zeolite A (Linde type 4A) composition is  $\text{Na}_{12}\text{Si}_{12}\text{Al}_{12}\text{O}_{48} \cdot 27\text{H}_2\text{O}$ <sup>(22)</sup>, making it one of the most aluminum-rich members of the family of zeolites. While an abundance of Si in the lattice assures stability under acidic conditions, the presence of aluminum has advantages as well -- specifically, it allows a higher mole fraction of intercalated ionic species and a resulting higher cation exchange capacity.

Because of its commercial applications, many studies have been made of zeolite A. It is known that the lattice does not degrade upon dehydration, and the ion-exchange capabilities can be manipulated, varying with the types of alkali metal or alkaline earth cations present. Although the cation exchange capacity of this material can be determined experimentally, the physical process of diffusion of the intercalated species is not well understood. For example, while sodium and calcium cations exchange readily in the presence of water vapor, no exchange occurs in the dehydrated crystal.

The objective of our study is to examine the electronic structure of zeolite A, and to estimate the binding energy of the cations to the lattice in the absence of water. We chose a structure with a chemical composition of  $\text{Na}_4\text{Ca}_4\text{Si}_{12}\text{Al}_{12}\text{O}_{48}$ , in which sodium and calcium cations were placed at what are termed site I positions, near the center of the 6-rings. The lattice constant is  $24.6\text{\AA}$ . We performed self-consistent-field (SCF) *ab initio* calculations using the program CRYSTAL, which implements the linear combination of atomic orbitals periodic Hartree-Fock (PHF) method<sup>(4,23)</sup>. The SCF energy was converged to  $10^{-7}$  hartrees, with the tolerances for the infinite Coulomb and exchange series set at  $s_c = t_M = 5$  and  $s_{ex} = p^{ex} = 5$  and  $p^{ex} = 11$ . We then calculated the physical properties of the system using the ground state wavefunction.

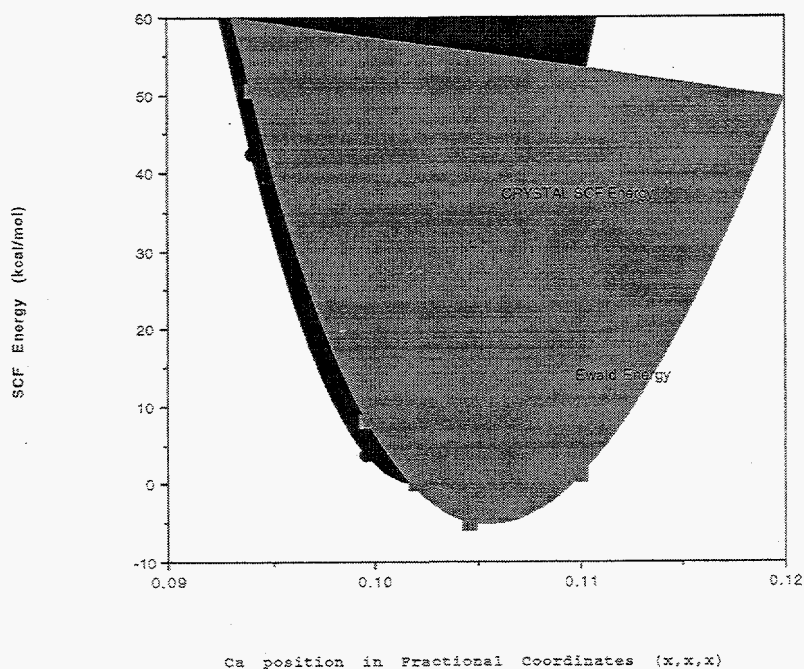


Figure 4 Energy of NaCaA as the calcium cations are moved through the crystal.

In the initial examination of the zeolite, termed NaCaA, we optimized the positions of the sodium and calcium cations by finding the minimum energy of the system as the cation moved within the fixed lattice. Figure 4 is a plot of the energy of the zeolite as a function of the position of  $\text{Ca}^{+2}$ . The energies are plotted relative to the minimum on the curve. Results indicate that the cations are situated slightly (less than 0.5 Å) above the 6-ring in site I, toward the center of the a-cage. This is in agreement with experimental observations of the dehydrated crystal. Using the Mulliken charges from each SCF calculation, the purely electrostatic contribution to the energy was calculated via the Ewald summation. As seen in Figure 1, the calcium cation is shifted further into the a-cage. The magnitude of the movement is minor, however. Although it was not possible to determine the binding energy of the cations in the lattice, we performed small cluster calculations to obtain an estimate. We based the cluster model on the coordinates of a 6-ring of NaCaA, terminated by hydrogens, giving  $\text{NaSi}_5\text{AlO}_6\text{H}_{12}$ . The small size of the cluster made it computationally tractable to investigate several basis sets. Interestingly, we found the binding energy to be very basis set dependent, with values ranging from about 75 to 175 kcal/mol. Preliminary calculations to determine the basis set superposition error show that the correction is only on the order of a few kcal/mol. Finally, the electrostatic potential and electric fields in the unit cell are currently being determined. We will also fit charges to the electrostatic potential and use these in molecular dynamics simulations of the behavior of intercalated species within the pores of the zeolite.

Investigations of bulk ZnO and the structure of the  $(1\sqrt{0}, \bar{1})10$  Surface & the Interaction of CO with this Surface.

We have used *ab initio* periodic Hartree-Fock (HF) all-electron total energy program CRYSTAL92<sup>(4)</sup> to compute the equilibrium atomic geometry of the  $(1\sqrt{0}, \bar{1})10$  surface of ZnO. The Gaussian atomic orbital set is the same one that was carefully optimized in a recent study<sup>(18)</sup> of bulk ZnO using CRYSTAL. That basis set has here been taken over without change, since reoptimizing the outer exponents on the surface atoms resulted in only very slight changes in the Gaussian exponents and total energy. Computational conditions were slightly less stringent than used in the earlier study but were adequate for the level of accuracy needed.

Our model of the semi-infinite solid bounded by the  $(1\sqrt{0}, \bar{1})10$  surface is a slab that is periodic in two dimensions and is terminated on both top and bottom by the same physical  $(1\sqrt{0}, \bar{1})10$  surface. This provides a tractable problem while avoiding the unphysical features of molecular or small cluster calculations (extra dangling bonds beyond those belonging to the surface in question, or artificial added hydrogens or other atoms to saturate those extra bonds) or three dimensional repeated slab calculations (where, especially with plane wave basis sets, much effort is required to make sure the vacuum gap is large enough to effectively isolate the layers without causing computational problems.) However, we still have to make sure that the slab itself is thick enough that the interaction between the two surfaces has negligible effects on the properties of each surface. We therefore began our study with total energy calculations of unreconstructed (truncated bulk) slabs containing two, four, and six atomic layers respectively. (Each layer contains an equal number of Zn and O atoms.) The bulk geometry was that which minimized the total energy in our earlier study of bulk ZnO. We then determined the surface energy per surface atom by comparing the energies of the various slabs to the bulk total energy calculated at the same level of precision. We find that the surface energy per surface atom is 0.82, 0.91, and 0.92 eV for the

two, four, and six-layer slabs respectively. We see that this energy appears to be already converging to its infinite-thickness limit at the four-layer thickness, at least to within the numerical uncertainty of our calculation which we estimate at 0.02 eV per atom based on convergence tests on bulk ZnO. The four-layer slab, which we use hereafter, is the thinnest one that has distinct surface and interior atoms; since we are relaxing the positions of only the surface atoms, the interior atoms maintain the bulk periodicity in the directions parallel to the slab.

We began our structure optimization in the rigid bond length model, in which nearest-neighbor distances remain constant, as suggested by the TB studies<sup>(19)</sup>. In this approximation there is only one free coordinate for relaxation of the surface layer which we take to be the tilt angle  $q$ , so that only a limited number of energy points needed to be computed. After minimizing the energy with respect to the tilt angle, we found that the surface dimers tilt by an angle of only 2.58° (positive angles signify that the anion, in this case oxygen, is furthest out from the slab). Also, the surface energy per surface atom was reduced by only 0.02 eV. This prediction of a nearly unrelaxed surface in our constant-bond length calculation suggests that the conservation of tetrahedral bond lengths is too severe a constraint to capture the essentials of the structural relaxation in this system.

Accordingly, we have recalculated the minimum-energy geometry for a general relaxation of the outer layers (top and bottom) of our four-layer slab. This involved self-consistently optimizing four coordinates ( $x_{\text{Zn}}$ ,  $x_{\text{O}}$ ,  $z_{\text{Zn}}$  and  $z_{\text{O}}$ ) by performing line minimizations on each coordinate in turn, going back to the first and continuing the process until a stable geometry was reached. The final values were  $x_{\text{Zn}} = 0.707 \text{ \AA}$ ,  $x_{\text{O}} = 2.581 \text{ \AA}$ ,  $z_{\text{Zn}} = 0.691 \text{ \AA}$ , and  $z_{\text{O}} = 0.766 \text{ \AA}$ . The tilt angle of the surface ZnO dimer changes very little under this full relaxation; we now find the angle to be 2.31°. However, the surface bonds shorten from a bulk value of 2.021 Å to 1.876 Å, and the geometric center of the bond moves towards the center of the slab by 0.221 Å reflecting the shortening of both surface and "backbonds" (the latter term meaning the bonds between surface atoms and the next layer). The bond centers are predicted to shift only a short distance parallel to the slab, in agreement with experiment. The energy cost of creating the surface is reduced to 0.72 eV per surface atom, which is 0.19 eV per atom less than for the unrelaxed surface. Tight-binding studies<sup>(19)</sup> have given quite different results, with only slight changes in bond lengths and a large surface dimer tilt (~18°) and consequentially a large shift along the  $c$  axis. The tight-binding results have been interpreted as a change in the surface cation's coordination from tetrahedral  $sp^3$  to approximately planar  $sp^2$  hybridization, accompanied by charge transfer to the surface anion which adopts a distorted  $p^3$  configuration, but our results are in closer accord with a qualitative description in which a double bond forms between the surface anion and cation functional (since exchange was already included at the HF level) derived from the Generalized Gradient Approximation. We recalculated the equilibrium structure of bulk wurtzite ZnO with correlation included in the total energy, then proceeded to reoptimize the surface geometry, starting from a suitably scaled version of the previous HF energy minimum. A stable equilibrium geometry was found in which the surface dimers were tilted by 2.48° and the surface bonds were 1.839 Å in length versus 1.967 Å in the unrelaxed case. At our calculation's level of accuracy, these results for the surface relaxation are essentially identical to those of the uncorrected HF theory. Thus, *ab initio* periodic HF theory predicts a relaxation of the  $(1\bar{1}0(-,0)10)$  surface of wurtzite ZnO

Finally, we have recently completed a study of the orientation of the CO molecule on the ZnO (100) surface. There is very little experimental information with this level of detail on insulating systems and it is a unique opportunity to evaluate the accuracy of the periodic Hartree-Fock theory. Experimentally, Solomon, et al, observe the molecule to bind carbon end down over the Zn atoms rotated off from the surface normal by an angle of 30°. Our calculations predict the this tilt angle to be 32° with a bond distance of 2.6 Å, in excellent agreement with experiment.

### Adsorption of Substituted Amines on Models of Zeolite Acid Sites

Zeolites are important industrial catalysts with both Brønsted and Lewis acid sites on their internal and external surfaces. The Brønsted acidity is due to acidic protons on bridging hydroxyl groups located within the zeolite framework. The Brønsted acid sites are characterized by their tendency to protonate various bases, such as ammonia and pyridine. There is considerable controversy regarding the nature of the complexes formed by the interaction of the adsorbed bases with the acid sites. The adsorption complex could involve only weak physisorption, which, in the case of ammonia, we can represent as  $\text{Zeolite-OH}\cdots\text{NH}_3$ . Alternately, if the zeolite has sufficient acidity, adsorption could lead to the formation of a zwitterion or contact ion pair:  $\text{Zeolite-O}^{(-)}\cdots\text{H-NH}_3^{(+)}$ . Results of spectroscopic measurements tend to favor the existence of the zwitterionic form. We have studied the adsorption mechanism of a variety of amines on models of zeolite acid sites. We present results for the adsorption of  $\text{CH}_3\text{NH}_2$  on  $\text{H}_3\text{Si-OH-AlH}_3$ . The ab initio molecular orbital calculations were done at both the RHF and MP2 levels using a pseudopotential basis set of 6-31G\* quality. We found that there was very little difference in the optimized geometries of various possible adsorbed states, except for the position of the proton on the bridging hydroxyl. Thus, we use the O-H distance to define an adsorption "coordinate". For all the configurations, we performed full gradient optimizations, holding only the O-H distance fixed.

There are several interesting aspects of the results. As shown in Figure 5, there is a substantial difference between the RHF and MP2 energies and geometries. The RHF energy of the physisorbed state is stabilized by 13.9 kcal/mol relative to infinite separation of the zeolite and adsorbate. This difference is increased to 20.8 kcal/mol when electron correlation is included. The RHF calculation predicts that the zwitterion is less stable than the physisorbed state, while there is no difference at MP2. The 5.1 kcal/mol barrier between the neutral and zwitterion at the RHF level is reduced to only 0.4 kcal/mol at MP2. Thus, the higher level of theory predicts that there is rapid equilibrium between the two states. Both levels of theory indicate a significant transfer of the hydroxyl proton to the base in the physisorbed state; the O-H distance is increased by  $\approx 0.1$  Å from its value in the isolated zeolite cluster. While the O-H distance varies by  $\approx 0.3$  Å between the physisorbed and zwitterionic states, there is almost no difference in the O-N distance. Lastly, note the importance of performing optimization at the correlated level; the calculation of MP2 energies at the RHF geometries would give an inaccurate description of the process. We are currently doing calculations using larger clusters to represent the zeolite in order to determine the effect of increasing numbers of shells of atoms on the acidity of zeolite model.

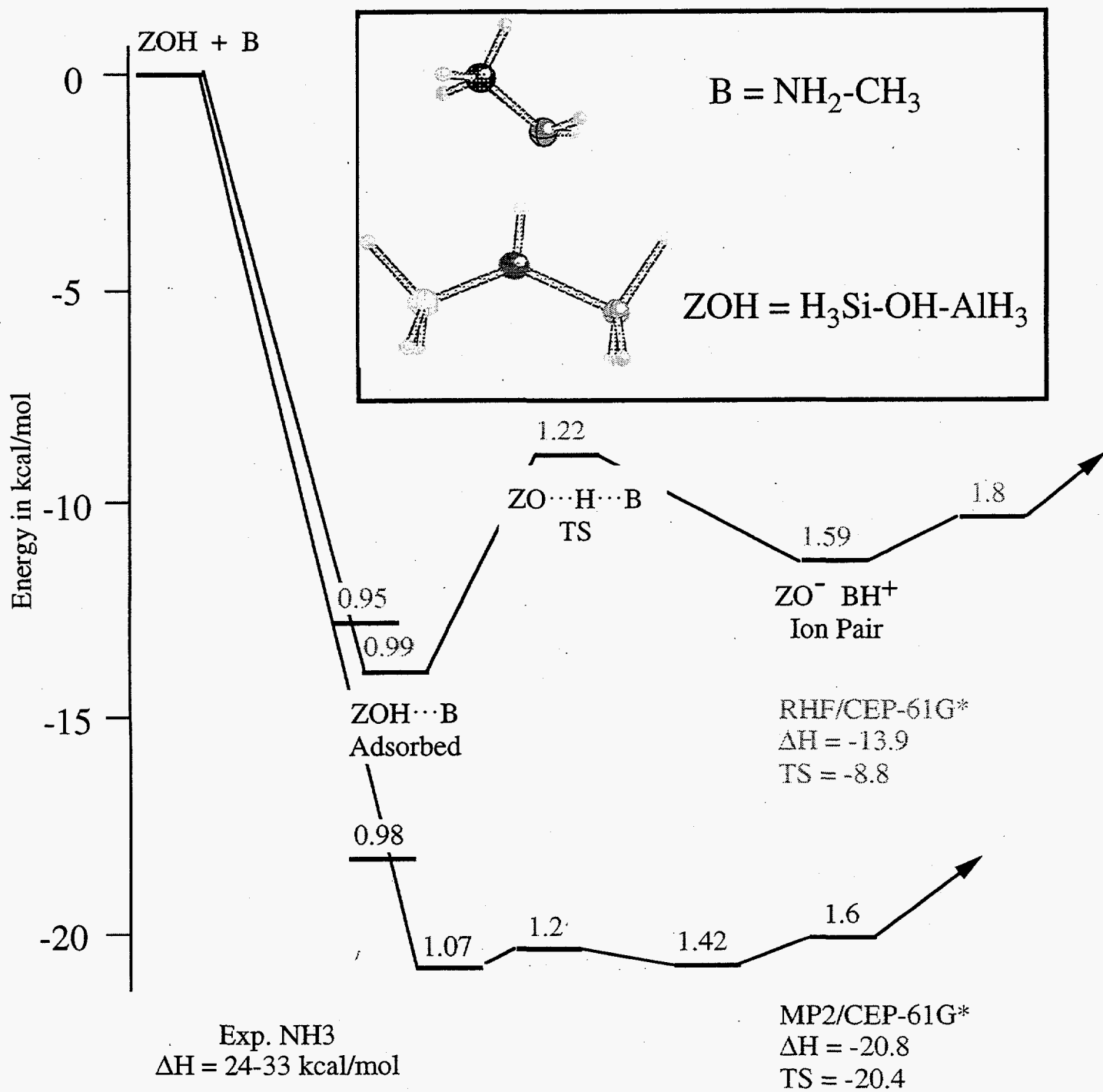


Figure 5: Potential energy levels for adsorption on a zeolite acid site using a cluster model. Reaction of interest  $\text{ZOH} + \text{B} \leftrightarrow \text{ZO}^- + \text{HB}^+$

## Molecular Dynamics Simulation of Transport in the Shape-Selective Cracking of Isomeric Hexenes in ZSM-5 Zeolites

(a) Argonne National Laboratory

Mass-transport molecular shape-selective catalysis arises when there exist large differences in the diffusivities of the molecules in the intracrystalline zeolite channels due to the variation in their effective sizes. On the basis of the crystallite size dependence of the reaction rates, Haag et al.<sup>(29)</sup> established that the cracking of the isomeric hexenes in ZSM-5 zeolite at 811 K exhibits mass transport molecular shape-selectivity. Reliable, direct measurements of molecular diffusivities under these (reaction) conditions are not feasible. However, we have shown that molecular dynamics (MD) computer simulation methods applied to hydrocarbon diffusion in zeolites have evolved to the degree that reliable determinations of these diffusivities can be obtained. The use of advanced concurrent supercomputing MD codes on the massively parallel Intel Delta computer permits the large size-scale and long time-scale simulation required to address these problems. These simulations allow the treatment of ensembles containing in excess of 100 hydrocarbon molecules at realistic loadings in the molecular sieve, with full inclusion of the framework dynamics and all degrees of freedom for the adsorbate molecules. The success of the force fields developed for both the (aluminum-free) silica framework and the adsorbed hydrocarbons has been firmly established in the previous work.<sup>(30,31)</sup>

Haag et al.<sup>(29)</sup> have used cracking reaction rate data on H-ZSM-5 zeolite to deduce the diffusivities at 811 K of the following series of isomeric hexenes (Table 1): Under the reaction conditions, the loading of the zeolite is very low. The average distance between the adsorbed hexene molecules at a pressure of 1 atm is estimated to be 400 Å in reference 1. Hence, we performed infinite dilution MD simulations with 108 non-interacting molecules in 27 MFI unit cells to provide reliable determinations of the diffusivities of these three isomers of hexene. Thus, there was no need for the simplifying assumptions about the topology of the diffusional process that the interpretation of the experimental data required. Since hydrocarbon diffusivities in ZSM-5 zeolite are unaffected by variation in its aluminum content, (aluminum-free) silicalite is the material of choice for the simulation.

Calculations in progress.

### Comparison of H<sub>2</sub> interactions with MgO and Li-doped MgO: Energy Decomposition and Population Analysis

MgO is as well known catalyst for a variety of organic reactions for which the rate limiting step requires the cleaving of a C--H or C--C bond. It is known that the catalytic rate of such reactions involving lithium-doped MgO (Li/MgO) can be two or three times as great as undoped MgO.<sup>(16)</sup> We employed (MgO)<sub>n</sub> and LiMg<sub>(n-1)</sub>O<sub>n</sub> clusters (n=4,6), and H<sub>2</sub> and CH<sub>4</sub> probe molecule to model molecular interactions with reactive surface sites of MgO and Li/MgO, (see Figure 6 for pictorial description of n=4 clusters). Although the H--H and C--H bond is readily cleaved on low-coordinated sites of such clusters, we found that the interactions of H<sub>2</sub> with Li/MgO were dramatically different compared to interactions with MgO. Specifically, on MgO H<sub>2</sub> was found to dissociate via heterolytic chemisorption on adjacent low-coordinated sites, but on Li/MgO we

observed that the reaction pathway involved the homolytic abstraction of a H atom from either H<sub>2</sub> or CH<sub>4</sub> with one hydrogen atom bound to a low-coordinated oxygen site and the partner hydrogen atom or methyl radical released into the gas phase, (see Figure 6). For MgO the active sites for chemisorption had to be no greater than three-coordinated. For Li/MgO we found that even four-coordinated sites supported the hydrogen atom abstraction reaction. Larger clusters will need to be employed to determine if Li/MgO can support abstraction at five-coordinated sites. In summary, the reason that Li/MgO is a more reactive catalyst than MgO is because it releases reactive intermediates directly into the gas phase, and it supports more reaction channels for H--H and C--H bond dissociation.

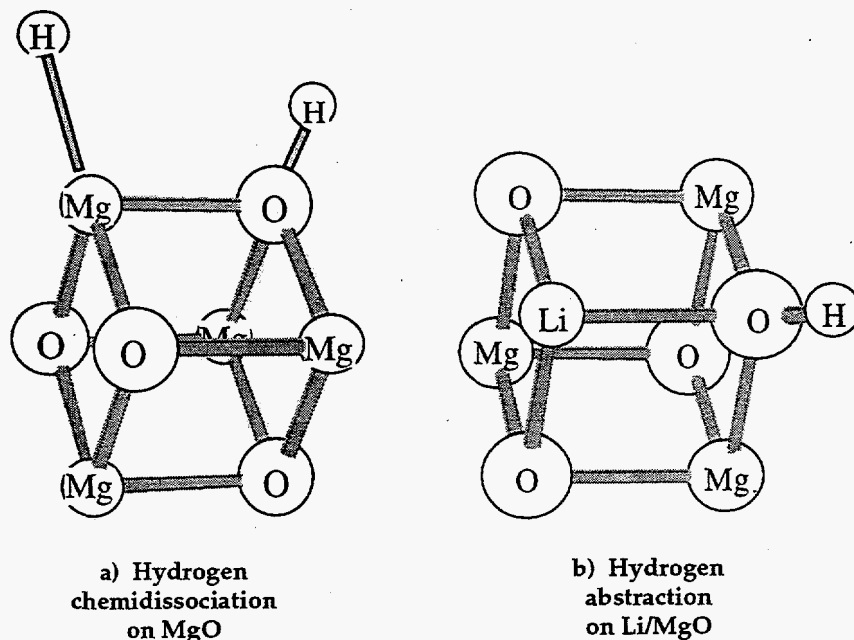


Figure 6: H<sub>2</sub> interactions with MgO and Li doped MgO clusters.

From the previous and other related studies there are a variety of observed phenomenon that our amenable to further explication by the Natural Bond Orbital and Natural Energy Decomposition analyses of Weinhold, Glendening, Reed and Carpenter.<sup>2</sup> The phenomena we chose to investigate are a) the propensity for H-H and C-H bond dissociation to occur at low-coordinated rather than high-coordinated sites, b) an observed cooperative effect when more than one chemisorptive or abstraction event occurs on neighboring catalytic sites, c) the absence of large transition state barriers in Li/MgO that appear in MgO, and d) the support of homolytic hydrogen atom abstraction on Li/MgO rather than heterolytic chemisorption that appears in MgO. We found that phenomena a) and b) are readily explained via purely electrostatic considerations. Briefly, the action of hydrogen atom adsorption at oxygen or magnesium catalytic sites changes that site from a doubly charged O<sup>-2</sup> or Mg<sup>+2</sup> to a singly charged OH<sup>-</sup> or MgH<sup>+</sup>. The penalty in units of electrostatic energy for replacing a -2 site with a -1 site in a sea of  $\pm 2$  point charges is reduced as the replacement-site coordination is reduced. Further, if MgH<sup>+</sup> or OH<sup>-</sup> are already present in the cluster, the penalty for additional singly charged sites is reduced. These facts explain the preference for abstraction and chemisorption to occur at low-coordinated sites and the observed

cooperativity effect. The explanation of phenomena c) and d) were found to depend on difference in the electron charge transfer in MgO and Li/MgO systems. In MgO, the orbitals of Mg and O are filled and the breaking of the H-H bond occurs when electron density on filled MgO orbitals is transferred to antibonding orbitals in H<sub>2</sub>. In Li/MgO there exists a half-filled O lone pair capable of accepting an electron from the H<sub>2</sub> bonding orbital. This difference in the charge transfer mechanism, and the orientation of the H<sub>2</sub> molecule necessary to achieve maximum overlap to support these mechanisms, explains the difference in the reaction pathways observed for MgO and Li/MgO.

**2. PROJECT TITLE:** Design, Characterization, and Testing of Multimetallic Catalysts for Pollution Control

**PRINCIPAL INVESTIGATORS:** T. S. King, A. E. DePristo

**PROJECT SITE:** Ames Laboratory

### **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, NO<sub>x</sub>, 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 NO<sub>x</sub>. 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 NO<sub>x</sub> 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.

NO<sub>x</sub> must be removed by reduction to nitrogen gas. The most active catalyst is Pt, but some NO<sub>x</sub> are reduced to ammonia, which is not desired. The most effective catalyst for the reduction of NO<sub>x</sub> 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.

#### **1994 ACCOMPLISHMENTS:**

Small metal clusters have been shown to have compact shapes [1,2]. An outstanding question about application of the entire range of methods in DeCal has been the compact shape of metal clusters. The above accomplishment validated the DeCal methodology. DeCal software parameters have been determined. A comprehensive data set has been produced for the simulation package of coordination-dependent site [2] and mixing energies [3]. The CEMBOS ("chem boss") model has been extended to accurately describe the variation of bond energy in bimetallics as a function of coordination [3]. A "seamless integration" of CEM, MD/MC-CEM and BOS codes has been produced. This effort is the first step in producing a user friendly software package. Further steps will be accomplished in FY 1995 and FY96, leading to software release in September 1996.

The surface composition of adsorbate-free supported Pt-Rh catalysts has been determined using <sup>129</sup>Xe NMR. We have demonstrated the utility of using <sup>129</sup>Xe NMR to determine the surface composition of clean, supported Pt-Rh catalysts [4]. Furthermore, because of the similarities of the surface (site) energies of Pt and Rh in the Pt-Rh system, <sup>1</sup>H NMR was used to experimentally determine the effect of an adsorbate [5]. The experimental results for both the adsorbate-free and the hydrogen covered Pt-Rh catalysts agreed well with the theoretical results from the CEMBOS model.

## 1995 PLANNED ACTIVITIES:

During this period work will be performed leading to full implementation of the chemisorption simulation capacities including the site dependent energetics of adsorption. We will begin with a simple adsorbate such as hydrogen for which the CEM method is quite accurate. This will enable us to parametrize the partial bond energy model and also to test the MD/MC-CEM method. Either of the faster methods will be used to investigate structures of H/Pt-Rh clusters. More complex adsorbates such as oxygen will be parametrized using self-consistent field non-local density functional calculations performed in cooperation with Dr. David Dixon, a DuPont industrial collaborator.

We will develop a graphical user interface, GUI, for application of the DeCal software and write user guidelines. This will come much closer to accomplishing one important goal, namely combination of three levels of modeling capability into one software package. The software, operating on a desktop workstation, will produce a simulation using the BOS model of CEMBOS that will be fast enough to use in real time testing of various combinations of metals. More computationally demanding MD/MC-CEM and full CEM simulation capabilities will be included in order to treat systems with metal atoms of various sizes. These are much more computationally demanding, especially the full CEM method. We will demonstrate the range of new scientific capabilities with a model reaction study.

In addition, the experimental validation studies coupled with standard catalytic characterization methods will continue. The focus of this work will be to assimilate the previous experience and extend it to the study of gaseous environments, support and promoter effects. This approach will serve to close the "gap" between understanding obtained by model studies and experience with commercial catalysts.

We plan to continue the experimental validation program and establish the viability of the  $^{129}\text{Xe}$  NMR approach for the determination of surface composition for systems other than clean Pt-Rh particles. In addition, the determination of the surface composition of an oxygen-covered supported bimetallic particle will be determined via  $^{129}\text{Xe}$  NMR. Several important advances in the ability to simulate and predict catalysts under operating conditions can be made. Clearly, the influence of adsorbates resulting from chemisorption of reactants and products is an important factor in catalyst surface properties. The surface perturbs adsorbing species in a manner that causes them to react, and the adsorbate changes the surface properties of the catalyst. Simulations are difficult, especially for adsorbates such as oxygen, due to the level of sophistication needed in the theoretical procedures to model highly directional bonding and the transition from covalent to ionic bonding.

The energetic effects of chemisorption must be determined for input into the BOS model. The microcalorimetric studies (currently underway) will serve as an experimental basis for testing the initial input parameters determined from collaborative efforts with DuPont theoreticians.

## ANNUAL TECHNICAL SUMMARY REPORT:

During this year we have devoted considerable effort to increasing the range of applicability of the theoretical models 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  $^{129}\text{Xe}$  and  $^1\text{H}$  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 partial bond energy model with the CEM method using actual simulations on model clusters instead of on extended surface planes. This has been accomplished for the metals Ni, Cu, Rh, Pd, Ag, Ir, Pt and Au. The results of partial bond energy simulations are in much better agreement with those of the full CEM values on small clusters than when the parameters were determined from extended surfaces.

We have developed the needed extension of the BOS model to treat coordination dependent bonding in bimetallics. The site energy for center atom of A-type surrounded by N of B-type and Z-N of A-type can be written explicitly as:

(1)

$$\epsilon_{Z, N \text{ of } B}^A = \epsilon_Z^A + N \Delta E_{Z, A-B}^A + \frac{N(N-1)}{2} \lambda_{Z, A-B}^A$$

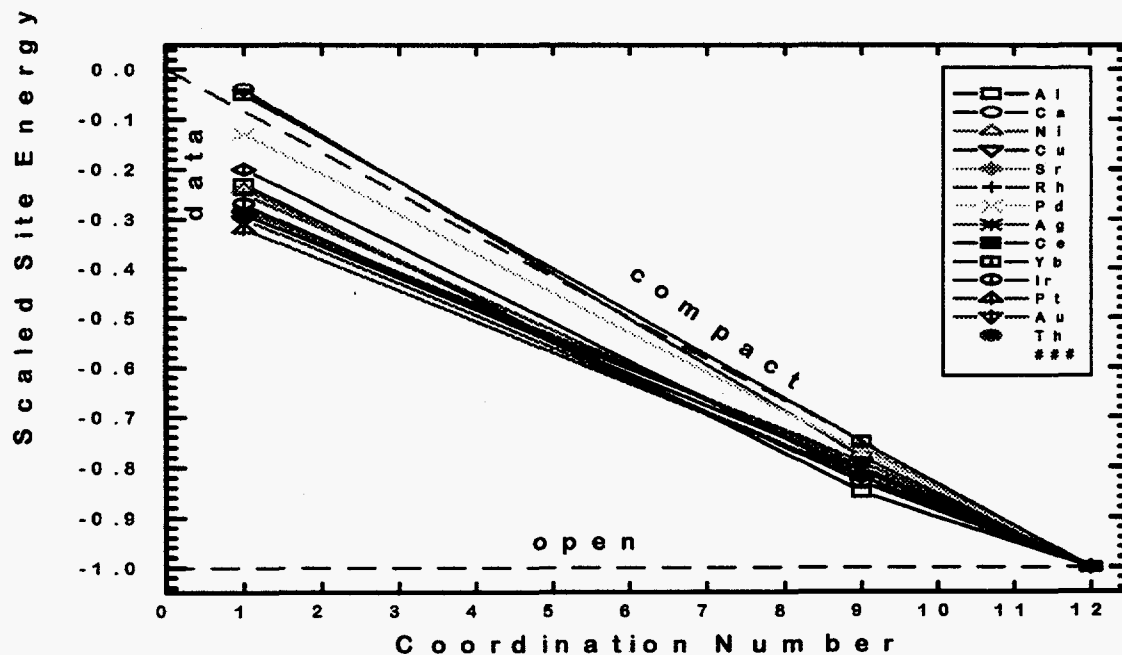
where is the 'bond energy change' during the first exchange and, the incremental change. This equation allows for accurate treatment of the asymmetric mixing energy for bulk systems as a function of composition. An analogous expression for a B type atom is:

(2)

$$\epsilon_{Z, N \text{ of } A}^B = \epsilon_Z^B + N \Delta E_{Z, A-B}^B + \frac{N(N-1)}{2} \lambda_{Z, A-B}^B$$

The new parameters have been determined for all combinations of Ni, Cu, Rh, Pd, Ag, Pt and Au.

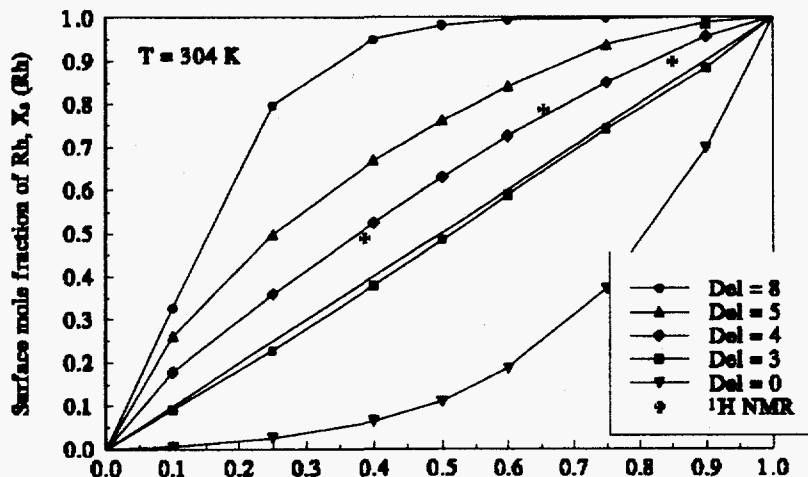
We have also shown that clusters are compact. We used experimental data on the dimer binding energy, the surface energy and the bulk cohesive energy to determine site energies for all 15 fcc metals: Al, Ca, Ni, Cu, Sr, Rh, Pd, Ag, Ce, Yb, Ir, Pt, Au, Pb, and Th. The results are shown for the scaled site energies. Elements for which some data is unavailable are shown as solid symbols.



The two extreme limits are: 1) the site energy is directly proportional to the coordination number; and, 2) the site energy is a constant. In the former case, each metal-metal bond has the same energy irrespective of the number of metal-metal bonds, producing 'compact' structures (as labeled). In the latter case, the metal-metal bond weakens inversely proportional to the number of neighbors (i.e., the total bond strength of any atom is constant), producing an 'open' and fluxional structure since each atom has the same energy irrespective of the number of neighbors, and is so labeled in the figure. Experimental data lies very close to the 'compact' line, indicating real fcc metal clusters will have relatively compact structures and thus providing firm validation for application of the CEMBOS model.

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.

## Alumina supported Pt-Rh catalysts BOS validation via $^1\text{H}$ NMR



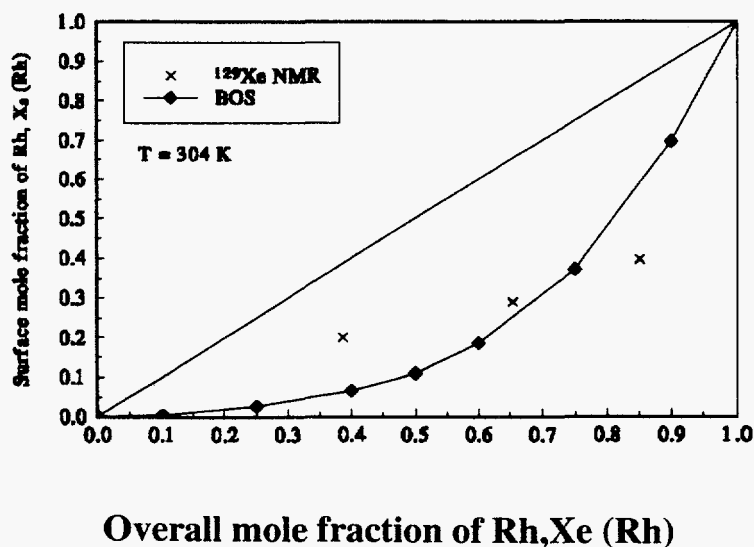
### Overall mole fraction of Rh, $X_g$ (Rh)

We have successfully employed  $^1\text{H}$  NMR to determine the surface composition of supported Pt-Rh bimetallic catalysts. Note that the surface site energies of Pt and Rh are similar and the difference in the energy of adsorption of hydrogen on these metals is sufficient to alter the surface composition. The results from the BOS simulation of supported Pt-Rh catalysts assuming various differences in the heat of adsorption of hydrogen are compared with the  $^1\text{H}$  NMR results in the following figure. Whereas the adsorbate free-surface is enriched in Pt, the hydrogen saturated bimetallic particles are enriched in Rh. The experimental results agree with the prediction when a difference in the heat of adsorption of about 4 kcal/mol is used in the BOS model, a value consistent with published data.

The fact that adsorbate probes such as hydrogen perturb the surface composition of bimetallic particles led us to pursue other experimental methods of surface composition determination. We have initiated studies using  $^{129}\text{Xe}$  as a probe of surface composition for two reasons: 1) it binds only weakly (physical adsorption) to metal surfaces and therefore will not greatly influence the relative surface composition; and 2) it has a large number of easily polarized electrons that produce large shifts in the NMR experiment. Even though  $^{129}\text{Xe}$  NMR has been used for surface characterization of zeolites, for example, it has not been used for the purposes intended in this program.

In the following figure is the surface composition determined by  $^{129}\text{Xe}$  NMR of the same Pt-Rh bimetallic catalysts as investigated via  $^1\text{H}$  NMR noted above. Also shown is the comparison to the BOS model results for the bimetallic with no adsorbates (i.e., no difference in energetic interaction of an adsorbate with Pt and Rh). Within the uncertainties of the experiment the agreement with the prediction is quite good.

**Alumina supported Pt-Rh catalysts  
BOS validation via  $^{129}\text{Xe}$  NMR**



3. **PROJECT TITLE:** Hydrocarbon Catalysis on Zeolites and Metal Oxides

**PRINCIPAL INVESTIGATORS:** P. J. Hay and A. 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.

#### **1994 ACCOMPLISHMENTS:**

**Zeolite catalysis.** Modeling capabilities to describe the chemistry of hydrocarbon cracking processes in zeolites are being developed using various levels of theory. The goal of these activities is to aid in the modeling of fluid catalytic cracking (FCC), the largest industrial catalytic process that converts gas oil to gasoline, diesel fuel and other products. The thermochemistry of  $C_1 - C_6$  hydrocarbon species including neutral molecules and positive ions has been systematically examined using semi-empirical techniques. More detailed studies of  $C_4$  and  $C_5$  species have been carried out using *ab initio* methods. Processes leading to fragmentation of saturated and unsaturated hydrocarbons upon protonation have been investigated using  $C_5H_{13}^+$  and  $C_5H_{11}^+$ , respectively, as prototypes for paraffins and olefins.

Using finite cluster models to represent the zeolite, the binding of small hydrocarbons such as butene to the acid site has been studied and several intermediates have been found. In addition, the environmental factors affecting the binding of small molecules such as  $NH_3$  have been studied systematically by varying the electron donating and withdrawing properties of the acid site.

Coupling of semi-empirical quantum chemical methods with molecular dynamics has resulted in a quantum molecular dynamics (QMC) approach which permits studies of hydrocarbon dynamics and chemistry that does not require the development of classical force fields. For proton transfer reactions with hydrocarbons, fragmentation processes involving breaking of C-C and C-H bonds have been carried out on several species.

Partial oxidation of hydrocarbons. A combination of molecular mechanics and quantum chemistry are being used to investigate a catalytic cycle for partial oxidation of hydrocarbons (as in conversion of methane to methanol) with  $O_2$  using bimetallic metal compounds. The initial step

involves the reversible binding of O<sub>2</sub> to a compound with a M-M bond, where M stands for a metal atom, to yield a M-O-O-M species. The geometric features which aid in this step have been extensively explored using the universal force field (UFF). The succeeding steps in the cycle involve O-O scission and oxidation of C-H bonds and are currently being explored.

A series of molybdenum-bismuth oxides have been synthesized as candidates for studies of selective hydrocarbon oxidation catalysis such as propylene to propylene oxide using both conventional and electrochemical reactors. The ionic and electronic conductivities of these compounds have been investigated and the results have been used to optimize the relative compositions of the components for the appropriate partial oxidation reactions.

### **1995 PLANNED ACTIVITIES:**

**Zeolite catalysis.** We will continue our focus on acid site cracking of hydrocarbons involving proton transfer from the zeolite to the hydrocarbon which subsequently breaks into smaller fragments. The three phases of this project involve (1) benchmarking of quantum chemistry methods, both semi-empirical and ab initio, for treating the gas phase thermochemistry and reactions of hydrocarbons and their positive ions, (2) modeling of the hydrocarbon chemistry in the presence of the zeolite lattice, and (3) employing Monte Carlo reaction models for the overall cracking process. This work will be carried out in collaboration with W. R. Grace and Amoco Oil companies. In particular, the former collaboration should enable us to have access to experimental data on cracking processes with which to calibrate the overall model.

**Partial oxidation of hydrocarbons.** Modeling efforts will continue on the current bimetallic prototype systems as potential candidates for carrying out processes such as converting methane to methanol. Following on last year's study of O<sub>2</sub> addition to the metal centers, the next phase would involve studies of the reaction of the resulting metal oxide centers with C-H bonds using molecular mechanics and quantum chemical methods.

In collaboration with Arco Chemical we plan to carry out theoretical studies of metal-substituted zeolites (e.g., TS-1, which is a titanium substituted zeolitic material based on zeolite ZSM-5) in partial oxidation reactions. The researchers at Arco Chemical will carry out a parallel experimental effort funded with internal company funds.

In our next regular meeting with Arco Chemical researchers and our collaborators from the University of Houston, we plan to evaluate the potential of the electrochemical reactor experiments and their possible incorporation into commercial processes. At this point, a decision will be made on whether or not to continue this research approach and if so, to what extent.

### **ANNUAL TECHNICAL SUMMARY REPORT:**

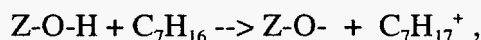
#### **1. Zeolite catalysis**

**a. Hydrocarbon cracking.** [A. Redondo, P. J. Hay, Y. Guo (LANL)] The major activity in zeolite catalysis involves examining the chemistry that occurs during hydrocarbon cracking in faujasite (zeolite Y). Currently, commercial fluidized catalytic cracking processes employ

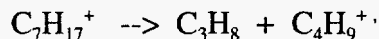


this catalyst to carry out cracking of hydrocarbon feedstocks in the refining industry. The goal of these activities is to develop, validate and implement computational models to describe the hydrocarbon chemistry in such catalysts and how this chemistry is influenced by modifications of the catalyst. Because of the complexity of this process, we are employing a combination of molecular level quantum chemistry approaches and more macroscopic Monte Carlo reaction modeling. The work is being carried out in collaboration with researchers from W. R. Grace Co. and Amoco Oil Company.

According to currently accepted mechanisms, the acid site in the zeolite serves to protonate either saturated (paraffins) or unsaturated (olefins) hydrocarbons. For example, in the case of the paraffin heptane, we have



where Z stands for the zeolite. These products subsequently crack into smaller fragments, e.g.,



which can undergo further reaction or eventually terminate the cycle by deprotonation of the positive ion to the zeolite leaving neutral hydrocarbons.

The study of these processes will eventually require three phases of modeling: gas phase thermochemistry and reactions, thermochemistry and reactions of hydrocarbons in zeolites, and Monte Carlo reaction and kinetics modeling of the overall cracking process. During the past year we have initiated activities in the first two phases as described below.

The first phase of our activity has been to benchmark semi-empirical and ab initio quantum chemistry techniques for typical gas phase hydrocarbon species typically encountered in cracking processes. To date essentially all the neutral and positive ion species from C<sub>1</sub> to C<sub>6</sub> hydrocarbons have been treated using MOPAC, from which heats of formation and geometries have been determined. These have been compared to existing experimental data and overall the errors in absolute heats of formation is  $\pm 10$  kcal/mole but, more importantly, only a few kcal/mole for relative errors for different forms of the same molecule (primary, secondary and tertiary cations of a C<sub>n</sub> species). For C<sub>5</sub> hydrocarbons these species are being examined in greater detail using ab initio techniques. While heats of formation are less accurate compared to experiment than MOPAC at the Hartree-Fock level, overall reaction energies for processes such as protonation, C-C bond-breaking and C-H bond-breaking reactions are reasonably well described. Estimation of barriers for processes are expected to be considerably more reliable using ab initio techniques.

The second phase involves the comparison of the gas phase thermochemistry and reaction energies with that of hydrocarbon species bound to acid sites in the zeolite using semi-empirical and first principles quantum chemistry techniques with cluster models of zeolites. Studies of butene (C<sub>4</sub>H<sub>10</sub>) at model acid sites have found several types of intermediates including hydrogen-bonded neutral species and a covalently bound alkoxide ion.

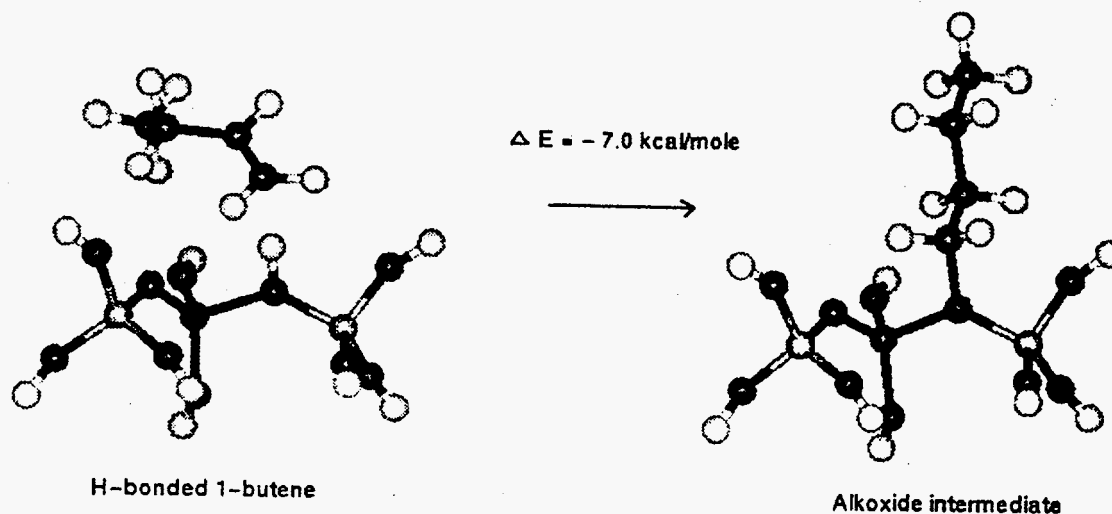


Figure 1

**b. Acid site properties.** [A. Redondo, P. J. Hay , P. MacDougall (LANL)] . One widely used method to measure acidity in zeolites involves the introduction and subsequent desorption of ammonia, pyridine and other bases--as measured by temperature-programmed desorption (TPD) or microcalorimetry experiments. This can often be correlated with other measures of acidity such as relative cracking rates of hydrocarbons. We have completed *ab initio* electronic structure studies of ammonia adsorbed at acid sites using small cluster models and assessing environmental factors that alter the acidity of the zeolite in specific ways.. In these studies, one issue concerns the relative stability of the hydrogen bonded form  $Z-O-H-NH_3$  and the protonated form  $Z-O-NH_4^+$  where Z denotes the remainder of the zeolite cluster and O denotes the Al-O-Si bridging O atom. When electron withdrawing groups are bound to the Si in the lattice, or when  $Na^+$  ions are located on the opposite pore walls, the acidity is increased as measured by the protonation of ammonia.

**c. Quantum molecular dynamics** [A. Redondo (LANL)]. We have developed a technique to carry out molecular dynamics calculations using semiempirical quantum mechanics to obtain the instantaneous forces on atoms in the system under study. This method avoids the need for predetermined potential energy functions among the atoms by calculating the forces from the solution of the Schrödinger equation at the instantaneous configuration of the molecules. The most important advantage of this technique over the standard molecular dynamics simulations is that we can now study the dynamics of chemical reactions as the bonds between atoms are broken and created. We have now used this technique in initial studies of hydrocarbon cracking.

**d. Hydrocarbon diffusion in zeolites.**[N. Henson, A. Cheetham (UCSB); A. Redondo]. Molecular dynamics techniques have been implemented for carrying out simulations of the motion of hydrocarbons in zeolite lattices. Initial calculations have been carried out on to

determine the diffusion of benzene in zeolite Y using existing literature potentials for the zeolite and adding benzene-zeolite interactions. Since  $\text{Na}^+$  ions are also present, we have also developed potentials for Na-benzene interactions from quantum mechanical calculations using the Möller-Plesset approximation. Our results indicate excellent agreement between the calculated adsorption sites for benzene in zeolite Y and the neutron scattering experiments carried out in Prof. Cheetham's group but point out the need to refine the current benzene-zeolite potentials to better reproduce the experimental diffusion and thermodynamic data.

## 2. Selective oxidation of hydrocarbons

**a. Mixed metal oxide catalysts for hydrocarbon oxidation** [Y. Yang, T. H. Lee, A. Jacobson (U. of Houston)]. The aim of this project is enhance the selectivity of chemical processes leading to the partial oxidation of hydrocarbons. We have focused on metals oxides with the general formula  $\text{Bi}_2\text{O}_2(\text{V}_{1-x}\text{Mn}_x)\text{O}_{3.5-2x}$ . They show excellent polycrystalline properties and high ionic conductivities when compared to other ionic conductors, such as  $(\text{Bi,Er})_2\text{O}_3$ . Their catalytic properties are currently under study. We plan to evaluate them at our next regular meeting with our University of Houston and Arco Chemical collaborators and a decision will be made at that point regarding their commercial viability.

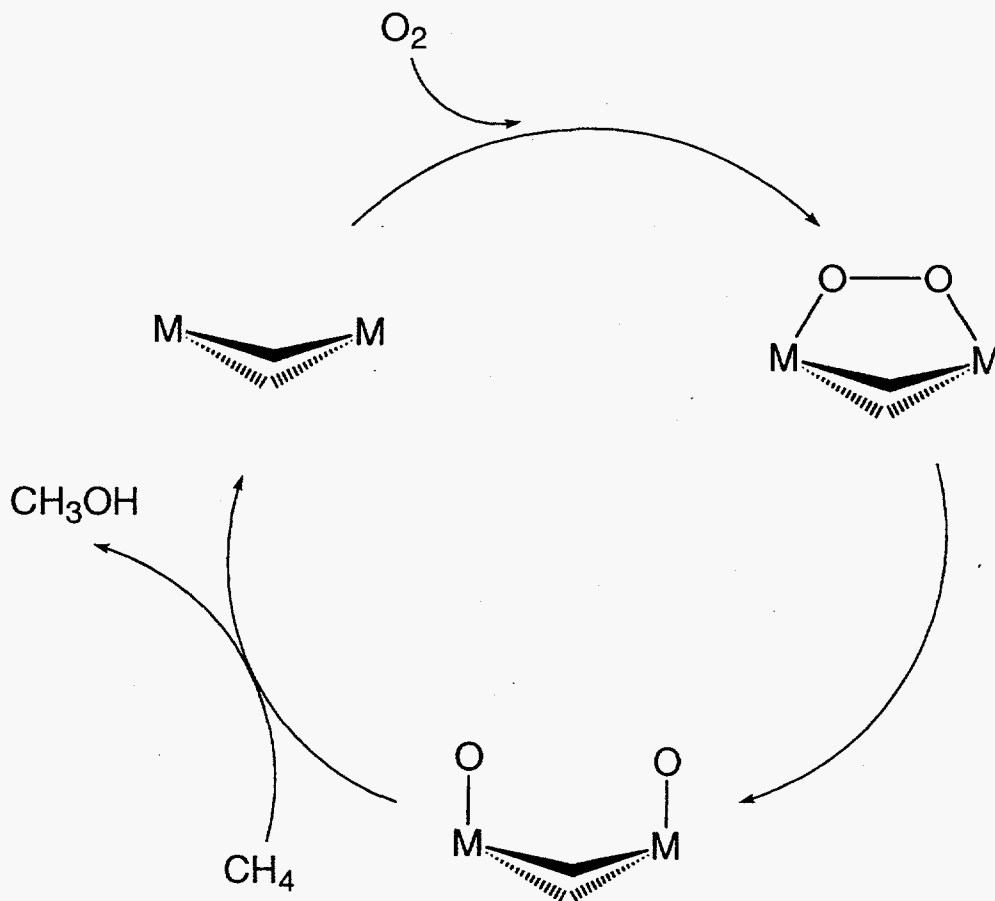


Figure 2

**b. Modeling of selective oxidation of C-H bonds** [A. K. Rappe, Colo. State Univ.] The selective oxidation of organic substrates by oxygen wherein all four oxidizing equivalents of  $O_2$  are used productively remains an unsolved problem in catalysis. Even nature does not provide a solution to this problem, enzymes such as methane monooxygenase and cytochrome  $P_{450}$  waste one of the oxygen atoms of  $O_2$  by generating an equivalent of water for each equivalent of substrate oxidized. An idealized catalytic cycle that could solve this problem has been developed in collaboration with Sergio Gorun at Exxon Research and Engineering. The first step of this catalytic cycle (see Fig. 2) involves the reversible binding of  $O_2$ . A correlation of M-M distance with M-O-O-M dihedral angle has been developed which should aid in the design of metal complexes or the choice of metal oxide surfaces for the binding and activation of  $O_2$ . The theoretical methodology used (the UFF force field) was benchmarked on a triply bridged, octahedral Mn  $\mu$ -peroxo complex  $[(1,4,7\text{-triazacyclononane})_2Mn_2(O)_2(O_2)](ClO_4)$  (as well as numerous metal complexes in the published UFF papers). The UFF force field was then used to understand the relationship between M-O-O-M dihedral angle and other molecular structural coordinates. A strong correlation between M-O-O-M angle and M-M distance was found.

The next step in the catalytic cycle is the cleavage of the  $\mu$ -peroxo bond into two terminal M=O bonds. Molecular mechanics will be used to see if sufficient strain can be introduced into the O-O bond (by the choice of the other bridging ligands) to facilitate bond cleavage. Ab initio electronic structure techniques will be used (employing the MESA electronic structure codes) to test the molecular mechanics results and to study the reactivity of M=O bonds with methane. Calculations at the Hartree-Fock level tend to underestimate M=O bonds rather dramatically (approx. 0.06 Å). In order to reliably calculate the transition states for the reaction of methane with M=O bonds, electron correlation needs to be included explicitly. We are currently comparing results of Complete-Active-Space CAS (6 electrons/6 orbitals) calculations to describe the M=O bonds with less expensive pairwise CAS calculations where the bonds are described as the product of three 2 electron/2 orbital CAS descriptions. We wish to see if the pairwise CAS results are adequate for the structures of  $Cl_2TiO$ ,  $Cl_3VO$ , and  $Cl_2CrO_2$ . For  $Cl_2TiO$  we can also compare the full CAS results with the pairwise results for the reaction with  $H_2$  to see if the pairwise scheme is appropriate for transition states. Following the validation of the technology,  $Cl_3VO$  will be reacted with methane and the O-O bond of a triply bridged model vanadium dimer, containing two oxo and one O-O linkages, will be cleaved as well as reacted with methane.

### 3. Generic enabling technologies in catalysis.

**a. Positron studies in catalysis research** [D. Gidley, W. Frieze (U. Michigan)] The use of positron microscopy as a tool in characterizing catalysts has been investigated over the course of the past year as part of a new initiative commissioned by the BCTR program to aid in the development already underway at the University of Michigan of a second-generation positron microscope. The project is nearly completed with data collection expected by the end of the year. All components of the microscope have been designed and fabricated, and assembly process is underway. Alignment and testing of the beam will occur during the next stage.

In an application to zeolites, positron lifetime spectroscopy was used to determine pore diameters in zeolites H-Y, 3A, ZSM-5 ( $NH_4$  and H forms) and 4A. Only after heat treatment

did the deduced diameter agree with the value specified by the manufacturer. Not only is this an important calibration of the lifetime technique, but it also suggests that there is sensitivity to adsorbates on the pore surfaces. Literature reports have claimed that positron lifetime spectroscopy is sensitive to specific surface oxygenation, and these results suggest that there is sensitivity to adsorbates on the pore surfaces of the zeolites.

Other experiments to calibrate the use of these techniques to characterize materials have included (1) depth profiled Doppler-broadened spectroscopy (DBS) of positron annihilation radiation in the investigation of epitaxial cobalt silicide films grown thermally on Si substrates in situ and (2) three dimension imaging of positron lifetimes in glassy polymers such as polycarbonate, polystyrene, PMMA and epoxy.

4. **PROJECT TITLE:** Theory-Assisted Design and Metal and Zeolite Catalysts

**PRINCIPAL INVESTIGATORS:** A. T. Bell & Michel A. Van Hove

**PROJECT SITE:** Lawrence Berkeley 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.

#### **1994 ACCOMPLISHMENTS:**

1. *Calculation of the Structure and Electronic Properties of an Acid site in H-ZSM-5.* We have employed quantum mechanical local density functional theory (DFT) to characterize the structure and electronic properties of the Brønsted acid site in the zeolite H-ZSM-5, responsible for industrially important reactions such as the conversion of methanol to gasoline, alkylation of benzene, isomerization of xylenes, and the cracking of petroleum. The calculations were performed on a 34 atom cluster embedded in the electrostatic field generated by the zeolite framework. Our predictions for the bonded geometry around the aluminum atom and for the location and vibrational frequency of the proton are in very good agreement with available evidence from solid-state NMR and IR spectroscopy. The nature of Brønsted acidity was characterized using the hard/soft acid-base principle and perturbation molecular orbital theory. DFT calculations have also been performed to describe the structure and energetics of ammonia adsorption at Brønsted acid sites. We find that ammonia adsorbs preferentially in both bi- and tridentate forms. The predicted heats of adsorption for these species are in very good agreement

with experimental observation. The significance of this work is that the properties of Brønsted acid sites and the interactions of such sites with adsorbates can now be simulated with good quantitative accuracy.

*Sorption Thermodynamics of Aromatic Molecules in Silicalite.* The prediction of sorption isotherms for bulky molecules that experience a tight fit within zeolites pores is impossible with conventional molecular simulation techniques, as the insertion moves these techniques employ have negligible probability of being accepted. From the point of view of applications, however, bulky molecules are the most interesting, as they are most affected by the molecular sieving action of the host lattice. During FY 1994 we have developed a new energy- and cavity-biased Grand Canonical Monte Carlo (GCMC) strategy that enables prediction of isotherms for such molecules. While this approach accurately predicts adsorption isotherms and heats of adsorption for benzene and toluene, it is exceptionally demanding of computational resources. To circumvent this limitation, we have developed a "coarse-grained" lattice model, which assumes that adsorbate is localized in channel intersections, straight channel segments, and sinusoidal channel segments. The energies associated with adsorbate-site and nearest neighbor adsorbate-adsorbate interactions are obtained from short duration GCMC simulations. The coarse-grained model faithfully reproduces the GCMC simulation of the adsorption isotherms, but at a factor of 40 savings in CPU time. Using the coarse-grained model we were also successful in the adsorbate-induced ORTHO-PARA transformation of the zeolite lattice and its effects on the adsorption isotherm. The significance of this work is that it demonstrates a systematic hierarchical approach whereby information derived from a relatively fast, fully detailed atomistic calculations can be built into a more coarse-grained model that can be solved with modest computational effort to give essentially the same results in a rigorous but much more expensive atomistic simulation.

*Prediction of Transport Diffusivities in Zeolites.* We have been developing molecular simulation techniques that can predict the diffusion rates of organic molecules in a zeolite from an atomistic-level description of the host crystal and its interactions with guest molecules. Past dynamic simulation efforts have focused on predicting the self-diffusivity through equilibrium molecular dynamics (MD) simulations. This approach becomes impractical, though, for slowly diffusing adsorbates such as benzene, which "jump" infrequently between "states", in which the adsorbate spends most of its time. To handle such situations, we have employed a multidimensional transition-state theory. The interactions of benzene with the zeolite framework are described by a six-dimensional potential hypersurface. All minima (viz., states) on this surface are identified, as are the minimum-energy pathways between minima. The transition frequencies between minima are determined from TST, and the motion of an ensemble of adsorbates in the zeolite is determined from Monte Carlo calculations. This strategy is extremely efficient and leads to a determination of the mean-square displacement from which the diffusion coefficient can be obtained. The importance of this effort is that it lays the foundation for elucidating the mechanisms and predicting the rates of motion of bulky sorbate molecules with complex internal structure within microporous solids.

*Adsorption and Diffusion of Long-Chain Alkanes.* Knowledge of the adsorption and diffusion of long-chain alkanes ( $C_6+$ ) is of importance for the simulation of processes involved in the cracking and separation of such molecules. Conventional simulation Monte Carlo techniques for predicting adsorption parameters such as the Henry's law constant and the heat of adsorption are highly

inefficient, since they require random insertion of the adsorbate into the zeolite. We have developed a Continuous Configurational Bias (CCB) Monte Carlo technique for carrying out simulations of the adsorption of long-chain alkanes in ZSM-5. This method involves growth of the alkane within the channels of the zeolite, and subsequent accounting for the bias induced in this fashion. Excellent agreement between experiment and simulation is achieved, where comparison is possible. This work provides a rigorous, computationally efficient framework for the prediction of the low-occupancy sorption thermodynamics of long-chain molecules and for the quantitative characterization of the siting and conformational preferences of these molecules.

*Ethylene Hydrogenation on Pt Surfaces.* As a starting point towards mapping the mechanism of ethylene hydrogenation, we have computed various energy versus site curves for ethylidyne ( $-CCH_3$ ) on the Pt(111) surface through extended Huckel calculations. Our results are in favorable agreement with surface crystallographic findings.

### **1995 PLANNED ACTIVITIES:**

*Density Functional Theory Calculations of Metal-Containing Species in Zeolites.* The preferred siting and local energetics of extra-framework aluminum and transition metal cations will be investigated in ZSM-5. Small amounts of extra-framework aluminum, obtained by steaming of the zeolite, are known to enhance the acidity and activity of ZSM-5. The purpose of our simulations will be to establish the mechanisms by which these enhancements are achieved. Transition metal cations of Cu and Co in ZSM-5 have recently found application for the decomposition and reduction of NO. We will explore the electronic states of such metallic substituents, as well as the structure and energetics of various species (e.g., OH, NO,  $N_2O$ ,  $CH_3$ ) attached to these cations.

*Prediction of the Diffusivities of Long Chain Molecules in ZSM-5.* The rate of diffusion of  $C_8$ - $C_{16}$  alkanes in ZSM-5 is of great importance for understanding the extent to which zeolite crystals are used in fluid catalytic cracking. The characteristic times for diffusion in these systems exceed by far the time scales that can be addressed with conventional MD techniques. We will develop a coarse-grained approach, based on Brownian dynamics and transition state theory with parameters extracted directly from atomic-level information, to predict diffusivity and at the same time elucidate the configurations of these molecules in the sorbed state.

*Transition State Theory of Aromatics Diffusing in ZSM-5.* The diffusivities of aromatics in ZSM-5 are a key to the shape-selectivity observed in alkylaromatic transformations. Predicting the diffusivity of these tightly fitting molecules is a frontier problem for molecular simulation. We will continue our work on multidimensional transition state theory, with the objective of incorporating the effects of lattice and adsorbate vibration into the model. This effort will establish the extent to which the diffusion coefficient for tightly fitting adsorbates is affected by the vibrations of the zeolite and the adsorbate.

*Interactions of Templating Agents with Zeolite Frameworks.* The synthesis of highly siliceous zeolites requires the presence of a templating agent, such as a tetraalkyl ammonium 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 have initiated a computational effort aimed at identifying the best template structure for the synthesis of a given zeolite. This work is being undertaken with the docking algorithm contained in the Biosym catalyst simulation software.

*Decomposition of Organic Molecules on Pt Surfaces.* Efforts on this part of the program will not be continued in FY 1995. Work completed in the preceding year will be written up for publication.

## **ANNUAL TECHNICAL SUMMARY REPORT:**

### Extended Hueckel Calculations of the Energetic of Ethylene Dehydrogenation

#### **A. Metals:**

We have continued our studies of the dehydrogenation process of ethylene on single-crystal platinum surfaces: molecular modeling of ethylene decomposition on both Pt(111) and Pt(100) has been performed. An important goal of this project is to determine suitable empirical or semi-empirical parameters to model atomistic molecular processes on surfaces representative of real catalysts.

We use a modified version of the extended Hueckel theory within the tight-binding formalism to investigate the hydrogenation of ethylene on platinum surfaces. This version of the original programs, modified by Calzaferri, Forss and Kamber, employs a distance-dependent extended Hueckel constant  $\mathbf{K}$  and also includes two-body repulsions to allow a more reliable investigation of the intermediate surface species present in the hydrogenation process, an important aspect of the project. The model uses the distance-dependent Wolfsber-Helmholtz formula,  $\mathbf{K} = 1 + \kappa \exp(-\delta(\mathbf{R}_{ab}-d_0))$ . The aim is to describe the energetics of each intermediate step of interest in the process, and trace other factors that govern the structure; the contributions of bonding and electronic factors, steric influences and reactivities, and the influence of adsorption sites are a particular interest.

Initially, we have concentrated on a set of carbonaceous species which are thought to be dominantly present as intermediates on Pt(111) and Pt(100) crystal surfaces during the process of ethylene hydrogenation. We restrict ourselves to the ones which include a C-C bond, namely acetylene, vinylidene, ethylidyne, vinyl, di- $\sigma$ -bonded and  $\pi$ -bonded ethylene, and ethylidene. We made calculations in order to verify which is the most stably bound on the two platinum surfaces. We thereby first confirmed earlier theoretical and experimental results, according to which ethylidyne ( $\text{CCH}_3$ ) is the most stable species, by at least 2.5 eV on each surface.

Next, we studied this most stable ethylidyne species on the two platinum crystal faces. We started by locating it on the "fcc" 3-fold site of Pt(111), normal to the surface, in the exact geometry that was determined from experiment (by automated tensor LEED in this same research group). Accordingly, the C-C and Pt-C bond lengths were fixed at 1.49 Å and 2.01 Å, respectively. We ran a charge iteration on the valence-state ionization energies ( $H_i$ ) of platinum and determined better, refined parameter values that we should use for this composite system. In this manner we



reduced the excessive electron flow of electrons in the metal slab, one of the drawbacks of the non-self-consistent extended Hueckel method. And thus we calculated the amount of charge shifted to the platinum surface to be 0.215 of an electron charge, for ethylidyne located at the fcc hollow site as described above. Such a charge transfer is consistent with experimental data.

Then we examined the relative stability of ethylidyne on different sites of Pt(111): the fcc hollow (3-fold coordinated), the bridge (2-fold), the hcp hollow (3-fold), and the on-top (1-fold) sites. In each case, at least one Pt-C bond length was kept at 2.01 Å and the C-C bond length at 1.49 Å. Also, the C-C axis was held perpendicular to the surface, and the methyl group of ethylidyne was kept rigid, with the C-C-OH angle at 109.5° and each C-H bond length at 1.10 Å. This confirmed that the most stable sites for ethylidyne are the fcc and the hcp hollows, with very little difference between them. The bridge site ranks third, destabilized by 0.85 eV with respect to the fcc hollow, and last the on-top site, 2.47 eV above the binding energy of the fcc hollow position.

We also optimized the Pt-C bond length at every site, keeping the internal geometry of ethylidyne constant. We found the following values for each site:

fcc hollow:	1.84 Å
hcp hollow:	1.84 Å
bridge:	1.74 Å
on-top:	1.51 Å

It is not surprising that our method is underestimating the Pt-C distance, relative to experiment: this is due to the underestimation in the calculated core-core repulsion energies, a familiar feature of extended Hueckel theory, which is always kept in mind when discussing bond lengths.

We proceeded to construct the potential energy curve that characterizes the rotation of the methyl group (-CH<sub>3</sub>) of ethylidyne around the C-C axis, when ethylidyne stands in the fcc hollow, at a distance of 1.21 Å from the platinum surface. We calculated an energy barrier of 0.032 eV for rotation of the methyl group, with a local maximum corresponding to the orientation of methyl group in an eclipsed fashion with respect to the 3-fold arrangement of the three nearest platinum atoms on Pt(111). This barrier is equal to about 3kT at 90K, and implies rather large angles of rotational vibrations, on the order of 20° of rotation or 0.3 Å hydrogen displacement. This is compatible with our surface crystallography findings of quite large hydrogen vibrations at 90K.

We introduced into our extended Hueckel program a conjugate-direction-set minimization routine, based on Powell's method, in order to allow us to fully optimize molecular adsorption geometries with respect to energy. This is necessary to energetically and geometrically characterize the diffusion barriers and pathways of ethylidyne and other species on the metal surface, in view of the wide range of possible configurations which a molecule can take as it diffuses from one position on the surface to another.

Using the standard values of Calzaferri et al. ( $\kappa = 0.75$ ,  $\delta = 0.13$ ) for all interactions in our system, we calculated an energy barrier of 0.28 eV for the diffusion of ethylidyne from the 3-fold fcc site to its nearest neighbor 3-fold hcp site on Pt(111). We allowed the geometric optimization of

ethylidyne at every intermediate sampled position (especially tilting of the molecule as it climbs and descends past the transition point at the bridge site).

This energy barrier value is too large, however. Also, the locally optimized geometries generally produced unsatisfactory interatomic distances, particularly between Pt and C atoms. Inadequate results are to be expected with a single set of  $\kappa/\delta$  values used to describe such diverse bonds as Pt-C, C-C and C-H. Therefore, we allowed the  $\kappa/\delta$  pair to take different pairs of values for the three different atom-atom interactions. An extensive optimization of these three pairs of values, was carried out, based on the known structure of ethylidyne on Pt(111). As a result, the diffusion energy barrier was lowered to a more reasonable 0.22 eV. This low value strongly suggests that the ethylidyne species is sufficiently mobile on the Pt(111) surface to allow unimpeded ethylene adsorption and subsequent hydrogenation.

An important result of this work is the determination of these  $\kappa/\delta$  value pairs: they have general value far beyond ethylidyne and allow the realistic simulation of a wide variety of reaction intermediates containing Pt-C, C-C and C-H bonds. Similar interaction models for other atoms (O, N, S, etc., and various metals) could be readily obtained in the same fashion, based on known surface geometries, to extend the modeling to many types of surface reactions and catalysts.

## B. Zeolites

### A Hierarchical Atomistic/Lattice Simulation Approach for the Prediction of Adsorption Thermodynamics of Benzene in Silicalite.

In FY 93 we formulated and implemented a combined energy/cavity bias MC strategy for the prediction of the sorption thermodynamics of aromatic molecules in silicalite using detailed atomistic models. Whereas conventional grand canonical MC (based on random insertions) fails to equilibrate these tightly fitting, bulky molecules in the zeolite, our bias technique gave reliable results in the whole range of relevant occupancies, in excellent agreement with experiment. By conducting separate simulations in the ORTHO (Pnma) and PARA(P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>) forms of silicalite, we established that the steps observed in experimental isotherms are due to a phase transition of the zeolite framework induced by the aromatic sorbate at an occupancy somewhat higher than 4 molecules per unit cell.

Although very successful, our atomistic energy/cavity bias MC simulations were quite intensive computationally, requiring access to a supercomputer or fast dedicated workstations. Thus, in FY 94 we set out to explore whether the detailed predictive qualities of atomistic simulations could be combined with the computational efficiency of more "coarse-grained" model representations. The localization of sorbed benzene molecules in channel intersections (I), straight channel segments (S) and sinusoidal channel segments (Z) suggested the coarse-grained picture of a lattice of sites. Thus, we developed a hierarchical simulation strategy, in which detailed atomistic calculations are used to obtain parameters that are then fed into a lattice model. The multitude of center-of-mass positions and orientations assumed by a sorbate molecule within a site are replaced by a single point in three-dimensional space in the coarse-grained representation.

The link between atomistic and lattice representations is provided by a set of five temperature-dependent free energy parameters. Three of those,  $A_K$ , are singlet free energies, characterizing a benzene molecule that resides in an isolated I, S, or Z site. The remaining two,  $A_{KL}$ , are pair contributions; they express the free energy that an isolated pair of molecules occupying adjacent (I,S) or (I,Z) sites possesses over and above the sum of their singlet free energies. Values of  $A_K$  and  $A_{KL}$  are computed via atomistic Monte Carlo integration with importance sampling over all degrees of freedom of a single benzene molecule or a pair of neighboring benzene molecules.

Separate sets of free energies were obtained for the ORTHO and for the PARA structures of silicalite. The grand partition function of the lattice model was written in terms of  $A_K$  and  $A_{KL}$  through a derivation that becomes exact in the limit of zero occupancy, and a simple rule was introduced for three-body interactions in the PARA structure, based on evidence from our atomistic calculations. The coarse-grained model constructed in this way was found to reproduce faithfully the atomistically derived sorption isotherms, although its CPU time requirements were lower by a factor of 40 relative to the energy/cavity bias atomistic simulation. Furthermore, by assuming a physically reasonable value for the free energy of phase transformation of the zeolite matrix from ORTHO to PARA and conducting grand canonical simulations of the coarse-grained model in which this transformation was allowed, we predicted isotherms that exhibit steps over the same range of temperatures and pressures where such steps are observed experimentally.

The significance of this work lies in that it introduced a systematic, hierarchical approach whereby information derived from relatively fast, fully detailed atomistic calculations can be built into a more coarse-grained model that can be solved with modest computational effort to give essentially the same results as a rigorous but much more expensive atomistic simulation. The computational difficulties in dealing with the thermodynamics of bulky sorbates that experience a tight fit in zeolite pores are thus circumvented. Although tested for benzene, our coarse-graining approach is readily applicable to any mixture of aromatic molecules, and will be applied to study the thermodynamics of such mixtures in the future.

#### Prediction of the Dynamics of Benzene in Silicalite by Multidimensional Transition State Theory in the Harmonic Approximation

The transport rates of aromatic hydrocarbons within zeolites of the ZSM-5 family are of great interest in many industrial applications. The self-diffusivity of benzene, the fastest of these molecules, in silicalite is by at least three orders of magnitude too low to be predicted safely through "brute force" MD simulations. On the other hand, the localization of sorbed molecules at equilibrium within well-defined sites (see above) suggests that diffusion occurs mainly through infrequent "jumps" between "states" (i.e., domains in configuration space), in which the molecule spends most of its time. A transition-state theory-based approach to diffusion is thus appropriate. By focusing on the energy bottlenecks that control the motion of the molecule, TST can serve as a basis for estimating the rate constants of infrequent jump events, however low these rate constants may be. Thus, it is not subject to the severe time scale limitations of "brute force" MD.

We have employed multidimensional TST to analyze the mechanism and predict the rates of motions of benzene in silicalite. Exactly as in our thermodynamic work (see above), the molecule was represented as a rigid body consisting of 12 Lennard-Jones interaction sites and bearing an

equal number of partial charges within a rigid zeolite framework. The TST analysis was conducted in six generalized coordinates (the three center-of-mass coordinates and the three Eulerian angles of benzene) and took advantage of the symmetry of the molecule and of the zeolite lattice. Our calculation started with an exhaustive identification of all potential energy minima and all saddle points of the potential energy using a quasi-Newton minimization technique and Baker's saddle point algorithm, respectively. 27 distinct minima and 100 saddle points were found in the asymmetric unit of the silicalite unit cell. The intrinsic reaction coordinate (IRC) approach of Fukui was invoked to construct "transition paths", i.e. steepest ascent/descent trajectories in the space of mass-weighted generalized coordinates that connect pairs of neighboring energy minima through a saddle point. The rate constants  $K_{i \rightarrow j}$  and  $K_{j \rightarrow i}$  for transitions between the minima (i,j) were calculated for each path at 200, 300, 400, and 500K. A simple harmonic approximation was invoked in the latter calculation, wherein the potential energy is assumed to be a quadratic function of the generalized coordinates in the regions around the minima and the saddle points.

The transition rate constants determined in this way correspond to all elementary motional processes of a benzene molecule in silicalite at low occupancy; they span a very wide spectrum on the frequency scale. At each one of the temperatures studied, diagonalization of the matrix of transition rate constants yields three eigenvalues corresponding to slow motions, then a break of three to four orders of magnitude followed by a near-continuum of fast motions.

Computer graphics was used to visualize the paths of fast motions, whose rates are relatively insensitive to temperature. In agreement with recent interpretations of nuclear magnetic resonance (NMR) and quasielastic neutron scattering (QENS) spectra, a number of these paths correspond to rotations of the benzene molecule about its  $C_6$  axis by 15, 30, and 60 degrees within the environment of a channel intersection. Most of these paths also involve some re-tilting of the plane of the aromatic ring. The time scale of the  $C_6$  rotational motions is on the order of  $10^{-12}$ s, in excellent agreement with QENS measurements. Flips of the aromatic ring by 90 and 180 degrees are also observed, on time scales of  $10^{-10}$  to  $10^{-9}$ s. The slower paths correspond to infrequent transitions which take the molecule from a channel intersection to the interior of a straight or sinusoidal channel, or vice versa. They are the ones that limit the diffusive progress of the molecule. For the purpose of calculating diffusivity, energy minima connected by fast paths were lumped into "macrostates" and the sequence of infrequent hops between macrostates was simulated by a continuous-time, discrete-position-and-orientation kinetic MC, utilizing the TST rate constants. This strategy is extremely efficient, allowing one to track the motion of benzene over several seconds within an hour of workstation time. The diffusion tensor was found to be strongly anisotropic, its fastest component being directed along the straight channels. According to our predictions, the orientationally averaged diffusivity follows an Arrhenius temperature-dependence, with preexponential factor  $2.5 \times 10^{-6}$  cm<sup>2</sup>/s and activation energy of 36.7 kJ/mol. This is by one to two orders of magnitude slower than experimental values, most of which are well described by a preexponential factor of  $5.3 \times 10^{-6}$  cm<sup>2</sup>/s and an activation energy of 25.2 kJ/mol. The reason for this discrepancy will be explored in future work.

The importance of this effort lies in that it laid a foundation for elucidating the mechanisms and predicting the rates of motion of bulky sorbate molecules with complex internal structure within microporous solids. The multidimensional IRC TST approach, applied here for the first time to a zeolite problem, can provide lucid information about the motion of systems that can be described

in terms of a handful of generalized coordinates subject to highly restrictive potential hypersurfaces over an extremely wide range of time scales (picoseconds to seconds) with relatively small computational effort.

### Low-Occupancy Sorption Thermodynamics of Linear, Long-Chain Alkanes in Silicalite

Despite its great importance for separations and catalytic applications (e.g., fluid catalytic cracking), the sorption thermodynamics of long, flexible alkane molecules in zeolites of the ZSM-5 family has been addressed with molecular simulation only for chain lengths up to  $C_6$ . Conventional simulation methods for the prediction of sorption isotherms employ insertions of molecules within the microporous material. Such insertions are needed to estimate the chemical potential by the Widom test particle method in canonical and microcanonical techniques, or to equilibrate at a correct mean occupancy in grand canonical and Gibbs ensemble techniques. Random insertions of long, flexible alkanes within the highly ordered and confining environment of the intracrystalline pores, however, have very low probability of success.

We have designed a Monte Carlo method which circumvents the problems of random insertion and allows the reliable prediction of the sorption thermodynamics of linear long-chain alkanes in narrow zeolite pores. We have used this method to study chains as long as pentacosane ( $n-C_{25}$ ) in silicalite, with spectacular efficiency. This method employs biased insertions, in the spirit of the Continuous Configurational Bias (CCB) Monte Carlo techniques that were proposed in recent years by Suter et al. and Frenkel et al. for polymer liquids. The sorbate molecule is "threaded", bond-by-bond, through open or energetically favorable regions of the zeolite so as to minimize repulsive overlaps with the surrounding walls or with itself. The bias associated with this highly selective process is removed during averaging. Using CCB MC, we have computed the Henry's law constants, which quantify the thermodynamic affinity of the zeolite towards alkanes of various lengths, as well as the heat and entropy of sorption at low occupancy. Our predictions are in very favorable agreement with the correlations of Hufton and Danner and of Abdul-Rehman et al., which summarize experimental thermodynamic evidence of chain lengths up to  $C_{10}$ . At 300K, the Henry's constant rises practically exponentially with chain length. The isosteric heat  $Q_{st}$  of sorption increases linearly with the number of carbons  $n$  for short chain lengths, in excellent agreement with experimental evidence. At 300K, a slight break is seen in the  $A_{st}$  versus  $n$  plot at about  $C_8$ , the isosteric heat rising somewhat faster with chain length for  $n > 8$ . Note that  $n-C_8$  in a fully extended conformation is roughly as long as the distance between two channel intersections. A direct analysis of conformations reveals that, at low temperature, chains longer than the distance between channel intersections align preferentially along the straight channels of silicalite. (Roughly 75% of  $C_{12}$  chains are aligned along the straight channels at 300K.) Straight channels constitute the lowest energy region of pore system, which explains the slight rise in the isosteric heat per carbon number beyond  $C_8$ . At higher temperature, entropic factors become more important and long-chain configurations become more randomly distributed. (For example, at 650K only about 23% of  $C_{12}$  chains are aligned along the straight channels.) As a consequence, the break in the  $Q_{st(n)}$  curve disappears at high temperature, the dependence of  $Q_{st}$  on  $n$  being practically linear up to  $n = 25$ .

To distill the large amount of information gleaned from our CCB MC simulations about the equilibrium distribution of sorbed chains in a manner that will be directly useful for our planned

studies of dynamics (see section 20g below), we have categorized the atomistic chain configurations encountered in each simulation into macrostates. A macrostate is defined by the sequence of environments (Straight channels S, zig channels Zi, zag channels Za, channel intersections I) traversed by the contour of the chain from head to tail. Macrostates provide a coarse-grained description of the equilibrium disposition of chains in a "wire-frame" representation of the channel system, wherein channels are reduced to lines running along the channel axes and intersections are reduced to points in three-dimensional space. Within a channel-terminated macrostate, the coarse-grained configuration of a chain is specified by two scalars,  $x_1$  and  $x_2$ , corresponding to the positions of the two chain ends along their respective channel segments. The equilibrium probability of coarse-grained configurations within a given macrostate is dictated by a potential of mean force  $U(x_1, x_2)$  is a free energy arising from integration of all microscopic configurations that are consistent with given values of  $x_1$  and  $x_2$ .

We have employed CCB MC to accumulate the potential of mean force for all macrostates of the chain species we have examined in silicalite. Our results indicate that sorbed chains prefer to assume highly extended configurations.  $U(x_1, x_2)$  rises very steeply upon further extension from this preferred contour length and exhibits a softer rise upon compression. In this respect, the potential of mean force is somewhat reminiscent of the finite-extensibility nonlinear elastic springs used in rheological modeling. A chain sliding along its contour at fixed contour length experiences a relatively featureless potential of mean force. There do not appear to be significant energy barriers preventing the chain from entering the intersection regions. In addition to the highly extended configurations, our calculations reveal the presence of some configurations with unusually low contour length, in which the middle section is coiled inside a channel intersection, with two short terminal sections protruding into the straight channel segments flanking the intersection.

The work provided, for the first time, a rigorous, computationally efficient framework for the prediction of the low-occupancy sorption thermodynamics of long-chain molecules with a multitude of torsional degrees of freedom and for the quantitative characterization of the sating and conformational preferences of these molecules. It constitutes the starting point for a computationally tractable study of the dynamics of such molecules (see below).

#### Interaction Energies, Structure, and Dynamics of Ammonia in H-ZSM-5

A major goal of our efforts is to develop an understanding of the sorption and diffusion of organic molecules in real zeolites that contain acid sites. Our interest is motivated by the fact that these are the systems that are used in industrial applications involving catalytic conversion and separation processes. Developing an understanding of the thermodynamic and transport characteristics of organic molecules and ions in acidic zeolites also presents significant scientific challenges that must be confronted and overcome. The first step toward developing such an understanding involves elucidating the nature of the acidic site. In FY93 we characterized the structural and electronic features of the acid site in H-ZSM-5. In FY94, we have completed two studies that enhance our knowledge of the behavior of organic molecules in acidic sites. Firstly, we have carried out a more detailed study of the nature of the acid site in H-ZSM-5. Secondly, and more importantly, we have investigated the structure and energetics of ammonia adsorbed on the acidic sites of H-ZSM-5. We have also initiated studies that aim to shed light on the dynamical

behavior of ammonia in H-ZSM-5. In the following, we note the highlights of results obtained from our investigations in FY94.

We have carried out an exhaustive study of the nature of the interactions of ammonia with the T12 acid site of H-ZSM-5. We have employed a direct SCF Kohn-Sham method that was developed in-house. This method is very efficient. We represent the zeolite as a cluster of 34 atoms that is embedded in a classical electric field. One methodological advance that we have made pertains to the embedding procedure. First, the embedding procedure is found to be very sensitive to subtle differences in the way in which the electrostatic field is represented. We have refined the way in which the field should be represented in order to obtain very accurate results. Secondly, and more importantly, we have shown unequivocally that the electrostatic field is merely a small perturbation in the Hamiltonian and that first order perturbation theory is remarkably accurate. This finding makes self-consistent-field calculations with the field incorporated in the Hamiltonian unnecessary for future work: first order perturbation theory is adequate! We find many interesting results pertaining to the structure and energetics of ammonia adsorptions in H-ZSM-5. We find that the energetics of adsorption are very sensitive to the structural arrangement of the adsorbate. Configurations wherein the adsorbed molecule interacts with three lattice bridging oxygens (tridentate) is energetically preferred over monodentate and bidentate configurations. The energy of adsorption for the tridentate structure is 150 kJ/mol, while that for the bidentate structure is 126 kJ/mol. These values are in agreement with experimental results using microcalorimetry. Our results also shed light on the structural and electronic properties of adsorbed ammonia molecules. These results are in agreement with recent NMR experiments. We also elucidate details of the nature of bonding that are not accessible by experiment.

We have also initiated studies that seem to investigate the dynamical behavior of ammonia in H-ZSM-5. We wish to calculate the diffusion coefficient for ammonia subject to interactions with the acid sites. The nature of the ammonia-acid site interactions that we have now been able to compute via our quantum mechanical calculations in FY94 are necessary inputs to the calculation of adsorbed ammonia dynamics and transport. We are employing a hybrid scheme that uses both transition state theory and a field - theoretic treatment of diffusion in random media (developed by Arup Chakraborty with David Chandler) to study the dynamical behavior.

## 4.2 Computer Aided Catalyst Design - Biological

5. PROJECT TITLE:	Chemistry and Immunology as Tools for the Rational Design of Stable Active Enzymes
PRINCIPAL INVESTIGATOR:	P. G. Schultz
PROJECT SITE:	Lawrence Berkeley Laboratory

### 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<sup>1</sup> 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<sup>2</sup> 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.

### 1994 ACCOMPLISHMENTS:

(a) A substituted piperidine, cyclic amidine and pyran were used as haptens in an effort to generate antibodies that catalyze the hydrolysis of a series of cyclic acetal substrates. An antibody specific of the piperidine hapten was found to catalyze the hydrolysis of a corresponding substrate with a  $k_{cat}$  of  $0.904 \text{ h}^{-1}$  and  $K_M$  of  $324 \text{ mM}$  (pH 5.5). The antibody-catalyzed reaction was inhibited by hapten with a  $K_i$  of  $35 \text{ mM}$ . The rate of the background reaction catalyzed by AcOH is  $1.3 \times 10^{-1} \text{ M}^{-1}\text{h}^{-1}$ . No antibodies specific for the cyclic amidine or pyran were found to have catalytic activity. These results are an important step toward the generation of antibodies that catalyze the sequence-specific hydrolysis of oligosaccharides. Second generation haptens are currently being pursued to generate more efficient glycosidases.

(b) An antibody raised against aminophosphonic acid hapten **3** was found to catalyze the sodium periodate-dependent oxygenation of sulfide **1** to sulfoxide **2**. The  $k_{cat}$  value was  $8.2 \times \text{s}^{-1}$  and the  $K_m$  values for **1** and  $\text{NaIO}_4$  were  $43 \text{ mM}$  and  $252 \text{ mM}$ , respectively, similar to those of the

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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.

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



naturally-occurring monooxygenase enzymes. A  $K_d$  of 52 nM was determined for the hapten using fluorescence quenching. No change in the  $V_{max}$  was observed after more than 25 turnovers. The antibody showed high selectivity for the nitrophenyl substituent of **1** but tolerated a range of alkyl side chains. This result suggests that it may be possible to generate antibodies that catalyze a wide array of synthetically useful oxygenation reactions using inexpensive, unnatural cofactors.

(c) Progress has been made toward the generation of catalytic antibodies for two additional oxygenation reactions: the long wavelength light dependent oxygenation of olefins and the Baeyer-Villiger oxygenation of ketones and aldehydes (see Technical Summary).

(d) Progress has been made toward the generation of antibodies for two chemically difficult reactions of commercial interest: the stereospecific synthesis of syn or anti oximes and the stereospecific hydrolysis of tertiary esters (see Technical Summary).

### 1995 PLANNED ACTIVITIES:

My intent is to pursue two programs in 1995.

(a) *Catalytic Antibodies* The first is a continuation of the catalytic antibody program with an emphasis on generating biological "environmentally safe" catalysts for reactions of commercial interest that are currently difficult to carry out. Specifically, we will:

- (i) Characterize the ability of an additional panel of antibodies generated against an aliphatic N-oxide to catalyze the asymmetric reduction of simple dialkyl ketones.
- (ii) Generate and characterize the ability of antibodies generated against an alkyl phosphonate to catalyze a Baeyer-Villiger rearrangement.
- (iii) Characterize the ability of antibodies generated against a aminophosphinate hapten to catalyze light dependent oxygenation reactions.
- (iv) Characterize the ability of antibodies raised against a tertiary phosphonate to catalyze the hydrolysis of tertiary esters.
- (v) Characterize the ability of antibodies raised against a cyclic amine to catalyze stereospecific oxime formation.
- (vi) Generate antibodies against a metal containing hapten in an effort to catalyze regio and stereoselective oxygen insertion into CH bonds.

(b) *Combinatorial Synthesis*. We would like to undertake a second major initiative in catalyst design, specifically the combinatorial synthesis and screening of zeolites. Specifically, one goal is to develop technology that will allow for the more effective, systematic and economical discovery of solid state materials with advanced properties. This will be accomplished by developing methodology that allows the parallel synthesis and analysis of new materials with novel electronic, magnetic, optical and mechanical 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 solid state materials composed of previously unexplored combinations of elements. Moreover, the synthesis and analysis of large numbers of diverse chemical structures should add significantly to our understanding and ability to predict the structural and physical properties of solid state materials. Although this technology will be generally applicable to a whole series of materials of interest to the Department of Energy (such as high temperature superconductors, light weight, high strength permanent magnets, conducting polymers, etc.) we will initially focus our efforts on zeolites.

*Background.* The discovery of new materials with novel physical and chemical properties often leads to the development of useful new technologies. For example, the preparation of single crystal semiconductors over forty years ago transformed the electronics industry. Currently, there is tremendous activity in phenomena such as superconductivity, supermagnetic alloys, nonlinear optics and high strength materials. However, even though the chemistry of extended solids has been extensively explored, few general principles have emerged that allows one to predict the composition, physical properties and reaction pathways for the synthesis of such solid state compounds. Consequently, the discovery of new material depends largely on the synthesis and analysis of new compounds. Given approximately 100 elements in the periodic table that can be used to make compositions consisting of three, four, five or even six elements, the universe of possible new compounds remains largely unexplored. The question then arises whether there is a more efficient, economical and systematic way to discover new materials with novel properties.

One of the processes whereby nature produces molecules with novel functions involves the generation of large collections (libraries) of molecules and systematic screening of these libraries for those molecules with a desired property. One such example is the humoral immune system which in a matter of weeks sorts through some  $10^{12}$  antibody molecules to find one which specifically binds a foreign pathogen. Recently, this notion of generating and screening large collections or libraries of molecules has been applied to the drug discovery process. The discovery of new drugs can be likened to the process of finding a key which fits a lock of unknown structure. One solution to the problem is to simply produce and test a million different keys in the hope that one will fit the lock. Methods have recently been developed for the synthesis of large libraries (up to  $10^{12}$  molecules) of peptides, oligonucleotides and other small molecules on a timescale of days. This approach promises to have a huge impact on the pharmaceutical industry. Biotechnology companies are being founded to pursue this new opportunity (and currently represent investments of hundreds of millions of dollars and hundreds of new jobs) and most major pharmaceutical companies are building research programs in this area.

*Approach.* We propose to apply this same combinatorial approach to the discovery of new solid state materials, specifically in this instance, zeolites. Although, theory can provide some guidance in the design of such material, trial and error discovery remains the major route. Consequently, by increasing the rate of synthesis and characterization some  $10^4$  to  $10^5$  fold we should dramatically enhance the rate of the discovery of zeolites with interesting new properties.

We anticipate carrying the synthesis of a library of zeolites in a spatially addressable format. An array of  $100 \times 100$  microreactor wells will be fabricated and a series of inkjets will be used to

deliver predefined quantities of each starting material to individual reaction vessels. Piezoelectric devices in which a small liquid volume is ejected through a tiny nozzle at very high accelerations (100,000 g) are able to deliver droplet volumes as small as 5 picoliters with very high reproducibility. Up to 10,000 drops per second can be delivered and liquids can be dispersed in single or accumulated drops to handle a wide volume range. Inkjet technology is well established and has been previously used to deliver concentrated solutions of metal salts in the xerographic process. The use of one inkjet per synthetic precursor requires delivery times on the order of 10 msec/well for compounds composed of seven elements (such that each individual inkjet can deliver a fixed amount of reagent to each individual reaction well).

A scanning detection system will be developed to analyze the properties of each reaction well (i.e., crystallinity, catalytic properties). By coupling mass spectroscopy with spatially addressable methods for ionization we hope to directly analyze catalysis turnover and products. Scanning Raman spectroscopy will be used to fingerprint the zeolite in terms of structural morphology.

## ANNUAL TECHNICAL SUMMARY REPORT:

### (a) Oxidative Reactions.

(i) *Heteroatom Oxidation*. Biological oxygen transfer reactions are essential for the biosynthesis of steroids and neurotransmitters, the degradation of endogenous substances, and the detoxification of xenobiotics. The monooxygenase enzymes responsible for these transformations all require biological cofactors such as flavin, cytochrome P-450, copper, or pterin and typically utilize NADPH for cofactor regeneration. Given their biological and chemical importance, oxygenation reactions have long been targets for antibody catalysis, and in a few cases, redox-active heme and flavin-dependent antibodies have been characterized. More recently, a new strategy has emerged for the generation of catalytic antibodies which utilizes unnatural, chemical cofactors. We have used this strategy to generate an antibody-catalyzed sulfide oxygenation reaction mediated by the chemical cofactor sodium periodate, with turnover numbers similar to those of the corresponding enzymatic reactions.

Sodium periodate ( $\text{NaIO}_4$ ) was chosen as the oxidant, since sulfoxide formation occurs under mild aqueous conditions with minimal over-oxidation to the sulfone. Furthermore, compared to the flavin and cytochrome P-450 cofactors required by the monooxygenase enzymes,  $\text{NaIO}_4$  is very inexpensive, obviating the need for cofactor recycling. Antibodies were raised against aminophosphonic acid hapten **3** in order to catalyze the oxidation of sulfide **1** to sulfoxide **2**. Since hapten **3** contains a protonated amine at physiological pH, antibodies specific for **3** were expected to stabilize the incipient positive charge on sulfur present in the transition state. A phosphonic acid moiety was introduced into the hapten to provide a binding site for the periodate ion.

Thirty monoclonal antibodies were generated using standard protocols and purified by protein A affinity chromatography as described previously. Eight antibodies were found to accelerate the  $\text{NaIO}_4$ -dependent conversion of sulfide **1** to sulfoxide **2** at a significant rate above the uncatalyzed reaction. Antibody 28B4.2 showed the highest rate enhancement and was characterized in further detail.

The oxidation reaction catalyzed by antibody 28B4.2 displayed saturation kinetics consistent with a random-binding, sequential mechanism and a true maximum velocity ( $V_{\max}$ ) of  $49.0 \mu\text{M s}^{-1}$  and a catalytic constant ( $k_{\text{cat}}$ ) of  $8.2 \text{ s}^{-1}$ . Michaelis constant ( $K_m$ ) values of  $43 \mu\text{M}$  and  $252 \mu\text{M}$  were determined, respectively, for **1** and  $\text{NaIO}_4$ . For comparison, the uncatalyzed second-order rate constant ( $k_{\text{uncat}}$ ) was measured to be  $8.5 \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$ , resulting in a rate enhancement  $[(k_{\text{cat}}/K_m)/k_{\text{uncat}}]$  of  $2.2 \times 10^5$ . The turnover number and rate acceleration for antibody 28B4.2 reveal that this antibody is as efficient as numerous monooxygenase enzymes, including the flavin adenine dinucleotide (FAD) dependent monooxygenases and the microsomal P-450 isozymes. Prolonged exposure of the antibody to sodium periodate at the concentrations used in this study had little effect on its specific activity. In addition, no change in  $V_{\max}$  was observed after more than 25 turnovers. The antibody-catalyzed reaction was completely inhibited by the addition of hapten **3**, consistent with catalysis occurring in the antibody combining site. Fluorescence quenching experiments performed in the reaction buffer at  $22^\circ\text{C}$  afforded a dissociation constant ( $K_d$ ) of  $52 \text{ nM}$  for the hapten.

(ii) *Photooxidation.* The selective addition of oxygen to olefins is a reaction of considerable synthetic interest. The selective photooxidation of alkenes by  $\text{O}_2$  to form hydroperoxides proceeds via excited charge-transfer states. Stabilization of these states have recently been demonstrated inside the cages of zeolite NaY and has been attributed to electrostatic interactions inside the charged cages. Since catalytic antibodies have been shown to stabilize transition states via highly specific electrostatic interactions (among other interactions), it may be possible to generate antibodies capable of stabilizing charged-transfer states. Such antibodies would be much more selective catalysts than the corresponding zeolites. Based on earlier studies with 2,3-dimethyl-2-butene in zeolite cages, the photooxidation of alkene **1** is anticipated to occur via the charge-transfer complex **2** to form hydroperoxide **3**. Since hapten **4** mimics the charged character complex **2**, antibodies raised against **4** may be capable of stabilizing this complex. It is anticipated that the synthesis of chiral hydroperoxides may be possible, since antibodies have been shown to perform highly selective chemical transformations. Forty monoclonal antibodies against hapten **4** have been elicited and alkene substrate **1** is currently being synthesized to test if the antibodies stabilize the light-induced alkene- $\text{O}_2$  charge-transfer state.

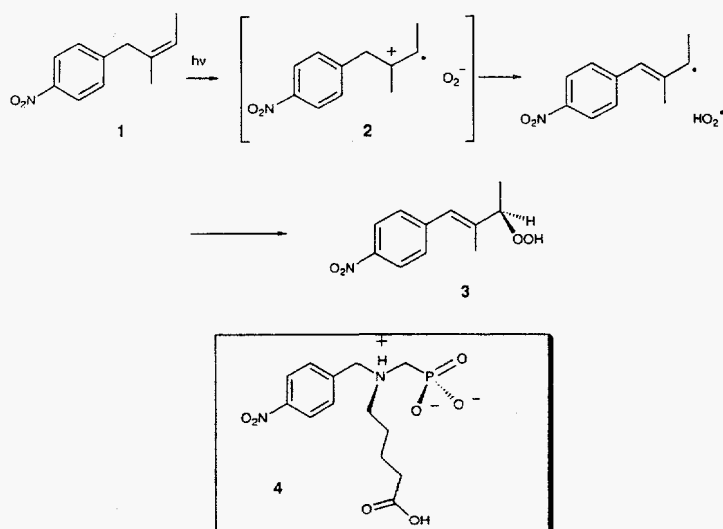


Figure 1

(iii) *Baeyer-Villiger*. The Baeyer-Villiger reaction of ketones or aldehydes and peracids is a useful reaction for the synthesis of esters. This reaction is catalyzed in nature by flavin dependent monooxygenases. In an effort to ask whether antibodies could be generated that are capable of oxygen insertion into carbon-carbon bonds we are generating antibodies against I. These antibodies are expected to catalyze the conversion of para-substituted benzaldehydes to the corresponding ester. The rate determining state of this rearrangement is the addition of the peracid to the carbonyl group which should be mimicked by the phosphonate moiety of hapten I. The synthesis of the hapten is complete and antibodies are being produced.

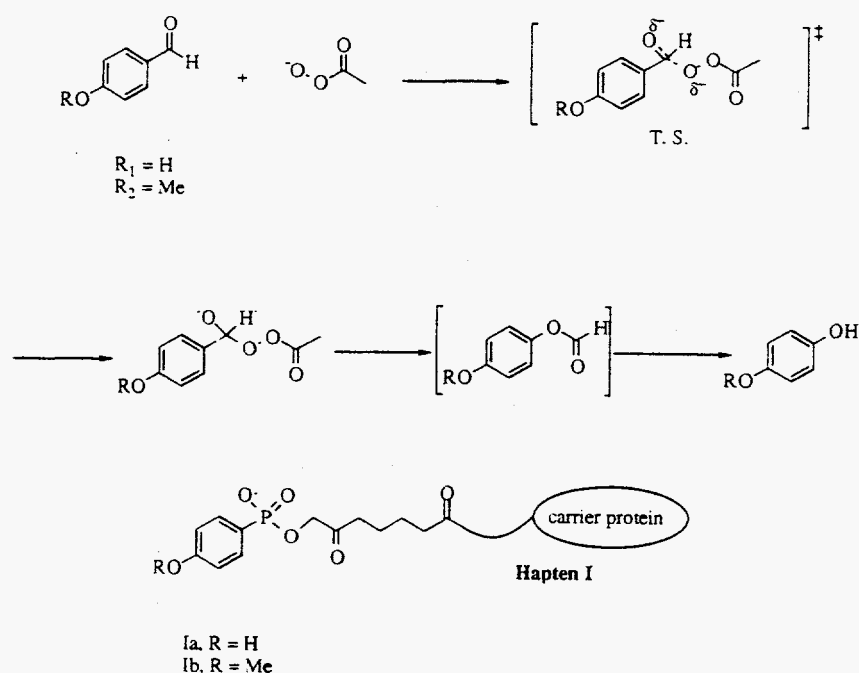


Figure 2

### (b) Stereoselective Addition of Hydroxylamine to Carbonyl Groups.

The addition of hydroxylamine to aldehydes and ketones to form oximes is a reaction of considerable commercial interest and is useful in the production of many important chemical intermediates. Unfortunately, there are no practical routes for controlling the stereochemistry of this reaction. Consequently, lengthy separation procedures are required to isolate the disfavored adduct. We have previously demonstrated that antibodies raised against haptens that mimic the charged transition state for the loss of water from the initial tetrahedral adduct will catalyze the addition of hydroxylamine to the corresponding ketone. In addition, antibodies were isolated that catalyze the formation of either the anti or syn products in ratios of 9:1. In an effort to increase the stereospecificity of this transformation we are generating antibodies against a conformationally restricted analogue of the transition state shown below. The synthesis of the corresponding hapten is underway.

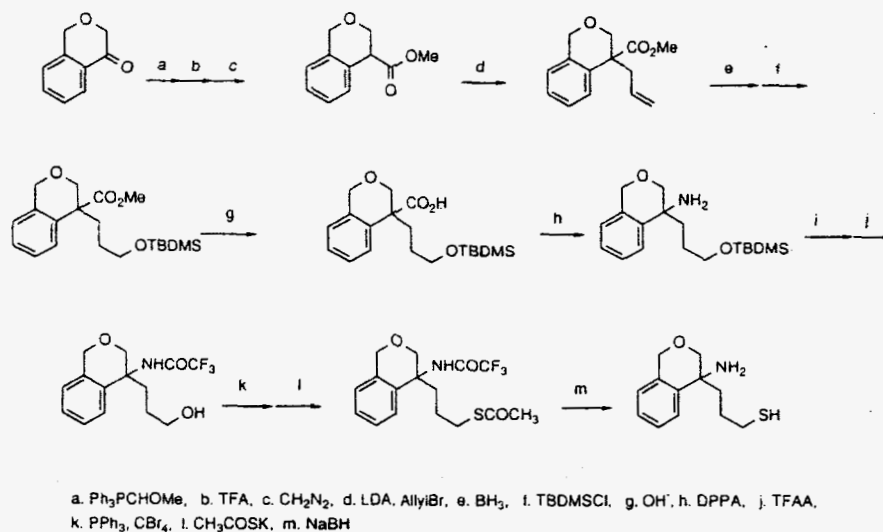
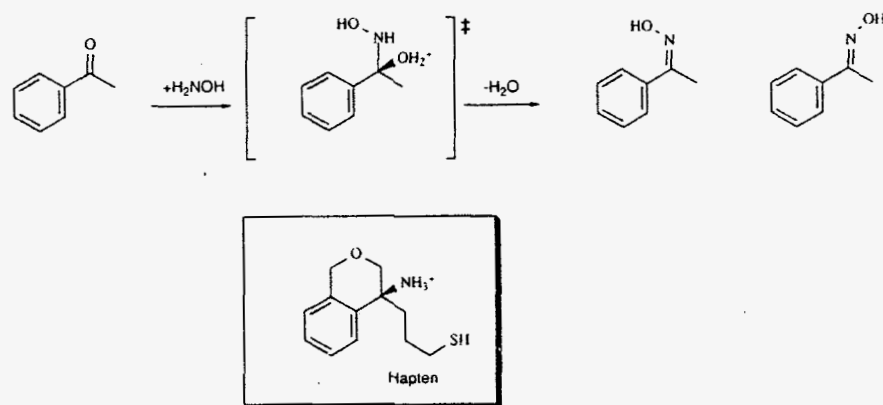


Figure 3

(c) Hydrolysis of tertiary esters.

The hydrolysis of tertiary esters is a reaction of considerable use in the synthesis of a number of chiral intermediates. Unfortunately, no good chemical or enzymatic catalysis exist for this reaction. Consequently, we are asking whether antibodies raised against phosphonate **1** will catalyze the hydrolysis of the corresponding tertiary ester. Generation of antibodies is currently underway.



**6. PROJECT TITLE:** Genetic Engineering of Methanogenic Bacteria for Industrial Chemical Applications

**PRINCIPAL INVESTIGATOR:** L. Baresi

**PROJECT SITE:** Jet Propulsion Laboratory

**DESCRIPTION:**

New breakthroughs in genetic engineering and knowledge of fundamental synthetic pathways in biological systems have made the control of methanogenic microorganisms for industrial applications feasible. Methanogenic bacteria may be used to decarboxylate acetic or higher molecular weight acids and/or metabolize other carbon compounds and acting as a carbon dioxide sink to produce methane. To this end, this project seeks to induce anaerobic methanogenic bacteria to operate under abnormal and potentially toxic conditions (viz., in the presence of oxygen). This will be accomplished by using genetic engineering techniques, shuttle vector, to introduce this new property. A shuttle vector carries genetic information from one organism to another. Phasmids are shuttle vectors which replicate independently of the chromosomal DNA and are composites of host plasmid and bacteriophage DNA. The project will use the model anaerobic organisms *Methanobrevibacter smithii* along with *Escherichia coli* to construct a *M. smithii* phage/*E. coli* pBR322 phasmid.

The project will develop strategies for the engineering of methanogenic bacteria for commercial purposes by employing recently developed software (Metabolic Pathways Systems (MPS)) to analyze the effects of genetic manipulations on metabolism. The MPS program uses new techniques of artificial intelligence programming to evaluate possible solutions for complex intracellular strategies. When coupled with the capability to genetically alter the metabolism of an organism by genetic manipulation this computer program will enunciate potentially useful pathways in a time- and cost-effective way, avoiding time-consuming empirical experimentation.

**1994 ACCOMPLISHMENTS:**

Work on the generation of phasmid continues. The *E. coli* vector, pBR322 was modified with the PAC gene introduced into the ECO RI site. *E. coli* host cells can now be transformed producing resistance to tetracycline, ampicillin, and puromycin. The puromycin gene will be used for selection in the methanogen host. To insure a higher degree of success the project has divided the final construction of the vector into two parts. The first part was the construction of a vector to test for the conditions most conducive for transformation in the methanogen. A test vector was constructed by the insertion of methanogen, *M. smithii*, DNA into the ECO RV site of pBR322 (lose of tetracycline resistance). Optimal conditions for transformation are being elucidated using DNA homology, integration, and puromycin selection. Once the optimal conditions for transformation are secured then methanogen phage DNA is inserted into pBR322 ECO RV. Using the optimal conditions for transformation, self replication of the newly constructed vector will be tested. If successful one should have a vector that can replicated and selected in both *M. smithii* and *E. coli*.



## 1995 PLANNED ACTIVITIES:

A new LISP program will be employed that will run under Windows. In this new environment the MPS program should be less memory constrained. New information on the methanogens enzymatic capabilities will be added to a new data base for analysis. Work on generating a shuttle vector will continue by the establishment of optimal transformation conditions and the introduction of phage DNA into the tetracycline site of the pBR322 keeping the original *ORI* site intact. The shuttle vector will then have an active ampicillin resistance marker as well as an active puromycin resistance marker. The composite of these two resistance markers along with the *ORI* site of both the methanogen phage and the original pBR322 should allow for transformation and selection in both *M. smithii* and *E. coli*.

7. **PROJECT TITLE:** Theory of Biocatalysis - Electron Transfer Reactions

**PRINCIPAL INVESTIGATOR:** D. N. Beratan

**PROJECT SITE:** University of Pittsburgh

### 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.

### 1994 ACCOMPLISHMENTS:

- ▶ developed method to calculate electron transport rates in DNA and in starburst dendrimer structures
- ▶ developed methods to calculate electronic coupling interactions in proteins, including all valence orbitals of the protein
- ▶ initiated study of role played by the protein/protein interface in electron transfer reactions
- ▶ initiated study of spin-state effects on electron transport rates
- ▶ initiated study of multiple electron transfer events

## 1995 PLANNED ACTIVITIES:

- ▶ continue recently initiated studies of protein/protein interface, spin-state effects, and multiple electron transfer events
- ▶ initiate industrial collaboration related to the DNA electron transfer calculations
- ▶ initiate studies of coupling electron transfer chemistry to chemical bond activation

## ANNUAL TECHNICAL SUMMARY REPORT:

In the first phase of this research project we focused on two new types of macromolecule electron transfer systems, nucleic acids and starburst dendrimers. These were chosen to broaden the understanding of electron transfer in macromolecules that was gained from our earlier studies of proteins. I am especially excited about the idea of exploiting electron transfer reactions to probe subtle intra- and inter-molecular interactions in these novel materials. For example, I believe that electron transfer reactions in polymer and dendrimer films will eventually be used to reveal information about the materials' structure and dynamics. And such information will aid our ability to design biocatalytic systems. Similarly, I believe that electron transfer rates in nucleic acids, and in their complexes with proteins, will be diagnostic of base sequence and the nature of intermolecular interactions. The possibility of using the simple "one-dimensional" nucleic acids as frameworks for charge separation is being considered for the first time at present. The area of DNA electron transfer experiments is about to expand drastically (from 2 or 3 groups with uninspiring preliminary data, to about 6 active groups with important new experiments), and there is essentially no theoretical work in this area going on outside of my own research group. Our preliminary results indicate important dependence of electron transfer rates on the sequence of intervening bases and on the disposition of the major and minor grooves of the double helix with respect to electron donors and acceptors. These results are being prepared for publication.

Having completed our exploratory research on electron transfer in DNA, our focus has turned to starburst dendrimers. Starburst dendrimers have the connectivity of Bethe lattices or Cayley trees. These species are of particular interest because they are partially ordered. That is, their backbones consist of identical repeating units (just like in linear polymers), but the materials fold into somewhat disordered 3D structures. Because starburst dendrimers have three or more branches coming from each repeat unit (conventional linear polymers have only two such units), the electronic coupling characteristics of these materials are quite unique.

We have found that because of the branching in these structures, the wave function amplitude splits at each branch leading to more rapid decay of coupling with distance than in the corresponding linear materials. This effect could be used to control the degree of charge localization in macromolecule based electron transfer processes. This localization is even seen in the "band" states of the materials, which in the corresponding idealized 1D linear polymers would correspond to fully delocalized Bloch states. These results are being prepared for publication.

With preliminary calculations on DNA and starburst dendrimers completed, we have returned to our work on protein electron transfer coupling calculations. Here our goal is to investigate the importance of secondary structure effects and quantum interference on protein electron transfer reactions. We demonstrated that a full 5,000 orbital (all valence orbitals, independent electron

level) calculation of long range electronic couplings (at the tight-binding level) is accessible using modern supercomputers (Pittsburgh Supercomputer Center Cray YMP-C90). These new methods allow the calculation of long-range electronic couplings (and rates) in proteins the size of cytochrome *c*. With improved independent electron parameters (coming other quantum chemistry groups), reliable calculations of electronic couplings involving *all* pathways and summation of "scattering" interactions to all orders should become accessible in the near future. Preliminary results suggest the nature of quantum interference effects in protein electron transfer will be qualitatively different in  $\alpha$ -helix vs.  $\beta$ -sheet structures. This effect could be used to control the directionality and specificity of electron transfer in biocatalytic systems.

8. **PROJECT TITLE:** Predictive Models and Effects of Structure on Catalytic Properties

**PRINCIPAL INVESTIGATOR:** W. A. Goddard III

**PROJECT SITE:** California Institute of Technology

#### **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.

## 1994 ACCOMPLISHMENTS AND ANNUAL TECHNICAL SUMMARY REPORT:

A central problem in developing new biotechnology is the difficulty in predicting the structures of new proteins or of old proteins in new environments. This is referred to as the *protein folding problem*. Long believed to be impossible by many researchers, a great deal of progress has been made in the last year. Our approach uses a general hierarchical framework for first principles predictions of protein conformations (tertiary structure) from primary sequence. This Hierarchical Protein Folding Strategy (HPFS) involves five levels

- i. Secondary structure: determine which residues are most likely to be  $\alpha$ -helix,  $\beta$ -sheet, coil, etc.)
- ii. Folded conformation: based on (i) use a coarse grain description involving only  $C_{\alpha}$  atoms to predict the conformations which fold the protein into a globular structure.
- iii. Optimal  $C_{\alpha}$  structure: starting with (ii) predict the best coordinates for the fold  $C_{\alpha}$  description
- iv. All atom structure: description for each  $C_{\alpha}$  structure, fill in the main chain atoms and side chains atoms to obtain the best all-atom description, given the  $C_{\alpha}$  positions.
- v. Solvent optimized: optimize the all-atom description of the protein while solvated to water (or other appropriate solvent).

We have developed an optimum program for (v). This involves the new CMM and NEIMO technologies implemented into a highly parallelized molecular dynamics program that runs on the Kendall Square Research Parallel Supercomputer.

We have also developed an efficient program for step (iv).

We have also developed a  $C_{\alpha}$  force field that can optimize the structure in (iii) given plausible conformations from (ii). However until recently our methods for predicting secondary structures (i) and folded conformations (ii) have been inadequate. This has obstructed applications to industrial problems.

A long standing strategy has been to use the  $C_{\alpha}$  - *description on a lattice* to predict both (i) and (ii). J. Solomon (independently supported by a DOE Computational Biology grant) of Caltech has made great progress on this, and we are collaborating to start with the lattice prediction of the conformation and secondary structure and use our techniques for (iii), (iv), and (v) to predict the full structures.

A second strategy developed over the last year is Protein Folding Using *Evolutionarily Derived Secondary Structures* (EDSS). A new strategy for first principles prediction of tertiary structure, EDSS starts with the structural elements deduced from the mutation-structure correlations derived by S. Benner (ETH, Zurich) from exhaustive matching of the protein sequence data base. This leads to assignments of

- (1) secondary structure ( $\alpha$ -helix,  $\beta$ -sheet, coil)
- (2) surface versus interior, and
- (3) active site.

Thus EDSS provide (i) plus a little more. We have been using NEIMO dynamics to fold the protein (ii) and (iii) based on these constraints. Starting with (iii), we then build the main chain, side chains, solvate, and optimize to obtain a final structure.

A third strategy uses an approach, Boltzmann Biased Monte Carlo, (BBMC), developed over the last year at Caltech to predict thermodynamics of polymer solutions (by Goddard and an industrial visitor). Combined with EDSS it appears that we can readily obtain well-folded conformations (ii) as the input to (iii).

### Protein Design

We have continued developing our strategy, *Protein Stitchery*, for designing proteins to recognize specific sites of DNA. We have now used this strategy for designing a protein to bind to a specific DNA sequence with 16 base pairs, and we carried out the gel retardation assays and DNase I footprinting that proved the design.

Most regulatory proteins involve binding a dimer to a palindromic DNA binding site. Using protein stitchery we have shown that the *monomers* bind strongly to the dimer DNA site and that at normal concentrations the normal pathway is to bind two monomers separately to the DNA and then to dimerize while attached to DNA (rather than forming the dimer in solution and then binding). This should have ramifications for developing strategies for regulatory controls.

### High Capacity Molecular Dynamics

The dynamical motions of a protein are described using Newton's equations. This is referred to as in Molecular Dynamics. Our focus is on high capacity molecular dynamics - simulation of 100,000 to 1,000,000 atoms for finite molecules or 100,000 to 1,000,000 atoms per unit cell for crystals and amorphous systems. Important developments here include:

### Methods

- i. The Cell Multipole Method (CMM) for macromolecular simulations which dramatically reduces the cost of long-range Coulomb and van der Waals interactions while retaining high accuracy. This makes the cost scale linearly with size and opens the way to atomic-level simulations for million atom systems.
- ii. The Reduced Cell Multipole Method (RCCM) for simulations of crystals. This handles the special difficulties with long range Coulomb interactions for crystals by combining a reduced unit cell plus CMM for interaction of the unit cell with its adjacent cells. This makes the cost scale linearly with size while retaining high accuracy and opens the way to simulation of crystals having a million atoms per unit cell (the major use is for models of amorphous and semi-crystalline materials).
- iii. The Newton-Euler Inverse Mass Operator (NEIMO) method for internal coordinate dynamics (e.g. torsions only). This allows the solution of the dynamical equations for internal coordinates without inverting the mass tensor (moment of inertia tensor). The

cost of NEIMO is linear to the number of degrees of freedom and small compared to other costs for million atom systems.

### Parallel Supercomputer Implementations

A focus over the next few years will be toward developing the *algorithms* and methods suitable for massively parallel high performance computers (Kendall Square Research, Intel Paragon, etc.) and to develop optimal software for exploiting such computer environments.

- i. The new MD//CMM program has been written and optimized for the Kendall Square Research (KSR) parallel supercomputer. MD//CMM/KSR is now being used for production simulations on million atom systems .
- ii. The new MD//PT program for perturbation theory molecular dynamics has been optimized for the KSR. MD//PT is now being used for production simulations of protein/DNA interactions

### Force Fields

In order to describe a molecule using MD it is necessary to average over the electronic wavefunctions to obtain parameters involving only the atoms. This force field (FF) enables one to use molecular dynamics (Newton's equations) rather than the Schrodinger equation. Our focus is on using data from QM to predict the FF. Important new developments here include:

### Force Field Parameters

- i. FFOPT - This automates the optimization of force field parameters to fit the Hessian, polarizability tensor, and geometry from quantum chemistry and the vibrational frequencies, geometry, unit cell parameters, and elastic constants from experiment. It uses the Hessian biased approach .
- ii Universal Force Field (UFF) - A generic force field to treat all 103 elements of the periodic table (developed with Professor A. K. Rappe, Colorado State University and M. Skiff of Shell Development) .

### New Types of Force Fields

- i. Pseudoelectron force fields in which classical electrons are included in the force field to treat polarizability effects in polymers, ceramics and metals .
- ii GLFF - generalized London force field for describing quantum mechanical reaction surfaces using classical potentials .

## Process Simulation and Design (PSD)

A new focus in the MSC is to develop a new strategy for the PSD software in which the results of atomistic simulations are used to determine the activity coefficients, phase diagrams, and other properties required for chemical engineering simulation and design. Current focus is on separations.

### **1995 PLANNED ACTIVITIES:**

The progress report gives many details of the projects, each of which will continue during year 2. A summary is included here.

A major focus for year 2 will be to build on the progress in protein folding. Over the next year we expect some success in predictions for real systems. By the end of the grant period, we expect to have a tool for protein folding that would allow the biotech industry to use modeling as a key new strategy.

The design of new biocatalysts using ROP/4H as the foundation has progressed to the stage of experimental test. Over the next year we will focus on synthesis of molecules (using gene sequences inserted into *E. coli*) and testing properties.

The focus over the next year on Protein Stitchery will be to build on the new insight into regulatory proteins and to quantify the effect of sequential DNA binding.

We have been combining the MD/PT technology with the MD/CMM technology on the KSR parallel supercomputer. This is required in order to have an industrially useful tool for predicting structures and properties in solvents.

A major focus for the second year will be on catalytic activation of  $\text{CH}_4$  and  $\text{CO}_2$  by biological molecules.

Of particular importance to applications in industry are methods of predicting optimal process conditions. We will be developing fundamental tools for predicting solubilities and miscibilities useful in process simulation and design. In this activity we will set up collaborations with one to four companies in order that our development be tested for real applications.

9. **PROJECT TITLE:** Computer Aided Molecular Design of Biomimetic Catalysts

**PRINCIPAL INVESTIGATOR:** J. A. Shelnett

**PROJECT SITE:** Sandia National Laboratories

#### **DESCRIPTION:**

The goal of the research is to develop computer-aided molecular design (CAMD) methodologies using classical- and quantum-mechanical molecular modeling techniques and apply them to design synthetic analogs of enzymes. Particular emphasis has been placed on the biomimetic activation of CO<sub>2</sub> to produce methanol, methane, formaldehyde and other products, ultimately using sunlight as the energy source for the chemical reaction. The understanding of the biochemistry of carbon dioxide conversion has only recently reached a sufficient level for designing catalysts that mimic the C<sub>1</sub> catalysis of methanogens and other CO<sub>2</sub> metabolizing bacteria.

The activation energy barrier to produce an excited state of CO<sub>2</sub> and the large free energy increase that must accompany reduction of CO<sub>2</sub> to useful products have made the investigation of biomimetic synthetic routes attractive. To use CO<sub>2</sub> as a feedstock, energy must be used in the most efficient manner possible, as occurs in the biological use of sunlight as the energy source for the reaction. Specific elements of the research are: (1) the development of a photoredox cycle to provide the required chemical reducing agent, (2) studies of the enzymes that catalyze the activation and conversion of CO<sub>2</sub>, (3) computer-aided molecular design of catalysts that mimic the active sites of these enzymes, (4) development of improved molecular modeling techniques, (5) synthesis, structural characterization, and testing of the designed catalysts, and (6) integration of the system components into a solar-driven process for CO<sub>2</sub> reduction to high value products.

Several biomimetic catalysts systems have been examined within the scope of this project. Particular emphasis has been placed on the metalloporphyrins and related organometallic complexes. These molecules are known to catalyze CO<sub>2</sub> conversion and their activity can be improved by modifying their molecular structure to mimic the structural features of CO<sub>2</sub>-activating enzymes. The specific objective is to design metalloporphyrins with the desired structural features and, subsequently, synthesize and test the most promising of the designed catalysts.

#### **1994 ACCOMPLISHMENTS:**

Our accomplishments for FY94 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.* We have designed and implemented an electrochemical catalyst testing and characterization apparatus using gas-diffusion electrodes. We have begun applying these electrochemical testing methods to the designed Co-porphyrin catalysts. Efficient CO<sub>2</sub> reduction to CO and possibly other products has been observed. Up to 5 percent conversion of a CO<sub>2</sub> gas stream through the electrochemical cell has been measured. Many routes to



improve the electrodes and electrochemical testing cell have been found. We have tested one of the designed catalysts, CoDPP, in the gas-diffusion cell, demonstrating efficient conversion of CO<sub>2</sub> to CO and other products.

- *Synthesis of advanced catalyst designs.* One of our design goals is to be able to control the reduction potential of the metal at the active center of the cobalt-porphyrin catalysts. Toward this goal we have now synthesized a series of fluorinated derivatives of cobalt dodecaphenylporphyrin (CoDPP) catalysts in quantities suitable for testing. We have demonstrated the Co(II)/Co(I) reduction potential can be varied over a wide range by varying the degree of fluorine substitution from 0 to 36 fluorine substituents. We have synthesized catalysts with hydrogen-bond donor groups forming the CO<sub>2</sub>-binding cavity. We have also designed, synthesized, and characterized tetraalkylporphyrin derivatives with chiral substituents.
- *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*. These manuscripts complete the initial phase of model validation for the designed catalysts.
- A new collaboration with Prof. Bill Goddard was initiated to improve our force field for POLYGRAF/BIOGRAF molecular mechanics calculations by using a method developed by Sidharth Dasgupta and Goddard. The method at first order allows us to obtain a molecular mechanics force field that best fits the results of the classical normal coordinate analysis. Preliminary results suggest that this force field is close to the one currently being used; however, the optimized force field that result will make predictions of vibrational frequencies and how they vary with structure, a capability that is not part of the presently used force field. Further, improvements can possibly be made using the new method after good *ab initio* quantum calculations that are underway for nickel porphine are completed.

#### 1995 PLANNED ACTIVITIES:

Test New CO<sub>2</sub> Catalysts. Current testing procedures using gas-diffusion electrodes will be refined and optimized so that accurate comparisons of the activities of various catalysts can be reliably compared. Parameters to be varied are gas-diffusion electrodes of different construction, catalyst deposition methods, electrolytes, solvents, and solution conditions (e.g., pH). Also, a smaller electrode cell will be constructed so that smaller quantities of catalyst can be tested and thus more tests can be run.

Currently available CO<sub>2</sub>-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 preceding 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<sub>2</sub>-Activation Catalysts. We will complete the synthesis of Co derivatives of nitrated octaphenyl porphyrins (OPPs). Meso-nitro substituents will be converted to the amines to provide for hydrogen bonding to the bound CO<sub>2</sub>.

Other synthetic strategies aimed at introducing hydrogen bonding functionality will be employed. For example, a number of mono- and di-meso-substituted porphyrins will be synthesized, including the amino-, hydroxymethyl-, formyl-, and chloro-derivatives of OEP. Modeling calculations predict particularly strong (~30 kcal-mol<sup>-1</sup>) hydrogen bonding in the case of di-hydroxymethyl-OEP in aprotic solvents because of the optimum distances and angles of the hydrogen bonds formed with the oxygen atoms of O=C=O. The synthesis of these mono- and di-substituted porphyrins will be completed in the next year.

Finally, the chiral dodecaphenylporphyrin catalysts modified with amide linkages to amino acid residues will contain such hydrogen-bonding groups and, in addition, a hydrophobic cavity for CO<sub>2</sub>. These and other metalloporphyrin electrocatalysts will be synthesized for activity testing and CO<sub>2</sub>-binding studies.

Design and Synthesize Chiral Catalysts. Chirally substituted dodecaphenylporphyrins will be synthesized. In particular, DPPs with ortho-amino acid amide substituents will be synthesized. Water-soluble and charge-deficient analogs will be synthesized by substitution of octaphenyl-tetra(N-methylpyridinium- and dodeca(N-methylpyridinium)-porphyrins.

Ab Initio modeling of Catalysts. We will complete geometry optimization calculations of NiP 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 of the two methods give the best results in various circumstances. Subsequently, 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 NMR spectroscopy.

The quantum calculations for NiP and other porphyrins will also be used to derive a classical force field for use in molecular mechanics calculations. Normal modes of NiP will also be calculated and compared with experimental vibrational data from Raman and IR spectroscopic studies of NiP that are currently underway. Finally, we will investigate the effect of nonplanar distortion of the porphyrin macrocycle on the calculated vibrational frequencies and compare with the experimental frequencies.

**Characterization of New Catalysts.** We will complete the structural characterization of metal derivatives of the currently available and 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 tetraalkylporphyrins, mono- and di-amino-OEPs, hydroxymethyl-OEPs, formyl-OEPs, chiral porphyrins, metal octaphenylporphyrins, and metal octaisopropylporphyrins, a novel class of nonplanar porphyrins.

For the porphyrins expected to form hydrogen bonds with CO<sub>2</sub>, we will also carry out spectroscopic studies of the binding of CO<sub>2</sub> to the porphyrins.

#### **ANNUAL TECHNICAL SUMMARY REPORT:**

A summary of technical progress since August 1993 is provided. Technical details of the work can be found in the listed publications. Our recent research efforts, as summarized below, are primarily concentrated in the five task areas: *(Task 1) Design and synthesis of new catalysts, (Task 2) test CO<sub>2</sub>-activation catalysts, (Task 3) characterize advanced catalyst, (Task 4) quantum modeling of designed catalysts, (Task 5) develop new modeling methods.*

This work includes the following major accomplishments: (1) the first successful use of the Coporphyrin as CO<sub>2</sub>-reduction electrocatalysts, (2) the entire series of fluorinated dodecaphenylporphyrins containing iron and cobalt have been re-synthesized, this time in quantities sufficient for CO<sub>2</sub>-reduction testing, (3) metal derivatives of octa(isopropylphenyl)porphyrin (OIPPP), another electron-poor class of cavity-containing catalysts for CO<sub>2</sub> binding and conversion, have been synthesized and characterized (see attached abstracts), (4) NMR characterization of the effect of the cavity on metal axial ligand orientation for the designed cobalt-porphyrin catalysts has been described in a manuscript submitted to *J. Chem. Soc., Chem. Commun.*, and (5) molecular modeling has been used to design a series of catalysts that incorporate hydrogen-bonding functionality for binding CO<sub>2</sub>.

**Design and Synthesis of New Catalysts.** *Synthesis of Halogenated Dodecaphenylporphyrins.* The cobalt derivatives of the fluorinated dodecaphenylporphyrins (DPPs) that are needed for CO<sub>2</sub> reduction tests were synthesized this year; these include CoDPP, CoDPPF<sub>20</sub>, CoDPPF<sub>28</sub>, and CoDPPF<sub>36</sub>. Nickel derivatives were also synthesized for characterization studies and testing. The perfluoro-dodecaphenylporphyrin (DPP-F<sub>60</sub>) has not yet been synthesized but is of interest because of its predicted high stability to oxidation and demetalation and novel electronic and structural properties. CoDPP has been subjected to electrochemical measurements and electrocatalytic CO<sub>2</sub> reduction studies (*vide infra*). The synthesis of additional members of the DPP and TPP series is currently being attempted by a new synthetic procedure. Phenyl boronic acid has been made from bromobenzene in preparation for making DPPF<sub>40</sub> and DPPF<sub>60</sub>; octabromotetraphenylporphyrin which reacts with the boronic acid to give DPP has also been synthesized successfully. The octafluoro-tetraphenylporphyrin has also been made and promises to be a good

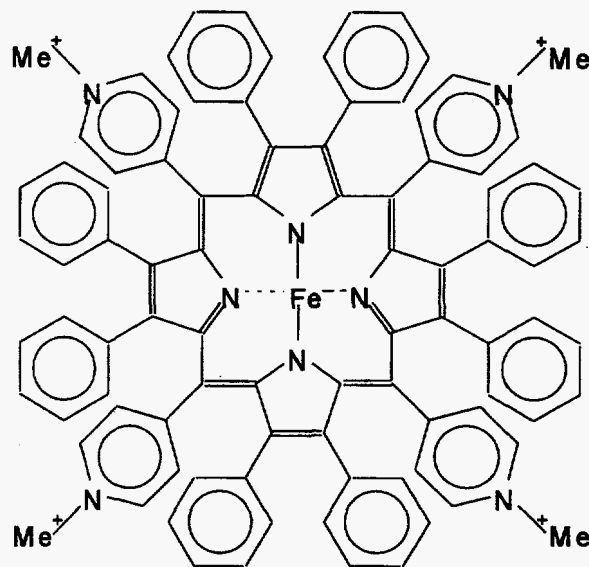
catalyst for CO<sub>2</sub> reduction because of the electron-withdrawing fluorine substituents directly on the porphyrin macrocycle.

Figure 1 shows the first example of a newly designed series of porphyrins using a new approach toward the synthesis of more electron-deficient porphyrins--octaphenyl-tetra(N-methylpyridinium)porphyrin. This porphyrin is synthesized as the tetrapyrrolyl derivative, which is not so electron deficient that the porphyrin cannot be synthesized, and the pyridine groups are subsequently converted to the highly electron withdrawing N-methyl-pyridinium derivatives. This procedure may allow us to synthesize dodeca(N-methylpyridinium)porphyrin, the ultimate target of this synthesis effort.

The redox potential of the metal is important in determining the electrode potential at which CO<sub>2</sub> reduction takes place and, thus, the efficiency of the electrocatalytic reaction. For the cobalt catalysts, the important reduction is the Co(II)/Co(I) couple. We found that this reduction potential of the Co couple also becomes more positive as the electron withdrawing power of the substituents becomes stronger. In fact, we have been able to make the potential for the Co(II)/Co(I) couple less negative than -0.5 V using the fluorinated DPP series and still less negative potentials are expected with the pyridinium porphyrins.

Recently, Dr. Miura at Brookhaven has synthesized the dodeca-carboxylic acid porphyrin with -C<sub>20</sub>H<sub>41</sub> ester groups attached in the para positions of the four phenyls of this porphyrin. The Ni, Zn, and Sn derivatives have now been synthesized. The Ni derivative of this lipo-porphyrin has novel solubility (in hexane) and has been incorporated into Langmuir-Blodgett films on electrodes. Also, the Zn and Sn derivatives are active photocatalysts.

*Hydrogen Bonding Functionality.* Earlier, we calculated significant CO<sub>2</sub> binding energies for several catalyst in a vacuum, but we measure much smaller relative binding energies in solution. Consequently, new porphyrin catalysts which incorporate the possibility of hydrogen bonding to CO<sub>2</sub> within the cavity have been designed and the binding energy calculated. The calculated structure of one of these catalyst-CO<sub>2</sub> complexes is shown in Figure 2. The catalyst has amino groups adjacent to the CO<sub>2</sub> binding cavity, permitting the CO<sub>2</sub> molecule to hydrogen bond to a pair of amino groups at each end of the cavity. As expected the binding energy is significantly enhanced by the hydrogen bonding interactions. Preliminary estimates of the hydrogen bonding contribution to the binding energy is over 28 kcal/mole in vacuum. The hydrogen bonding adds to the binding energy for CO<sub>2</sub>, but not for most solvents. In apolar solvents most of this hydrogen



*Fe(III) Octaphenyltetra(N-methylpyridinium)porphyrin*

Figure 1. New porphyrin catalysts that are the targets of recent syntheses.

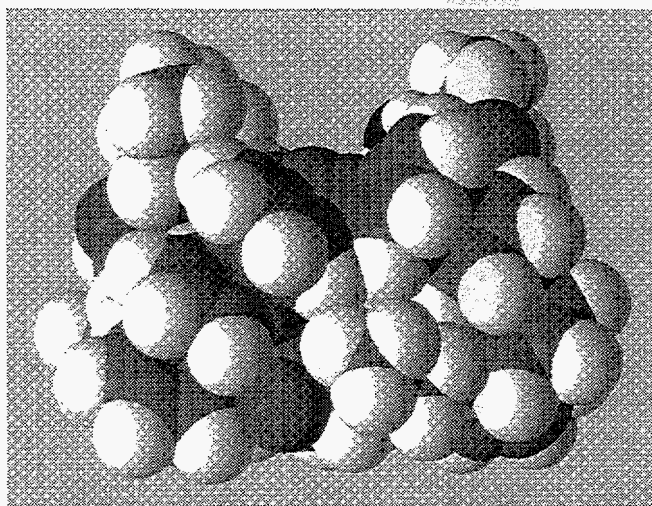


Figure 2. Structure of the  $\text{CO}_2$ -Ni(II) dodeca-(phenylvaline amide)porphyrin complex.

characteristic spectroscopic signatures for the two types of distortion. This is important from the standpoint of designing a rigid cavity for  $\text{CO}_2$ .

**Testing of  $\text{CO}_2$  Activation Catalysts.** Experimental testing of the designed porphyrin catalysts for  $\text{CO}_2$  reduction has been successful. We have now found electrode-electrolyte systems in which our catalysts carry out  $\text{CO}_2$  reduction. It is our immediate goal to use our molecular design methodologies to develop catalysts that selectively produce a desired  $\text{CO}_2$  reduction product at the minimum potential beyond what is thermodynamically required and with the greatest current efficiency possible.

An ASTRIS Quickcell is currently being used with  $\text{CO}_2$  gas flow at 5 ml/min at the reduction electrode and  $\text{H}_2$  at 100 ml/min at the oxidation electrode. The electrolyte is either 0.5 M  $\text{NaHCO}_3$  (pH 8.3) or 1.0 M KOH. The hydrogen electrode is a commercial electrode composed of Pt ( $0.5 \text{ mg/cm}^2$ ) on carbon cloth with Vulcan XC-72R from Electrosynthesis Corp. The  $\text{CO}_2$  electrode is an Alupower gas diffusion electrode modified with the porphyrin catalyst. The gas diffusion electrode is composed of a Black Pearls 2000 carbon catalyst and PTFE backing. The Co-porphyrin catalyst is added by soaking the gas diffusion electrode in a pyridine solution of the catalyst. The potential of the  $\text{CO}_2$  electrode is potentiostatically controlled between 0.0 and -1.5 V relative to a Ag/AgCl reference electrode. Product (CO) conversion is determined by gas chromatography given the known  $\text{CO}_2$  flow rate. No CO is observed from the cathode in the absence of the Co-porphyrin catalyst.

Figure 3 shows the current versus voltage curves for a CoTTP modified and an unmodified electrode. In the absence of the catalyst no CO is produced, but water is reduced to  $\text{H}_2$ , which is thermodynamically possible at potentials more negative than -0.76 V, although a sizable

bonding energy should be realized in the selective binding of  $\text{CO}_2$ . This chirally substituted dodecaphenylporphyrin should also serve as a catalyst for enantiomerically selective oxidation of olefins.

**Tetraalkylporphyrin Derivatives.** We have also synthesized Ni derivatives of a series of *meso*-tetrasubstituted porphyrins. These include tetraphenylporphyrin (TPP), tetramethylporphyrin (TmeP), tetraethylporphyrin (TetP), tetrapropylporphyrin (TprP), tetrapentylporphyrin (TpeP), tetraisopropylporphyrin (TIPrP), and tetra-*t*-butylporphyrin (TtBP). *Meso*-substitution invariably gives ruffled, as opposed to saddle, distortions of the macrocycle. The idea is to identify

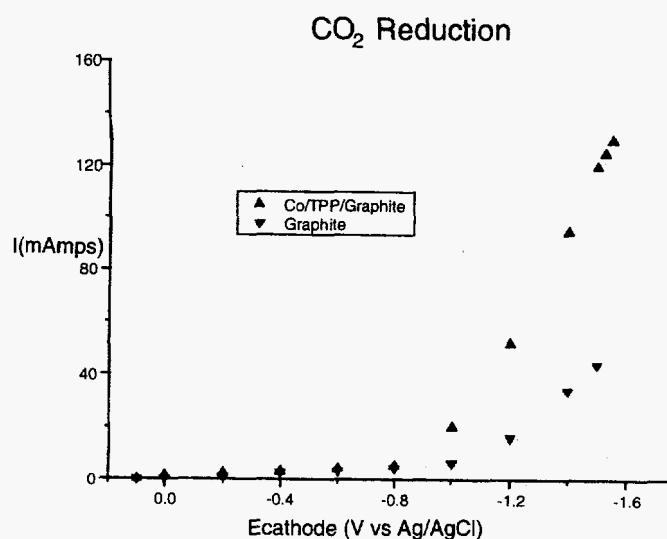


Figure 3. Current versus Voltage for Co-porphyrin modified electrode and unmodified electrode.

overpotential is required. In the presence of the CoTPP catalyst, CO<sub>2</sub> to CO conversion occurs along with H<sub>2</sub> generation above -0.82 V, the thermodynamic potential for CO<sub>2</sub> reduction to CO and by coincidence, the potential of the Co(II)/Co(I) couple. Conversions of up to 5 percent in the CO<sub>2</sub> stream have been observed. Similar conversions have been observed in 1.0 M KOH as well as 0.5 M NaHCO<sub>3</sub>. Finally, a CoDPP-modified electrode gives similar CO<sub>2</sub> conversion even at low catalyst loading on the electrode. Future work will focus on obtaining mass and current balance in the analytical methods, electrosynthesis electrode mass transfer limits, electrolyte make-up, and preparation of the electrode methods.

To aid in understanding the electrochemistry of CO<sub>2</sub> reduction in these cells we are investigating the solution electrochemistry of the porphyrin molecules being synthesized for this project. These experiments require varying solvent, electrolyte, electrode and other electrochemical parameters in order to make accurate measurements of the redox potentials of our catalysts which vary as a function of substituent. Making such structure/redox potential correlations allows us design porphyrin catalysts with optimal redox potentials.

Experiments on homogeneous solutions of our catalysts have also been carried out in search of shifts of the solution redox potential in the presence of CO<sub>2</sub> which would indicate binding of CO<sub>2</sub> to the molecule under study. Extensive testing of CoOEP, NiOEP, CoTPP and NiTPP in benzonitrile with either tetrabutylammonium perchlorate or tetrabutylammonium triflate electrolyte yielded no shifts in redox potential as a function of CO<sub>2</sub>. Preliminary experiments with two newer porphyrins which we expect to have a better "pocket" for CO<sub>2</sub> were also tried, but no CO<sub>2</sub> effects were observed. We now believe these negative results are caused by diffusion problems and that gas diffusion electrodes are required to evaluate the catalysts.

Because Japanese researchers have reported CO<sub>2</sub> reduction with porphyrins covalently attached to carbon electrodes, we have tried to make similar attachments. The surfaces of commercial glassy carbon electrodes were oxidized in a phosphate buffer at +2.5 V vs. SCE for 2 hours. It was then refluxed in thionyl chloride for 2 hours, soaked in a saturated solution of 4-amino pyridine in benzene overnight, and finally refluxed in a 0.3 mM solution of Co<sup>III</sup>TPP in a 4:1 mixture of benzene and CH<sub>2</sub>Cl<sub>2</sub>. Electrochemistry between -1.4 and +0.5 V vs. SCE was then performed in the phosphate buffer. As was reported in the literature, a reduction current was observed at more positive potentials in the presence of CO<sub>2</sub> than in its absence. It is not clear that this is CO<sub>2</sub> reduction, since CO<sub>2</sub> reduction products have not yet been identified.

Finally, other carbon supports and methods for binding porphyrins are being explored. In related experiments by others, phthalocyanines have been adsorbed from pyridine onto glassy carbon for the reduction of O<sub>2</sub>. In the application of our porphyrin catalysts for O<sub>2</sub> reduction we have been able to adsorb the Co OEP, Co OETPP and Co TPP onto glassy carbon and observe the catalytic reduction of O<sub>2</sub>. Moreover, the increasingly positive shift in reduction potential correlates with the  $\Sigma\sigma_m$  of these porphyrin macrocycles. These results indicate to us that it is possible to adsorb our porphyrins on glassy carbon, and that, at least for O<sub>2</sub>, the expected correlation occurs. We have obtained several different carbon materials, including highly ordered pyrolytic graphite (HOPG), two different vitreous carbons, and carbon foams and cloths. Each of these may be expected to have different interactions with porphyrin catalysts. In particular, the carbon cloths have been reported as good catalyst substrates, and HOPG would be expected to have unique support features.

**Characterization of Advanced Catalysts.** Characterization studies using NMR, resonance Raman spectroscopy, X-ray crystallography and other methods are described in the attached reprints and manuscripts.

**Quantum Mechanical Modeling.** We have recently grown a crystal of the simplest metal porphyrin, Ni porphine (NiP). The crystal was sent to Prof. W. R. Scheidt at Notre Dame University who has obtained the X-ray crystal structure at both low and room temperatures. This is the first crystal structure of a metal porphine, a molecule small enough that ab initio molecular orbital methods can be applied with large basis sets, electron correlation contributions, and geometry optimization. Both LDF and HF methods are being used to predict the structures of NiP for comparison with the experimentally obtained structure.

**Development of New Modeling Methods.** We have been Beta-testing Molecular Simulations new CERIU<sup>2</sup> interface to POLYGRAF, to which we have had significant input.

**Table 1. Calculated total energies for bis-axial ligand complexes with the plane of the ring of the ligand oriented at various angles about the z-axis of CoOETPP and the estimated and measured (NMR) rotational barriers.**

Rotation Angle (deg)	pyridine	1-methyl-imidazole	4-phenyl-imidazole
0	390.7	392.8	420.9
22.5	391.2	393.3	421.6
45	396.8	396.9	425.3
67.5	407.5	402.8	431.6
90	416.4	407.0	436.5
Est. Rotational Barrier	25.7	14.2	15.6
Measured Barrier	(3-Cl-py) 12.6	≤8	≤8

*Model CO<sub>2</sub>-Cavity Interactions.* Using NMR methods we have determined that the groove-shaped cavity of CoOETPP complex orients pyridine axial ligands, but not imidazole ligands. The barrier to rotation was experimentally determined from the temperature-dependent NMR studies for the pyridine ligands. The barriers to rotation were also estimated from the molecular mechanics calculations in which the bis-complex has the plane of the axial ligand ring constrained at various angles between 0 and 90° about the z-axis measured from the major axis of the groove. Table 1 shows the calculated energies for several angles for the ligands pyridine, 1-methyl-imidazole, and 4-phenyl-imidazole. From the lowest (ring parallel to the groove) and highest (perpendicular to the groove) energies, the rotational barrier is estimated (see Table 1). Clearly, the molecular mechanics calculations overestimate the rotational barrier, but the trend toward lower barriers for the 5- versus 6-membered rings is predicted.



### 4.3 Advanced Bioprocess Systems

10. **PROJECT TITLE:** Bioprocess Engineering: Immobilized Cell Systems for Continuous, Efficient, Biocatalyzed Processes

**PRINCIPAL INVESTIGATORS:** B. H. Davison, E. N. Kaufman, C. D. Scott and T. C. 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.).

#### **1994 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 biparticle FBR was operated continuously for a period of 48 h and demonstrated a productivity of 4.7 g/L-h, a 12-fold improvement over fermentations conducted without resin addition in the same reactor. An eight-fold increase in product concentration and a 1,734-fold enhancement in separation of product from substrate was realized when regenerating the sorbent particles used in the biparticle FBR. An initial economic assessment of the biparticle processing scheme as opposed to batch fermentation has revealed that the biparticle reactor should have equal chemical and operating costs, but lower reactor costs due to its continuous nature and higher productivity.

- The degradation of two 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.

Mass transfer coefficients have been measured for trickle bed and shake flasks. The trickle bed has achieved rates up to 40-60 g VOCs/h/m<sup>3</sup> using actively growing microbes. We confirmed

that the VOCs can be degraded by microbes as sole carbon and energy sources during stable operation for over 36 months in a bubble column.

- A fully predictive model for the multiphase FBR was developed for use in assisting scaleup of a FBR for ethanol production.

The model is the first fully predictive model for a fluidized-bed bioreactor incorporating mass-transfer resistances within the biocatalyst beads, changing phase holdups and axial dispersion due to CO<sub>2</sub> production. The model allows a semiquantitative fit of the experimental data and is being extended to a larger system. It was developed in collaboration with Washington State University.

- An economic analysis of the ethanol FBR by Fluor Daniel indicated that savings of up to 6¢/gal would be expected by using the FBR in place of a fed-batch system with yeast.
- The Symposium on Biotechnology for Fuels and Chemicals was partially supported.

#### **1995 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: (1) evaluation of the primary mechanisms; (2) integrated study of the new concept on a bench scale; (3) development of a predictive mathematical model; (4) establish technical feasibility at an adequate scale; and (5) 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. Mass-transfer coefficients and kinetics will be used to maintain increased biomass and experimentally evaluate gas-continuous trickle bed versus liquid-continuous bubble columns to determine feasibility. This understanding will be extended to other gas-phase reactions.

Advanced bioreactors for nonaqueous processing will be investigated in using biocatalysts in biphasic liquid systems. ORNL has begun an assessment document on nonaqueous multiphase biocatalysis for DOE. For our experimental effort, the biocatalysts (primarily enzymes, but some microbes) will be in the aqueous phase and reactor with substrates and product in the immiscible organic phase. This will examine systems of interest to other elements of the program. Aqueous-

organic reactors will be tested with a small effort using the solvent extractive FBR for butanol production. 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.

The use of attrition reactors for hydrolysis of lignocellulosic feedstocks will be supported at a low level.

The following bioprocessing systems will be completed and phased out during FY94 to emphasize the effort in nonaqueous bioprocessing. The development effort for the biparticle FBR for simultaneous fermentation and separation will be completed with continuous bench-scale operation for lactic acid production using immobilized *Lactobacillus*. Lesser parallel efforts will continue in resin regeneration. The modeling and analysis effort for the fluidized bed for ethanol production has been completed and is being continued for support scaleup and commercialization in another program.

#### **ANNUAL TECHNICAL SUMMARY REPORT:**

Advanced bioreactors can greatly increase the productivities and yields of microbial fermentations and enzymatic reactions for useful products such as chemicals and fuels. 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. We continue to extend the use of multiphase bioreactors with high concentrations and active biocatalysts.

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 the usual three (solid catalyst, liquid media, and gas coproduct). This approach is to add a fourth phase with extractive capability for the desired product. This will serve to enhance the fermentation by moderating reactor pH, while simultaneously accomplishing 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 fluidized bed. These particles progress downward through the fluidized biocatalysts, adsorb the inhibitory product, and are removed from the base of the columnar reactor. The feasibility of such a concept has been demonstrated in an unoptimized system for lactic acid fermentation using the polyvinyl-pyridine resin,

Reillex 425. In these experiments, resin was added to the fluidized bed in a batch-wise manner and the acid product was not recovered from the resin.

Ongoing research has focused upon the design and demonstration of a biparticle FBR with continuous resin addition and product recovery. We have screened a series of sorbents for their capacity, selectivity, ease of regeneration, and kinetic properties. The weak-base ion-exchange resin, Amberlite IRA-35, was selected for the following tests and regenerated with acid and base. We compared fermentation runs with continuous resin addition to those conducted without resin

addition and to fermentations conducted with identical biocatalyst and media in a batch reactor. The biparticle FBR was operated continuously for a period of 48 h and demonstrated a productivity of 4.7 g/L·h, a 12-fold improvement over fermentations conducted without resin addition in the same reactor. An eight-fold increase in product concentration and a 1,734-fold enhancement in separation of product from substrate was realized when regenerating the sorbent particles used in the biparticle FBR. An initial economic assessment of the biparticle processing scheme as opposed to batch fermentation has revealed that the biparticle reactor should have equal chemical and operating costs, but lower reactor costs due to its continuous nature and lower productivity. A base case scenario for the production of 1 kg of lactic acid in a 4 L reactor revealed that this process would require 7.5 d in a batch reactor as opposed to 2.2 d in the biparticle FBR.

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. Improvements in this technology could allow replacement of the current techniques of incineration or adsorption which are energy expensive for dilute streams. ORNL is also elucidating the engineering principles that govern the behavior of these systems for bioreaction of sparingly soluble gaseous substrates, which should have broader applicability.

The removal of dilute hydrocarbon gases from effluent gas streams is of particular interest, with the primary emphasis on *n*-pentane and isobutane. The degradation of these industrial VOCs was tested in various gas-phase reactor systems (bubble columns, trickle beds, and shake flasks) to confirm and elucidate mass transfer and kinetic limitations. These gases are sparingly soluble in water, therefore, good mixing and high-surface area between the gas and liquid phases is required. One of the liquid-continuous columnar bioreactors (or bubble column) has been operating for over 36 months with continued degradation of pentane and isobutane as sole carbon and energy sources for the microbial consortia. The maximum degradation rates in the bubble column so far are 2 g VOCs·h/m<sup>3</sup>.

Mass transfer was determined to be controlling under many conditions in each of the reactor systems tested. Frequently the reactors would change from reaction limited to mass-transfer limited when the biomass increased. A trickle-bed bioreactor (or trickling biofilter) was operated continuously for 9 months to provide a high-surface area for an active biofilm with increased rates. It is a gas-continuous columnar reactor with a trickling liquid film. The consumption rates have increased along with the increased biomass and the maintenance of the biomass in a growth state. During a three-month test, the consumption was about 2 g·h/m<sup>3</sup>, at 6 weeks it was 7 g·h/m<sup>3</sup> and at the end it had increased to above 60 g·h/m<sup>3</sup>. At this point the packing is covered with a thick biofilm and plugging is beginning to be a problem. Mass-transfer coefficients have been measured for trickle bed to be  $K_L a \epsilon_L = 30 \text{ h}^{-1}$ . Mass transfer was also measured in shake flasks.

In order to increase and control the biomass, batch experiments were performed that determined that nitrogen was limiting in the mineral salts media used. In closed batch systems, pH effects due to CO<sub>2</sub> production can also be significant. We are repeating experiments to elucidate the growth and nongrowth associated alkane consumption rates. It has been observed that the growth-associated consumption is higher than the nongrowth consumption rates. Microbial isolation has

indicated that we are dealing with a mixed culture, not all of which are actively consuming the alkanes.

Ethanol FBR: Economics, Modeling and Hydrodynamics - The demonstration at a larger scale of the high-productivity ethanol fermentation using immobilized *Zymomonas mobilis* in a FBR is progressing with funding through the National Renewable Energy Laboratory (NREL) in the Alcohol Fuels Program. The Advanced Industrial Concepts Division (AICD) Program funded the initial justifying work in this area and will support this effort through modeling and economic assessments. Under a subcontract from ORNL, Fluor Daniel, Inc. performed an economic and engineering assessment of the ethanol FBR system in direct comparison with conventional technology. This study analyzed the economic impact that a fluidized-bed reactor for using immobilized *Zymomonas mobilis* (as devised by ORNL) would have on a plant converting corn starch into ethanol. The study addresses substituting this new technology into an existing plant or using it for a new plant and used flow schematics and material balances to generate equipment pricing and capital and operating costs. These conceptual design results were then compared, allowing for an evaluation of the operating costs between the FBR and traditional technologies. The study results show that the FBR technology can provide a significant reduction in the production costs of ethanol – over 2¢/gal savings if inserted into an existing plant, and over 6¢/gal savings if used at a new plant. The study also examines the consequences of several FBR design parameters, using a series of sensitivity analyses.

Dr. J. N. Petersen of Washington State University and ORNL jointly developed a fully predictive mathematical description of a three-phase, tapered, fluidized-bed bioreactor with immobilized microorganisms. This model is to be used to assist the scaleup of the ethanol FBR. This model includes the effects of the coproduct CO<sub>2</sub> on the variable dispersion coefficient and solid holdup and the resulting concentration profiles in the bed. In addition, the effect of the concentration profile which is developed inside the biocatalyst bead is included by means of an effectiveness factor calculation. Using accepted correlations for the dispersion coefficient and for the liquid, gas and solid holdup in the bed, the model is fully predictive. The model was found to adequately predict experimentally obtained concentration profiles.

Biocatalysis in Nonaqueous Media - ORNL has begun an assessment document on nonaqueous multiphase biocatalysis for DOE. Experimental efforts were begun on biocatalysts will be investigated in biphasic liquid systems.

The dehalogenation of process byproducts is being investigated in a new confidential with Dow Chemical Co. The primary emphasis of work at ORNL has been divided into three areas: (1) conceptual reactor flowsheet; (2) development of analytical and enzymology techniques; and (3) culturing of two strains of bacteria obtained from Dow Chemical. The microbes have been transferred and grown at ORNL allowing ORNL to immobilize the dehalogenase.

11. **PROJECT TITLE:** Enzyme Catalysts for a Biotechnology-Based Chemical Industry

**PRINCIPAL INVESTIGATOR:** F. H. 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.

**1994 ACCOMPLISHMENTS:**

1. Subtilisins exhibiting improved activity in organic solvents.

Screening of randomly mutagenized subtilisins E for increased subtilisin activity in DMF carried out during the last quarter of the previous project resulted in the identification of a variant with high activity in organic media and high levels of expression. The gene for this enzyme (termed 13M) has been sequenced to identify the amino acid substitutions, and it has been further characterized. This enzyme is 800-fold more efficient towards peptide hydrolysis in 60% DMF than the wild-type enzyme and is expressed at much higher levels than previous variants.

2. Enzyme-catalyzed polymerizations.

Novel subtilisins have been used to catalyze the polymerization of natural and nonnatural amino acid esters (pentenylglycine and allylglycine methyl esters). We have investigated several factors that control the yield, overall reaction rate and the degree of polymerization achieved. In addition, the polymer products have been characterized using HPLC, fourier transform infrared

spectroscopy (FTIR), nuclear magnetic resonance ( $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$ ), plasma desorption mass spectroscopy and FAB-mass spectroscopy.

### 3. Random mutagenesis of pNB esterase for improved activity in organic solvents.

A significant accomplishment was made this period when Caltech signed a license agreement with Eli Lilly to engineer the p-nitrobenzyl esterase in order to increase the enzyme's activity in polar organic solvents. Thus far an efficient screening method has been developed, and two rounds of random mutagenesis and screening have already resulted in a 2-8-fold improvement in catalytic efficiency.

## **1995 PLANNED ACTIVITIES:**

### 1. Subtilisin engineering.

We will continue our efforts to develop subtilisin variants suitable for peptide ligation and polymerization. This part of the project involves the following specific tasks:

- a. Development of the rapid screening assay for peptide ligation.
- b. Subtilisin random mutagenesis and screening. PCR mutagenesis combined with the screening assay developed in part a will be used to engineer enzyme variants suitable for peptide ligation.
- c. Characterization of subtilisin variants. Promising variants will be purified and characterized.
- d. Characterization of polymer products. The products of peptide polymerization will be characterized and subjected to additional modifications to make potentially useful materials.

### 2. p-Nitrobenzyl esterase.

Random mutagenesis and screening of the pNB esterase will be completed in the 2nd year. The most promising variant(s) will be fully characterized (purification, sequencing, kinetic analysis), and their performance in organic solvents will be compared to that of the wild-type enzyme. This involves the following tasks:

- a. Finish random mutagenesis and screening to identify the most promising variants.
- b. Purify variants to homogeneity and determine their performance in HPLC assays using the antibiotic substrate.
- c. Sequence all interesting variants
- d. Prepare publication(s) and patent(s)

### 3. Identification of a new enzyme for modification.

We plan to identify an additional enzyme for further improvement by random and site-directed mutagenesis. The enzyme will most likely be proposed by an industrial collaborator.

## ANNUAL TECHNICAL SUMMARY REPORT:

This research to evolve new enzymes for technological applications relies on the premise that there is a great deal of room for improvement when enzymes are asked to perform jobs for which they were never evolutionarily optimized. Those of use 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.

Although ~ 3000 enzymes have been isolated and characterized, we know this represents a tiny fraction of the functions in the huge biological bank displayed by nature--the so-called natural diversity. Limiting oneself even to this much larger set is shortsighted, however, as these molecules, too, have evolved within the context of living organisms. The possible function space is much larger indeed! This DOE-sponsored research has been dedicated to probing the limits of this space and to identifying practical strategies for obtaining new and useful functions.

### **Subtilisin mutants exhibiting improved activity in organic solvents.**

Random mutagenesis and screening 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. The gene for this enzyme (termed 13M) has been sequenced to identify the amino acid substitutions, and it has been further characterized. 13M subtilisin E is the product of two sequential rounds of random mutagenesis and screening performed on 10M subtilisin E (10 amino acid substitutions). Three additional amino acid substitutions not found in the 10M variant were identified by gene sequencing. *This enzyme is 800-fold more efficient towards peptide hydrolysis in 60% DMF than the wild-type enzyme (~ 2.5 times more efficient than the 10M).* Because this variant is expressed at much higher levels than previous variants, it is much better suited to application in organic synthesis. Large-scale expression and purifications are now being carried out to prepare quantities of the catalyst for synthetic studies.

Variants of subtilisin significantly more active in organic solvents have been used to catalyze the polymerization of natural and nonnatural amino acid esters. We have investigated several factors that control the yield, overall reaction rate and the degree of polymerization achieved. In addition, the polymer products have been characterized using HPLC, fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance ( $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$ ), plasma desorption mass spectroscopy and FAB-mass spectroscopy.

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 efforts to develop a sensitive assay to screen random mutants directly for synthesis activity (rather than



hydrolytic activity) are underway. In this screening assay, an amino acid ester is attached to a filter paper. Another amino acid ester is labeled with fluorescein on a long, flexible linker. Colonies expressing an enzyme able to ligate the two esters will be identifiable on the filter paper through the fluorescent signal.

### **Evolution of an esterase for large-scale antibiotics synthesis:**

We are working in collaboration with a major pharmaceutical company to engineer an enzyme for use in antibiotics production. 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. Although the natural enzyme exhibits the desired activity, the enzymatic process is currently not economically feasible due to the very limited solubility of the antibiotic substrates in aqueous media. The rate of hydrolysis in polar organic solvents is very low. We are attempting to improve the enzyme's activity in the presence of polar organic solvents.

We started with the wild type gene from *B. subtilis* cloned into an *E. coli* expression vector. This system expresses the enzyme intracellularly at high levels in active form. Examining the sequence of the esterase, we found a high degree of similarity between it and acetylcholine esterase (greater than 34% sequence identity). Since acetylcholine esterase's crystal structure is known, we should be able to reasonably approximate this esterase's structure. If the natural substrate of the Lilly esterase is acetylcholine or a related substance, there is also reason to believe that there is a great deal of room for improvement in substrate selectivity as well as activity in the presence of the organic solvent. Thus we are using sequential random mutagenesis to evolve an optimized biocatalyst for this reaction.

From the sequence data single-stranded DNA primers flanking the esterase gene were designed. We used these primers, located outside of two unique restriction sites, to carry out PCR-based random mutagenesis. Expression of the pNB esterase gene is controlled by a temperature-sensitive repressor such that the transformant colonies are grown at 30 °C and then are heat treated at 42 °C for 8 hours to induce protein expression. Colonies are screened for expression of an enzyme variant that exhibits higher activity in the presence of dimethylformamide (DMF). We have developed two screening assays using substrates closely related to the desired reaction, both of which yield color changes upon reaction and are suitable for rapid assays. As of August, 1994, two rounds of random mutagenesis and screening have increased the enzyme activity several-fold, as in Figure 1.

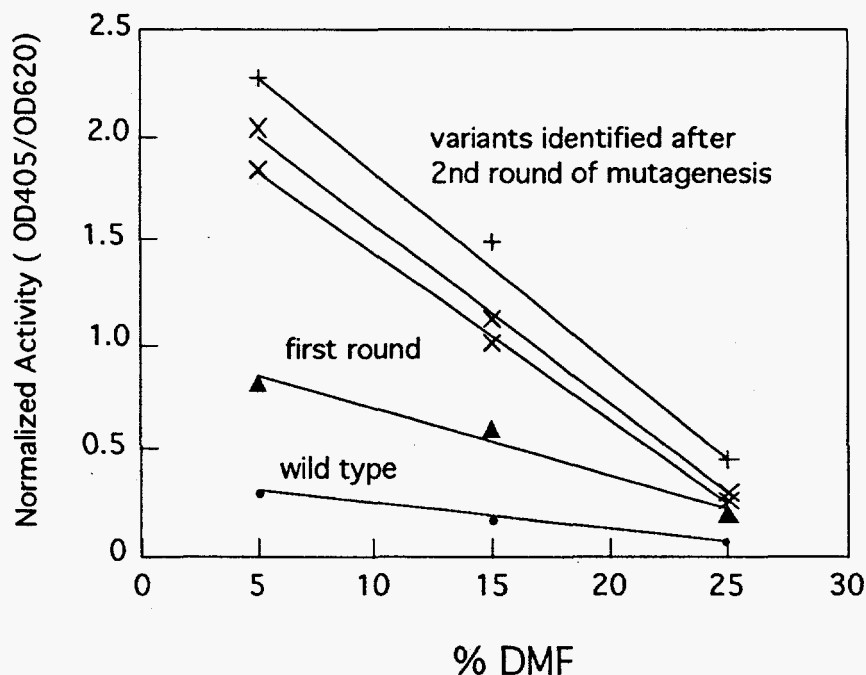


Figure 1 Results of two rounds of random mutagenesis and screening of esterase. Activity is improved over the entire DMF range measured (5-25 vol %)

Results of two rounds of random mutagenesis and screening of esterase. Activity is improved over the entire DMF range measured (5-25 vol %).

12. **PROJECT TITLE:** Rational Enhancement of Enzyme Performance in Organic Solvents

**PRINCIPAL INVESTIGATOR:** A. M. 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.

## 1994 ACCOMPLISHMENTS:

The overall goal of this project is to rationally improve the catalytic performance of enzymes in organic solvents and to employ biocatalysis in such media for potentially useful transformations. During the past year,

- We have demonstrated that the marked solvent dependence of substrate specificity of the protease subtilisin Carlsberg can be quantitatively explained on the basis of thermodynamic parameters, such as activity coefficients of the substrates in different solvents which we calculate using the UNIFAC computer algorithm. This achievement is a significant step toward controlling substrate specificity of enzymes at will, thus providing heretofore unavailable means of optimization of enzymatic processes.
- We have found that enantioselectivity in organic solvents of subtilisin Carlsberg covalently attached to macroporous polymeric supports may be controlled by support's aquaphilicity. Furthermore, the catalytic activity of immobilized subtilisin in organic solvents is strongly affected by the enzyme pretreatment. These findings suggest additional approaches to enhancing efficiency of enzyme-catalyzed processes.
- We have elaborated a new approach to enzymatic acylation of sugars in organic solvents which could lead to useful materials.

## 1995 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, several avenues of investigation, listed below, will be pursued.

The possibility of activating lipases in organic solvents by immobilization support-induced opening of the "lid" covering their active centers will be explored. Recent X-ray crystallographic studies have revealed that all lipases whose structures have been solved have such a "molecular door". In water, this lid is normally closed, thereby making the lipase active center inaccessible to substrates. When the lipase adsorbs on an interface, the protein molecule undergoes a conformational change, the lid opens, and substrate molecules can enter the active center. What is the status of the lid in organic solvents, i.e., is it closed or open? Can individual organic solvent molecules bind to the lipase macromolecule and open the lid? How does this process depend on the nature of the solvent? Alternatively, can the lid be opened by immobilizing lipases on hydrophobic supports? These are the questions to be addressed experimentally by us.

We will attempt to broaden our UNIFAC computational approach to rationalizing the solvent dependence of enzymatic substrate specificity (see the Annual Technical Summary Report below). In particular, we will extend it to the *nucleophile* (rather than the ester) substrates employed in subtilisin-catalyzed transesterifications in organic solvents. In addition, we will test the validity and predictive power of our computer-assisted calculations for a non-protease group of enzymes, namely lipases.

In order to put our studies of nonaqueous enzymology on a firm mechanistic footing, we will look into the structure of lyophilized proteins in organic solvents. Specifically, we will adopt the

methodology of hydrogen isotope exchange, followed by high-resolution NMR spectroscopy, to ascertain whether most protein molecules in lyophilized powders suspended in organic solvents are partially unfolded (as our previous BCTR-sponsored work suggests). Further insights into this issue will be obtained by examining enzymatic activity in protein-dissolving vs. non-dissolving organic solvents (stabilizing protein-protein contacts will exist in the latter but not in the former).

#### ANNUAL TECHNICAL SUMMARY REPORT:

The overall goal of this project is to rationally improve the catalytic performance of enzymes in neat organic solvents and to employ biocatalysis in such media for potentially useful synthetic transformations. During the project period reported herein, we have focused (i) on control of substrate selectivity of the protease subtilisin Carlsberg by the solvent, (ii) on control of catalytic activity and enantioselectivity of this enzyme in organic solvents by immobilization support, and (iii) on lipase-catalyzed acylation of sugars in anhydrous hydrophobic media.

(i) In our previous work, the substrate specificity of the protease subtilisin Carlsberg in the transesterification of *N*-Ac-L-Ser-OEt and *N*-Ac-L-Phe-OEt with propanol was found to be markedly dependent on the solvent and, in fact, directly proportional to the ratio of solvent-to-water partition coefficients of the substrates; a thermodynamic model was developed which explained this phenomenon. Now, the utility of this model has been broadened to include water-miscible solvents (for which the partition coefficients cannot be readily determined experimentally). Additionally, the need to experimentally measure the partition coefficients has been eliminated altogether through the use of a computer program which uses the UNIFAC algorithm to calculate the partition coefficient ratios. The calculated partition coefficient ratios satisfactorily predict subtilisin's substrate specificity in water-miscible, as well as in water-immiscible, solvents.

(ii) Subtilisin Carlsberg has been covalently attached to five macroporous acrylic supports of varying aquaphilicity (a measure of hydrophilicity). Kinetic parameters of the transesterification of *S* and *R* enantiomers of *sec*-phenethyl alcohol with vinyl butyrate, catalyzed by various immobilized subtilisins, have been determined in anhydrous dioxane and acetonitrile (these solvents have been previously found to afford extreme - the highest and the lowest, respectively, - enantioselectivities of subtilisin). Enzyme enantioselectivity in acetonitrile (but not in dioxane) correlates with the aquaphilicity of the support: the greater the latter, the higher the enantioselectivity; a mechanistic rationale for the phenomenon has been proposed. While the catalytic activity of immobilized subtilisin in anhydrous solvents is a strong function of the mode of enzyme pretreatment (for example, depending on whether the immobilized enzyme was dried under air vs. lyophilized, or dried with vs. without subsequent washing, or lyophilized with vs. without excipients, its catalytic activity in the same organic solvent may vary up to 50 fold), the enantioselectivity is essentially conserved.

(iii) By complexing glucose and other mono- and disaccharides with phenylboronic acid, they have been solubilized in many organic solvents, including hydrophobic ones. *Pseudomonas* sp. lipoprotein lipase readily acylates such solubilized sugars in these solvents (significantly, no reaction is observed without phenylboronic acid, i.e., when sugars are insoluble in the reaction medium). Activated esters of various carboxylic acids, a number of triglycerides (different plant oils), and even free fatty acids all can act as acylating agents in the enzymatic transesterification.

Solubilized  $\alpha$ -D-glucose has been enzymatically acylated with vinyl acrylate on a preparative scale, and the resultant 6-O-acryloylglucose has been chemically polymerized to yield a sugar-based polyacrylate material with a molecular weight of 14,000 Da.

13. **PROJECT TITLE:** Immobilized Enzymes in Organic Solvents

**PRINCIPAL INVESTIGATOR:** Haya Zemel

**PROJECT SITE:** AlliedSignal Research & Technology  
Des Plaines, IL

#### **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 immobilize 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.

#### **1994 ACCOMPLISHMENTS:**

A major factor controlling the immobilized enzyme performance in non-aqueous media was determined to be the aggregation of support particles at high water content.

Using a lipase catalyzed inter-esterification reaction as a model, we studied the controlling factors when water content in the organic solvent is such that a micro-aqueous phase is formed. In such an environment it was found that support particle aggregation is the major cause for decline in enzyme activity. We have shown that particle porosity, as well as the use of a particular non-woven fabric as an enzyme support, could alleviate this problem. These findings are being translated into a bioreactor design.

Enhanced performance of proprietary support was demonstrated also under dry conditions.

We have also studied two "dry" non-aqueous systems, where a water phase is not formed since the water content is below its solubility in the organic solvent. In one of the systems, Subtilisin catalyzed trans-esterification of vinyl acrylate with a chiral alcohol, we have demonstrated that the use of a proprietary fabric support provides a significant boost in enzyme activity. We suggest that this particular fabric with its hydrophilic fibers acts as a lyoprotectant in the process of drying the enzyme. The benefits of this material as an enzyme support and its use in a lab scale bioreactor are being studied.

An enzymatic synthesis of Allied Signal proprietary monomer was developed.

Preliminary experiments have also been performed with a second "dry" reaction. This is the lipase catalyzed synthesis of AlliedSignal's new product, VEctomer 4010. The enzymatic synthesis of this product could potentially provide a cleaner route to a better-performing material. We have so far demonstrated that the product can indeed be made enzymatically in a non-aqueous environment, and that rates could be improved by adding 3% water and using PEG as a lyoprotectant.

#### **1995 PLANNED ACTIVITIES:**

The coming year will be devoted to designing and building a lab scale reactor for continuous enzymatic production of a specialty monomer. The non-woven proprietary fabric will serve as an enzyme support. As a part of this effort we will conduct the following work:

1. We will continue investigating the proprietary fabric as a potential bioreactor enzyme support. The effect of fabric composition on the immobilized enzyme performance will be studied. In collaboration with our Fibers Business unit we will produce fabrics with various compositions and test their performance.
2. We will determine the commercial viability of enzymatic VEctomer 4010 production.
3. Depending on the viability of each of the monomers we have studied, we will make a selection and develop a bioreactor for the monomer production. Various reactor configurations for continuous operation will be examined. We will then study and improve the long term operating parameters of the enzymatic reactor. At the end of the period, enough material will be produced for polymerization.

## **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 by external factors such as water content, support, pH, etc.. We have investigated the interrelation between support morphology and water content, and its effect on overall enzyme performance.

Using a lipase catalyzed inter-esterification reaction as a model, we studied the controlling factors when water content in the organic solvent is such that a micro-aqueous phase is formed. In such an environment it was found that support particle aggregation is the major cause for decline in enzyme activity. We have shown that particle porosity, as well as the use of a particular non-woven fabric as an enzyme support, could alleviate this problem. These findings are being translated into a bioreactor design.

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Preliminary experiments have also been performed with a second "dry" reaction. This is the lipase catalyzed synthesis of AlliedSignal's new product, VEctomer 4010. The enzymatic synthesis of this product could potentially provide a cleaner route to a better-performing material. We have so far demonstrated that the product can indeed be made enzymatically in a non-aqueous environment, and that rates could be improved by adding 3% water and using PEG as a lyoprotectant. Although significant improvement has been achieved in the efficiency of this trans-esterification reaction, the rate of VEctomer production is still very low from the standpoint of a commercial process. In addition, under the best operating conditions, significant quantities of by-products are formed. We will be addressing these problems in the next few months.

14.    **PROJECT TITLE:**                                   Biological Separation of Phosphate from Ore
- PRINCIPAL INVESTIGATOR:**   R. D. Rogers
- PROJECT SITE:**                                   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.

#### **1994 ACCOMPLISHMENTS:**

- Provided technology transfer to CRADA partner. Planned "teaming" activities have been carried out with the J. R. Simplot Co. Separation of phosphate product from technology developed at the INEL has been made available to Simplot.
- Developed understanding of biomechanism responsible for separations. We have isolated the gene responsible for the biosolubilization of phosphate ore. This has led to the development of a working hypothesis on the part microorganisms play in the phosphate solubilization process.
- Developed a model "micro encapsulated" bioseparation process. Work based on the mechanism of solubilization provided data which indicated that sufficient microbial activity could be maintained in a 2 mm bead containing phosphate ore and an organic binder to allow for micro release of soluble phosphate.
- Patent application submittals: Two applications were submitted to the U.S. patent office. Subjects of the patents were the genetic pathway responsible for solubilization and encapsulation technology.

#### **1995 PLANNED ACTIVITIES:**

Proposed work will be developed around three critical hurdles identified for this project:

- Development of methods to obtain soluble phosphate concentrations which will satisfy manufacturing and marketing requirements as specified by our commercial CRADA partner.
- Reduce the number of separations steps required during processing in order to decrease both capital equipment costs and energy consumption.
- Development of methods to maintain a pure microbial culture without the need for extensive sterilization of process streams.

Research and engineering efforts which were started during FY 1994 will continue to be applied to overcoming these hurdles in order to commercialize a viable biosolubilization process.

#### **ANNUAL TECHNICAL SUMMARY REPORT:**

Work on the Mineral Phosphate Solubilization (Mps) gene and the mechanism of solubilization continued. Studies were conducted which indicated that the mechanism responsible for phosphate ore (RP) solubilization is due to proton substitution and not necessarily bulk H<sup>+</sup> concentration. This conclusion was reached after conducting abiotic work with high concentrations of gluconic acid (GA) and isolates of *Pseudomonas cepacia* with and without the Mps gene. Data from the



GA concentration studies showed that solubilization of RP was stoichiometrically related to the acidic protons provided by GA given a  $pK_a$  of  $\sim 3.6$ . There was no evidence of calcium chelation by the GA. Further, the data confirmed early reports that the presence of microorganisms boosts the quantity of RP that is solubilized by at least an order of magnitude.

Three isolates of *P. cepacia* were assayed in both liquid and with the solid agar bioassay plates to study the relationship between expression of the direct oxidation pathway, acidification of the external medium and mineral phosphate solubilization. The isolates were designated E-37, 249-VM (both wild type isolates), and 249-100 (a mutant of 249-VM). Both wild type isolates are capable of promoting solubilization of RP, however, 249-100 does not solubilize RP because it does not have quinoprotein (PQQ) glucose dehydrogenase (GDH) activity. Our work has shown that the direct oxidation pathway (GDH) for glucose catabolism is the mechanism responsible for solubilization in *P. cepacia*. When grown in modified M-9 minimal medium, all three strains showed approximately equivalent levels of medium acidification. However, on the bioassay plates only E-37 and 249-VM showed clearing zones (an indication of solubilization). Analysis of these zones or the equivalent region of agar around 249-100 showed that, as expected, clear zones had enhanced levels of soluble phosphate. However, the pH of the agar adjacent to all three strains was approximately the same. It was of interest, though, that approximately the same activity was obtained for all three strains when 10nM PQQ was added to the medium. These experiments conclusively demonstrated that the direct oxidation pathway is essential for expression of the Mps phenotype (solubilization of RP) in *P. cepacia* while at the same time showing that acidification per se is not the causative agent of biological solubilization of mineral phosphate.

Other studies have been devoted to the design of a system which can be used to produce a biosolubilized phosphate product. A compact bioreactor design was developed which could significantly reduce the quantity of water required for RP solubilization. The prototype of this system consisted of the components of the process (*P. cepacia* and RP) packed into dialysis tubing. The tube was placed into a column which was then filled with washed quartz sand. Sufficient M-9 medium was then passed through the column to activate the solubilization process. A preliminary test of the method showed that significantly more GA and solubilized phosphate were recovered in the treatments than in the controls.

The above work lead to the investigation of the possibility of encapsulating the component parts of the biosolubilization process into small, 2-3 mm diameter beads. Initial work on this process used alginate as an encapsulation matrix. Various studies using this material showed that it was possible to maintain activity RP solubilization when the microbial component E-37 was incorporated into alginate with a supply of RP. 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 will be subjects of future work.

#### 4.4 Feedstock/Process Interactions

15. **PROJECT TITLE:** Enhanced Carbon Dioxide Utilization for Biopolymer Production: an Evaluation of an Enzymatic Culture Devisocofication Approach

**PRINCIPAL INVESTIGATOR:** R. Kern

**PROJECT SITE:** Jet Propulsion Laboratory

#### **DESCRIPTION:**

The long term goal of this project is to construct CO<sub>2</sub> fixation plasmid genes to convert CO<sub>2</sub> and hydrogen to biodegradable polymers such as the polyesters (PHB) from the bacterium, *Alcaligenes eutrophus* or polysaccharides from a variety of other microbial sources. These polyester plastic and polysaccharide materials have a wide variety of applications, including the food and cosmetic industries, rheology modifiers, enhanced oil recovery, chromatography industry, the paper and metals finishing industries, and packaging industries. Currently many of these products are derived from plant sources outside the United States. It is estimated that the use of microbes for polymer synthesis can reduce the costs of manufacture from four to six times over that of conventional chemical synthesis. However, the cost of isolation of this product from bioreactors is still too high for the product to compete with larger volume film/packaging applications. ICI has the bulk of the patents in this area in the production of PHB and copolymers of PHB and polyhydroxyvalerate from glucose. Bacteria such as *A. eutrophus* grow rapidly (2-3 h generation time) in the presence of carbon dioxide as the sole carbon source, hydrogen for energy from some source, and oxygen as electron acceptor. These generation times are comparable to those in the biological production of commercial aminoacids. Nutrient requirements appear minimal (including nitrogen). High cell densities are achieved and about 70-80 wt% of polymer granules are produced intracellularly from a glucose-utilizing mutant. After cell disruption, the polymer is extracted with an organic solvent. For other systems, the polysaccharide is excreted making the broth extremely viscous and limiting the access of cells to substrates, especially CO<sub>2</sub>. In the short term, this project has two overall goals. One, examine ways to devisocofy media broths and enhance the utilization of CO<sub>2</sub> by microbes. Second, begin genetically modifying *A. eutrophus* to use CO<sub>2</sub> directly in the production of polyester synthesis. These two short range goals will be done sequentially.

#### **1994 ACCOMPLISHMENTS:**

The screening of a 64 strain collection of *Xanthobacter* species has been completed.

Genes encoding for a soluable hydrogenase *Alcaligenes eutrophus* have been introduced into 21 *Xanthobacter* strains. A typical strain X9 has been identified as highest yield strain and characterized for suitability as a polyhydroxybutyrate (PHB) production strain.

In collaboration with project advisor Kjell Andersen of the Laboratory of Microbial Gene Technology at the Agricultural University of Norway a broad host range hydrogen chemoautotrophic expression vector, pAEX2, has been designed and constructed for introducing new metabolic pathways into carbon dioxide utilizing *Alcaligenes eutrophus*.

A patent application for the synthesis of a pharmaceutical oligosaccharide based on the production of oligosaccharides from the bacterial polysaccharides by the action of deviscofication enzymes specifically (bacteriophage endopolysaccharases).

#### **1995 PLANNED ACTIVITIES:**

FY 95 activities are limited to the first quarter due to expiration of the current contract. Research efforts will focus the identification of those *Xanthobacter* strains, already demonstrated to host the soluble hydrogenase encoding plasmid pGE-15, that are capable of expressing soluble hydrogenase. The effect of soluble hydrogenase on the growth and polyhydroxybutyrate (PHB) production of these strains will be determined. *Xanthobacter* X9, the highest yielding of the naturally occurring *Xanthobacters* will be used as benchmark to aid in evaluating the potential benefits of a recombinant approach for improvement of polymer production by the species.

#### **ANNUAL TECHNICAL SUMMARY REPORT:**

The long term goal of this project is to manipulate  $H_2$  utilizing  $CO_2$  fixing organisms, or genes originating from those organisms, to produce useful biopolymers. This study has primarily focused on *Alcaligenes eutrophus* as a potential source of genetic material and *Xanthobacter autotrophicum* strains as a possible sources of the biodegradable polyester polyhydroxybutyrate (PHB) and polysaccharides. These biopolymers have a wide variety of applications including use as rheological modifiers, enhanced oil recovery, chromatography materials, additives for the paper and metal finishing industries, food and cosmetic industries and packaging industries. Emphasis on research for the past year has concentrated on three goals: 1) identification of possible PHB production *Xanthobacter* strain candidates based on their natural ability to produce the material and their ability utilize a second set of hydrogenase genes originating from *A. eutrophus*; 2) design and construction of a broad host range plasmid with a hydrogen chemoautotrophic expression promoter to provide *A. eutrophus* with the ability to produce a wider range of chemicals from gaseous substrates:  $CO_2$ ,  $H_2$  and  $O_2$ ; 3) study the properties of bacteriophage endopolysaccharases as a possible means of deviscofying media during the process of polysaccharide production thus preventing reduced rates of production due to slowed rates of gas diffusion.

The primary research emphasis during the past year has been the continued characterization of a collection of 64 *Xanthobacter* strains. We have identified on *Xanthobacter* strain X9 as capable of producing consistently producing polyhydroxybutyrate yields of over 90% of whole cell weight on fructose minimal media. Yields equivalent to 9.5 grams per liter were achieved in 2% fructose minimal media but growth rates were slow, in excess of 10 hours. Reduction of fructose levels to 0.4% resulted in a significantly increased growth rate of 4.2 hours suggesting this organism may have industrial application. Autotrophic growth rates were intermediate, 6.0 hours, but the level of PHB produced 26% of whole cell weight resulting in yields equivalent to 3.8 grams per liter.

Following the identification of X9 as the most promising strain for *Xanthobacter* PHB production effort has focused on characterizing strains for their suitability as recipients in gene transfer experiments. Primary emphasis focused on the transfer and expression of the soluble hydrogenase genes from *Alcaligenes eutrophus* to *Xanthobacter* strains. The presence of these genes is expected to directly increase the amount of intracellular reductant and indirectly increase the amount of polyhydroxybutyrate. As reported at the Principal Investigators meeting at Denver (July, 1994) we have successfully mobilized a pVK102 derived vector containing the *A. eutrophus* Soluble Hydrogenase genes (*hoxS* locus) into several *Xanthobacter* strains. However we have not yet assayed for expression of function soluble hydrogenase. The next round of experiments will focus on detection of soluble hydrogenase by the hydrogen dependent conversion of NAD to NADH.

To be specific a collection of 30 *Xanthobacter autotrophicum* strains has been evaluated to identify suitable recipient strains for the soluble hydrogenase genes in the current shuttle vector, pVK102. It has been determined that almost all *Xanthobacter* strains show sensitivity to the antibiotics whose resistance is conferred by the vector. Over 25 strains were readily and reproducibly found to be sensitive to Kanamycin (30 µg/mL) and Tetracycline (10 µg/mL) on both the media required for conjugal gene transfer and required for selection of the *Xanthobacter* exconjugants hosting the shuttle vector. An *E. coli* strain, obtained from the laboratory of Prof. B. Friedrich Freie Universität Berlin, which hosts a pVK102 derivative pGE-15 has been used in all hydrogenase gene transfer experiments. pGE-15 contains the genes encoding for the soluble hydrogenase of *A. eutrophus* inserted into the region encoding for Kanamycin resistance in pVK102. Initial attempts to transfer pGE-15 were unsuccessful. None of the 25 *Xanthobacter* strains was identified by acquisition of antibiotic resistance as a suitable recipients for functional soluble hydrogenase genes following standard conjugal transfer experiments.

Therefore experimental efforts were redirected toward demonstrating the transfer of the unmodified pVK102 shuttle vector into *Xanthobacter*. It was found that vector transfer can be demonstrated but only on antibiotic selective plates with low levels of fructose as a carbon source (0.5% or less). Higher fructose concentrations of 1% to 2%, while supporting growth of *Xanthobacter*, do not permit isolation of exconjugants. We attribute this observation to the osmotic sensitivity of the strains. Subsequent conjugal transfer experiments have demonstrated that the pVK102 can be successfully transferred to 21 of the 25 strains selected as potential recipients. However under no conditions could vector transfer be detected in type strain 7C. The frequency of vector transfer ranged from  $10^{-5}$  to  $10^{-4}$  per initial recipient in those strains where it did occur. These frequencies of successful transfer are among the lowest reported for pVK102 vector system. However, in each case the plasmid transfer was confirmed by screening presumed exconjugants for the co-transfer of tetracycline resistance, the rate of co-transfer was 100%. Recently pGE-15 has been transferred to the same set of recipient strains as judged by the appearance of Kanamycin resistant exconjugates. Screening for strains with active NAD hydrogenase reducing are in progress.

In collaboration with project advisor Kjell Andersen of the Laboratory of Microbial Gene Technology at the Agricultural University of Norway a broad host range hydrogen chemoautotrophic expression vector has been designed and constructed. The expression vector, pAEX2 (6.2 kb) is derived from has the same host range and transfer properties as pVK102. It

was constructed from pGL14, a pRK290 derivative which contains the *par* stabilization region of RK2 for enhanced genetic stability of the plasmid. The *A. eutrophus* strain ATCC 17707 Ribulose biphosphate carboxylase (Rubisco) promoter, plus a small synthetic DNA fragment, has been inserted into pGL14 as a EcoRI to HindII fragment. This permits genes to be cloned into down stream restriction sites (SphI, BamHI, XbaI, SalI, PstI, SphI, and HindIII) under chemoautotrophic regulated expression. It is intended as a means of introducing a broad range of new or enhanced metabolic capabilities into *A. eutrophus* strains. No specific application has yet been targeted.

At the sponsors request research emphasis focused primarily on PHB production, however some aspects of improving capsular polysaccharide production have been investigated. We have previously demonstrated, using *Klebsiella* strains, that bacterial strains producing large amounts of polysaccharide show relatively reduced and erratic growth when compared with strain variants producing no extracellular polymer. As should be the case for hydrogen chemoautotrophic bacteria polysaccharide production strains self limit their growth when producing viscous polysaccharide due to the impedance of gas transfer. For uses where the rheological properties of the polymer are not important the application of deviscofying enzymes would improve productivity by enhancing gas transfer rates. The ability to engineer enzymes capable of degrading specific polysaccharides should be very useful for this purpose. Based on previous studies of the interaction of bacteriophage bearing endoglycanase in *Klebsiella* strains proof-of-concept experiments have determined that the phage enzyme site can be altered without loss of phage viability. Our study of *Klebsiella* K63 and its associated bacteriophage, has shown that a class of spontaneous host range mutants can be generated which show a dramatic decrease, and in one case complete loss, of endolytic activity for the native capsular polysaccharide. This suggests that it may be possible to use direct selection to genetically alter enzymatic activity to other polysaccharide substrates of similar structure. A procedure for selecting bacteriophage bearing endopolysaccharases with altered substrate specificity has been designed but has not yet been tested.

A poster entitled "Bacteriophage Polysaccharases: A tool for the production of new oligosaccharides" was presented at a "The Second Annual Conference on Glycotechnology" held May 16-18 in La Jolla, CA. This represents one high value application of deviscofied polysaccharides, an intermediate in the production of oligosaccharides with pharmaceutical application. At the meeting presentations dealing with current methods of synthesizing oligosaccharide pharmaceutical agents, based on a combination of enzymatic and chemical synthetic steps, suggests that the action of endopolysaccharase bearing bacteriophage on polysaccharides have potential for the production of precursors to pharmaceutical oligosaccharides. CalTech has elected to patent one possible application of such a synthetic route for the production of a Sialic Lewis X Analog. Due to time and resources available this patent is based on an a yet to be performed "prophetic" experiment.

16. **PROJECT TITLE:** Photobiological Conversion of Synthesis Gas into Bioplastics

**PRINCIPAL INVESTIGATOR:** P. Weaver

**PROJECT SITE:** National Renewable Energy Laboratory

**DESCRIPTION:**

This effort addresses one phase of an overall larger project which involves the conversion of lignocellulosics into syngas which is then converted by a photobacterial system into a bacterial polyester, polyhydroxybutyrate (PHB). By manipulation of the culture, the production of PHB valerate can also be achieved. These bioplastics can be produced to simulate polystyrene or polyethylene like plastics. One of the major processing hurdles that prevents this work from being considered in industrial applications is good process engineering data that allows us to design an overall process. In particular, the data on mass transfer of gases, a critical processing step, needs to be determined. This effort is designed to obtain that data. Future work would be required to complete the total development of this project. Currently, the strains in use only produce about 33% of their biomass as the bioplastic. Future efforts should include engineering strains to increase their production of bioplastic.

**1994 ACCOMPLISHMENTS:**

About 400 distinct strains of a unique subclass of photosynthetic bacteria were isolated that are able to nearly quantitatively photoassimilate inexpensive synthesis gas (primarily CO and H<sub>2</sub>) or producer gas (primarily N<sub>2</sub>, CO, and H<sub>2</sub>) into new bacterial cell mass. A large fraction of the new cell mass is found as internal granules of poly-β-hydroxyalkanoates (PHA's). PHA's are linear, long-chain polyesters that have physical properties similar to polypropylene or polyethylene thermoplastics; however, the chiral bioplastics are completely biodegraded by common microbes found in aerobic or anaerobic soil or water. The economic production of PHA bioplastics using this method is dependent on developing inexpensive solar bioreactors that promote enhanced mass transfer of the gaseous substrates into the aqueous environment of the bacteria. Hollow fiber, bubble tower, sprinklered, floating bead, and mat forming bioreactor designs have been examined at a 100-200-liter scale. The bioreactors are driven by sunlight with natural diurnal cycles. Temperatures are controlled. On synthesis gas and ammonia as growth substrates and non-sterile conditions, some contamination, but usually not more than 10% of the biomass product, occurred from sulfate-reducing and methanogenic bacteria or green algae. With N<sub>2</sub> as the sole source of nitrogen, however, contamination was less than 1%. The maximal cell mass productivity rate so far observed is from a shallow bubble reactor, which generated 76 g cells·m<sup>-2</sup>·day<sup>-1</sup>.

**1995 PLANNED ACTIVITIES:**

A final report will be prepared either as a stand alone document or a publishable paper which will summarize the results of this work. This specific task will not be continued.

17. **PROJECT TITLE:** Photobiological Reactors  
**PRINCIPAL INVESTIGATOR:** E. 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<sub>2</sub> as the sole carbon 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 mutant, *Chlamydomonas* B4, capable of reducing atmospheric CO<sub>2</sub> using a single light reaction of photosynthesis.

#### **1994 ACCOMPLISHMENTS:**

Major technical advance in the discovery of a new single-light photosynthetic pathway for the conversion of atmospheric CO<sub>2</sub> to value-added chemicals in the genetically engineered mutant B4 of *Chlamydomonas reinhardtii*.

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 *Chlamydomonas reinhardtii* that was developed in the laboratory of Professor L. Mets, University of Chicago. We have shown that although mutant B4 lacks the Photosystem I light reaction, it is capable of converting atmospheric CO<sub>2</sub> 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<sub>2</sub> to value-added chemical compounds.

#### **1995 PLANNED ACTIVITIES:**

The research plan for 1995 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<sub>2</sub> 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 one electron from the positive oxidizing potential of water splitting and oxygen evolution

to the reducing potential of CO<sub>2</sub> 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 1995 experiments on the absolute energetic and quantum conversion efficiencies in mutant B4 and wild-type *Chlamydomonas* will be performed.

#### ANNUAL TECHNICAL SUMMARY REPORT:

Key progress for the current reporting period is based on a new discovery in the Chemical Technology Division of Oak Ridge National Laboratory utilizing a mutant of the green alga *Chlamydomonas reinhardtii* that was developed in the laboratory of Professor L. Mets, University of Chicago. We have shown that although mutant B4 lacks the Photosystem I light reaction, it is capable of converting atmospheric CO<sub>2</sub> 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<sub>2</sub> to value-added chemical compounds.

The key results described in this report are a demonstration of sustained simultaneous photoevolution of molecular hydrogen and oxygen and photoassimilation of CO<sub>2</sub> in mutant B4 of *Chlamydomonas reinhardtii*, which contains Photosystem II only. The results are discussed in terms of thermodynamic limit of photosynthesis and its implication for maximum conversion efficiencies of light energy into chemical energy and origin of the photosynthetic function in early and present-day atmospheres.

Mutant B4 of *Chlamydomonas reinhardtii*, deposited with the Duke University *Chlamydomonas* Genetics Center and available as Stock No. CC2933, was obtained as a photosynthesis-deficient mutant following metronidazole enrichment in a mutagenesis experiment described in detail elsewhere. Pre- and post-experimental analysis showed that the 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. P700 signals were undetectable as was the G = 2.007 EPR signal characteristic of PSI. Genetic analysis indicated that the B4 mutation is carried in a nuclear gene. Molecular studies indicated it was 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. In addition, *prima facie* data demonstrating a lack of PSI in mutant B4, and its presence in wild-type *Chlamydomonas* are presented in the  $\lambda > 700$ -nm data using Schott 730-nm cut-off filters. It is known that PSI absorbs longer wavelengths of light than PSII. Wild-type *Chlamydomonas* displayed a  $\lambda > 700$ -nm-dependent on/off hydrogen evolution pattern, whereas mutant B4 did not (*vide infra*).

Figure 1A demonstrates simultaneous photoevolution of molecular hydrogen and oxygen from mutant B4 in an atmosphere of 363 ppm CO<sub>2</sub> in helium; Figure 1B contains data for the simultaneous photoevolution of hydrogen and oxygen in pure-helium. Figures 1C and 1D contain corresponding data on simultaneous photoevolution of hydrogen and oxygen from wild-type



*Chlamydomonas*, obtained from the University of Texas Culture Collection of Algae. Data for mutant B4 and the control wild-type 137c were obtained simultaneously in a dual-photoreaction-chamber flow system. A comparison of the results of these two parallel experiments is intriguing and quite surprising in context of the generally accepted vectorial model of photosynthesis comprised of two sequential light reactions of differing thermodynamic properties.

ORNL DWG 94A-605

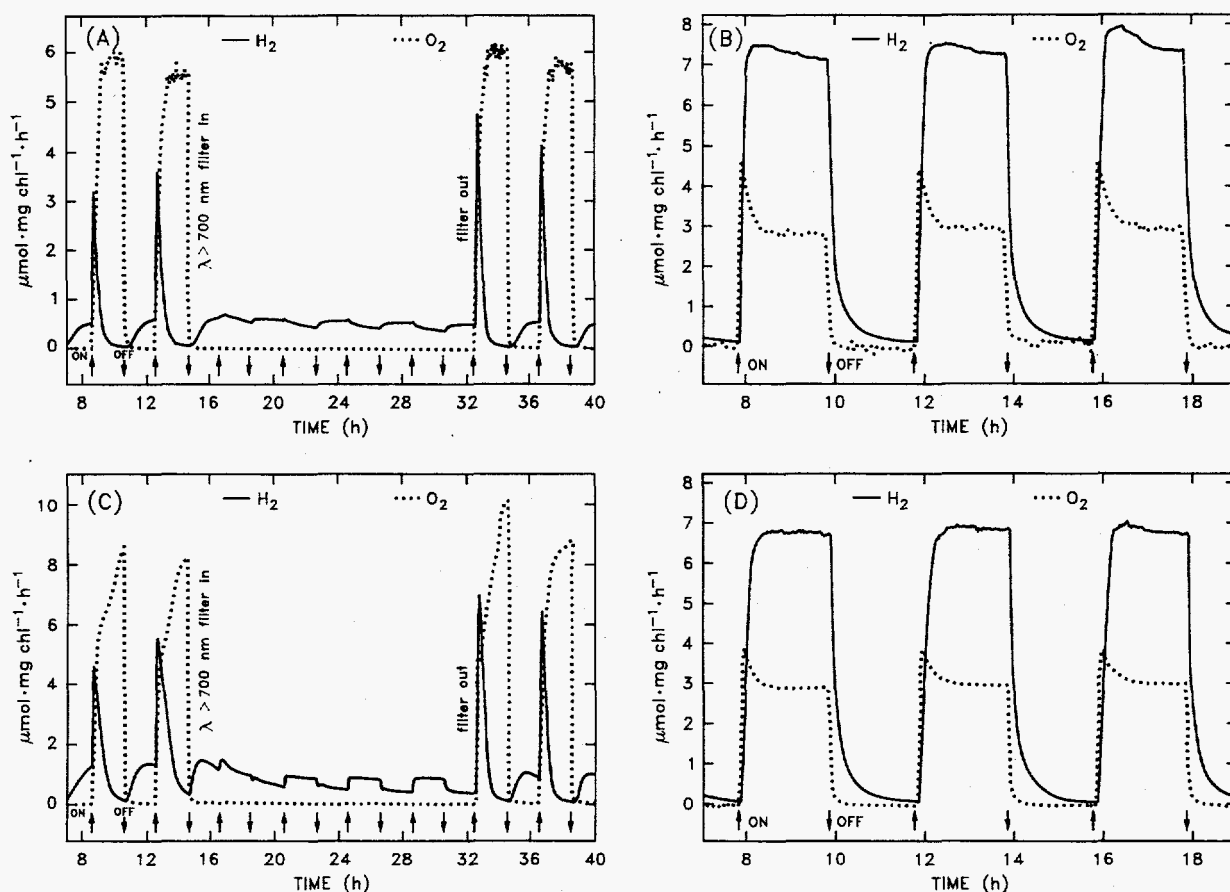


Figure 1

Simultaneous photoevolution of molecular hydrogen and oxygen by *Chlamydomonas* mutant B4 and wild-type *Chlamydomonas* 137c. (A) mutant B4, 363 ppm CO<sub>2</sub> in helium; (B) mutant B4, helium; (C) wild-type, 363 ppm CO<sub>2</sub> in helium; (D) wild-type, helium. As indicated in Figs. 1A and 1C, irradiation with light of wavelength  $\lambda > 700$  nm generated Photosystem I-dependent hydrogen evolution in 137c, whereas no such hydrogen evolution was observed in mutant B4. In B4 thermally-activated hydrogen production was slightly suppressed with far-red light, whereas PSI-dependent hydrogen production was observed with wild-type 137c. As also indicated in Figs. 1A and 1C, the peak and decline of hydrogen evolution while the light is still on represents the real-time activation of the Calvin-Benson cycle following a period of darkness. The chlorophyll concentration for the experiments of Figs. 1A and 1C was 15  $\mu\text{g}/\text{ml}$ ; for Figs. 1B and 1D it was 3  $\mu\text{g}/\text{ml}$ . The simultaneous photoevolution of hydrogen and oxygen was measured in a tandem flow system containing a tin-oxide gas-sensitive semiconductor for the hydrogen measurement and a Hersch electrogalvanic cell for the oxygen measurement. Absolute calibration of both sensors was achieved using an in-line electrolysis cell, a constant current source, and Faraday's Law of Electrochemical Equivalence.

Although mutant B4 was grown photoheterotrophically using minimal growth medium + acetate and totally lacks Photosystem I, sustained simultaneous photoevolution of hydrogen and oxygen was easily observed. The reaction itself was performed in minimal growth medium by successive washings to eliminate all soluble acetate. These data clearly demonstrate that a single Photosystem II-driven reaction can span the potential difference between water oxidation/oxygen evolution and proton reduction/hydrogen evolution. The reversible equilibrium thermodynamic potential for these two reactions is 1.23 V. Of course, since the reactions do not occur reversibly at thermodynamic equilibrium, the actual potential must be higher, perhaps 1.4-1.6 V.

The actual patterns of hydrogen and oxygen evolution in the presence and absence of CO<sub>2</sub> for both mutant B4 and wild-type *Chlamydomonas* are instructive. In Figs. 1A and 1C, with CO<sub>2</sub> present in the carrier gas, it can be seen that 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. Since the Calvin-Benson cycle is activated by light, it subsequently serves as 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<sub>2</sub> reduction cycle. When the light is turned off, thermally-activated hydrogen evolution is observed. A similar 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. This thermally-activated hydrogen evolution is dependent on growth history. If the algae experience extended periods of photosynthesis, thermally-activated hydrogen is greater than if they do not. *C. reinhardtii* grows quite well in an anaerobic atmosphere consisting of CO<sub>2</sub> and helium. In a pure helium atmosphere, illustrated by the data of Figs. 1B and 1D, the hydrogen time profiles are qualitatively different. They climb monotonically to an approximate steady state with the light turned on and return to zero when it is turned off. Since CO<sub>2</sub> is not present in this atmosphere, the Calvin cycle is not activated and does not serve as a sink for reducing equivalents. As expected, oxygen activity is less in helium due to the absence of CO<sub>2</sub> and the corresponding Photosystem II bicarbonate requirement. Irradiation data with  $\lambda > 700$  nm, as indicated in Figs. 1A and 1C, are consistent with the above results. In the wild-type alga, Fig. 1C, a long-wavelength Photosystem I component of hydrogen evolution is easily observed. However, the contrasting data for mutant B4 show no such component. For B4,  $\lambda > 700$ -nm light slightly suppresses thermally-activated hydrogen evolution.

Perhaps the most remarkable aspect of the data for mutant B4 for CO<sub>2</sub> in helium is that the Calvin cycle is evidently activated in this system. This is positively demonstrated by the data of Fig. 2 in which the photoassimilation of CO<sub>2</sub> is illustrated. It therefore follows that mutant B4 can perform photosynthesis using a single Photosystem II reaction.

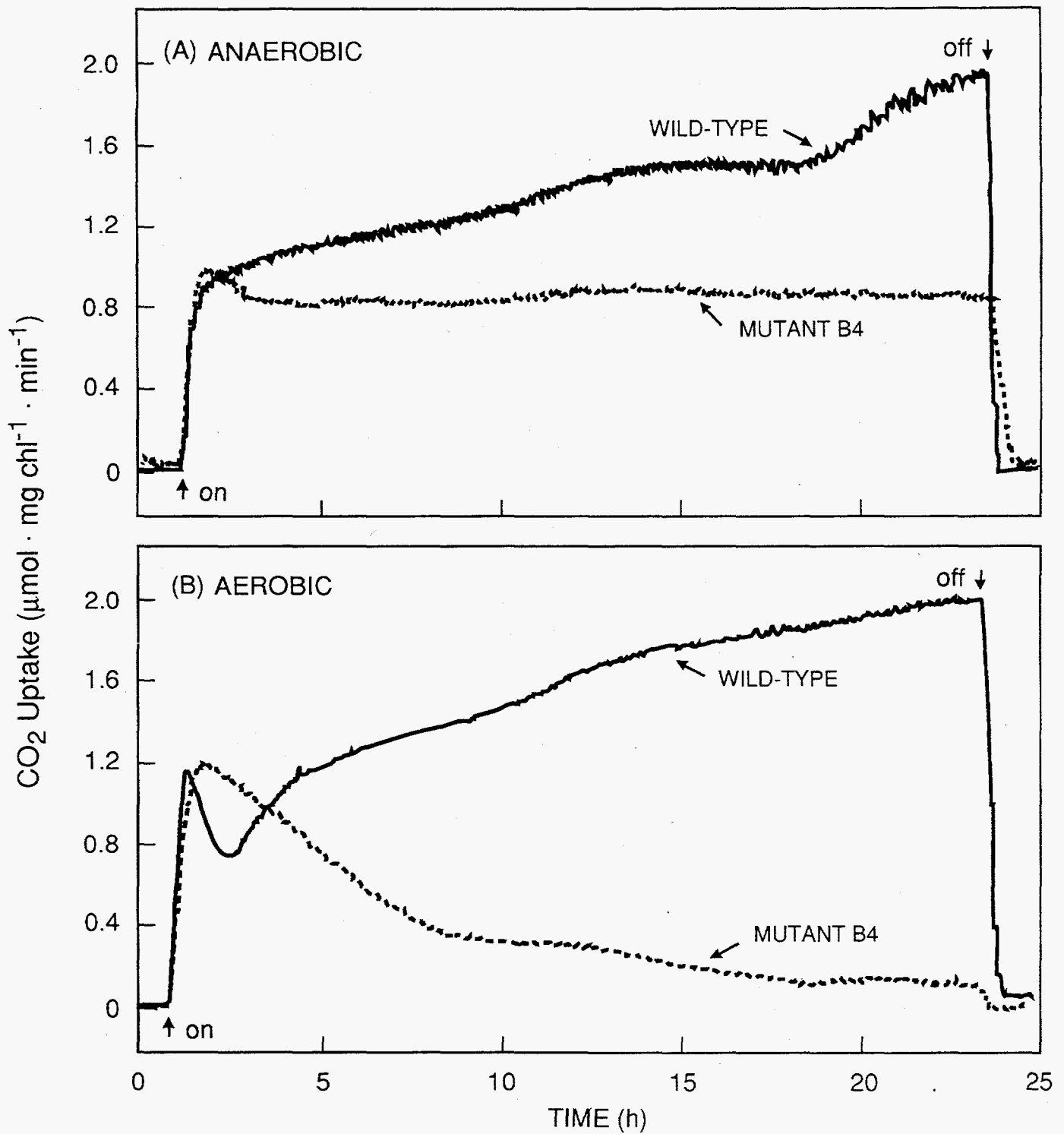


Figure 2

A comparative study of photosynthesis between mutant B4 and wild-type 137c in anaerobic and aerobic atmospheres. (A) 363 ppm CO<sub>2</sub> in helium; (B) 363 ppm CO<sub>2</sub> in air. The loss of photosynthetic activity by mutant B4 indicated in Fig. 2B was accompanied by a bleaching of its chlorophyll. Continuous measurement of CO<sub>2</sub> was achieved with an in-line infra-red spectrophotometer. The CO<sub>2</sub> detector was calibrated using an electronic gas blender with primary standard gas mixture of CO<sub>2</sub> in helium.

Working with *Chlamydomonas* ACC-1, a mutant that lacked Photosystem I, Klimov et al. observed the photoreduction of NADP<sup>+</sup>. However, this was interpreted as a non-physiological reaction, an alternative electron pathway occurring only at high light intensities. Arnon and Barber reported the photoreduction of NADP<sup>+</sup> by isolated reaction centers of Photosystem II. However, the reaction centers of this work were not functional oxygen evolution preparations. The PSII electron donor, 1,5-diphenylcarbazide, was used as the electron source. The key emphasis of the Arnon-Barber paper was the requirement for plastocyanin. The primary electron acceptor of Photosystem II is pheophytin, which has an E<sub>m</sub> of -0.61 V. 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. The unique feature of mutant B4 is that it apparently allows access by ferredoxin to the PSII reduction site. Since DCMU inhibits both hydrogen and oxygen evolution, access to Photosystem II reductant most likely occurs at the Q<sub>a</sub>/Q<sub>b</sub> level. This interpretation is consistent with the known redox potential of doubly reduced bacterial Q<sub>a</sub>.

These data clearly illustrate a new photosynthesis is being performed by the Photosystem II light reaction. They shed light on the origin of the photosynthetic function: which came first, oxygen evolution or two-light reactions? Blankenship has discussed the latter: "some sort of genetic fusion event took place between two bacteria." According to this scenario, the bacteria contained one each of the pheophytin-quinone and Fe-S-type reaction centers. Initially, the reaction centers of this chimeric organism were unlinked. Subsequently, the two photosystems were linked, and the oxygen evolution system was added. However, although single-light-reaction-center bacteria of both varieties are abundantly available today, no examples of their geminate descendants have ever been observed. Put another way, although the alleged geminate organisms were formed to confer survival advantage, none has survived. The present data support an alternative view: a pheophytin-quinone-reaction-center bacterium developed complete photosynthesis using a single Photosystem II-like reaction center with water as the source of reductant with a second light reaction, Photosystem I, added later for survival when molecular oxygen became a major component of the earth's atmosphere. Data which support this statement are presented in Figs. 2A and 2B. The data in Fig. 2A are for CO<sub>2</sub> in helium, those of Fig. 2B are for CO<sub>2</sub> in air. It can be seen in Fig. 2A that CO<sub>2</sub> photoassimilation is stable for both mutant B4 and wild-type *Chlamydomonas* in an anaerobic atmosphere. However, it is only the wild-type alga containing both Photosystems I and II that is stable in air. Within 24 hours, mutant B4 lost 90% of its photosynthetic activity.

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 or reduced carbon, is doubled from about 10% to 20%. The reason for this is that a single photon rather than two is now required to span the potential difference between water oxidation/oxygen evolution and proton reduction/hydrogen evolution. This assumes, of course, that a relatively high quantum yield is preserved. A measurement of the quantum requirement of photosynthesis in mutant B4 will answer this question.

18. **PROJECT TITLE:** Levoglucosan Polymers

**PRINCIPAL INVESTIGATOR:** L. Moens

**PROJECT SITE:** National Renewable Energy Laboratory

**DESCRIPTION:**

Levoglucosan, a monosaccharide that is the 1,6-anhydro form of D-glucose, is presently attracting much attention as a major product of the thermochemical degradation of cellulose and cellulosic feedstocks under pyrolytic conditions.

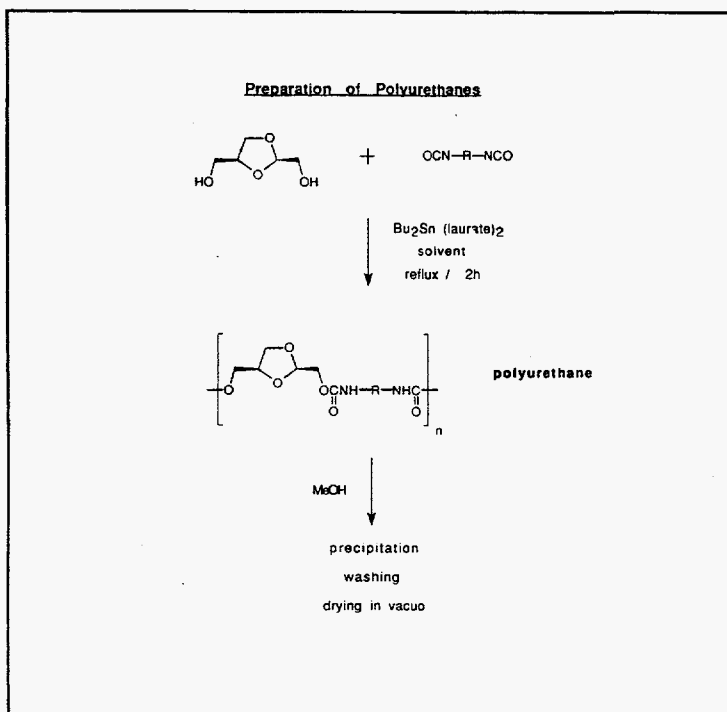


Figure 1

The preparation of levoglucosan through this process continues to be the focus of much research because it cannot be readily synthesized using more conventional technologies that start from petrochemicals. This monosaccharide is considered to be a potentially very useful starting material for a variety of chemical reactions and processes but its unavailability and extreme cost price make further R&D work on large scale virtually impossible. However, recent research efforts in many laboratories worldwide, but mainly in our laboratories at NREL, have led to new methods for the isolation of levoglucosan from (ligno)cellulose-derived pyrolysis oils. This is expected to stimulate the development of new applications for levoglucosan as a chemical feedstock.

The objective of this subtask is to prove the concept that levoglucosan can be converted into new types of polymers that have several interesting features. First, the polymers will be designed such that they will be enantiomerically (chirally) pure and thus optically active. The synthesis of chiral polymers continues to attract much attention since they can be used as support materials in enantioselective chromatography that allows for the preparation of enantiomerically pure

chemicals. Also, several examples are known of catalysts that contain polymer-bound asymmetric centers. Asymmetric catalysis with such optically active polymers also allows for further modification of the conformational rigidity around the asymmetric center depending on the chemical nature of the polymer backbone. Secondly, the optical properties of these polymers could make them useful as building blocks for liquid crystals. A third important feature is a highly oxygenated polymer backbone that is expected to promote biodegradability and/or acid sensitivity.

#### **1994 ACCOMPLISHMENTS:**

- Levoglucosan, a monosaccharide derived from cellulose through pyrolysis, has been converted into *cis*-1,3-dioxolane-2,4-dimethanol on a multigram scale.
- A new class of polyurethanes has been synthesized from *cis*-1,3-dioxolane-2,4-dimethanol, using methylenedi-*p*-phenyl diisocyanate (MDI) and hexamethylene diisocyanate (HDI)
- The thermal properties of the new polyurethanes were studied using Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA)

#### **1995 PLANNED ACTIVITIES:**

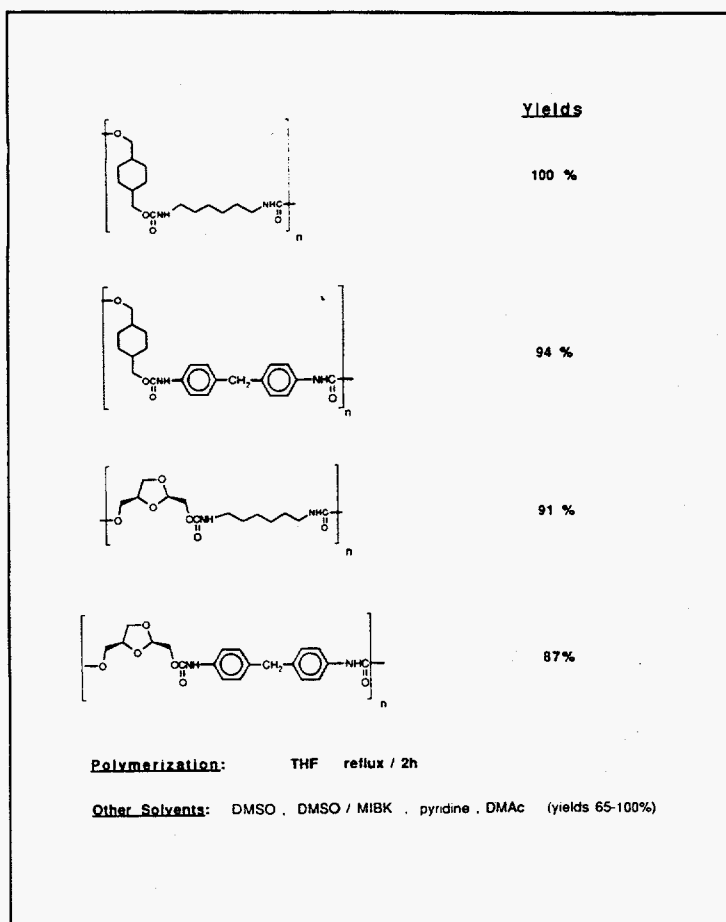
The availability of levoglucosan on a multigram scale through in-house pyrolysis and isolation technology makes it possible to further explore the synthesis of other novel levoglucosan-derived polymers. We are particularly intrigued by the possibility of using *cis*-1,3-dioxolane-2,4-dimethanol for the preparation of novel polyesters and polycarbonates. Another area is the synthesis of polyesters and polyamides from *cis*-1,3-dioxolane-2,4-dicarboxylic acid, an enantiomerically pure compound that, like the diol, can easily be prepared from levoglucosan. Any new polymer that will be synthesized will be studied using DSC and TGA in order to evaluate their physico-chemical properties.

#### **ANNUAL TECHNICAL SUMMARY REPORT:**

The following is a summary of the presentation given during the Annual Review Meeting. More details will be presented in the publication that is in preparation.

##### *a) Synthesis of the novel polyurethanes*

We have succeeded in the preparation of polyurethanes from *cis*-1,3-dioxolane-2,4-dimethanol using methylenedi-*p*-phenyl diisocyanate (MDI) and hexamethylene diisocyanate (HDI) as reaction partners. The polymerizations were carried out in a variety of solvents such as tetrahydrofuran, dimethylsulfoxide/methyl isobutyl ketone mixtures, pyridine and *N,N*-dimethylacetamide in the presence of dibutyltin dilaurate as a catalyst. Although the polymerizations were exothermic, the mixtures were heated at reflux for two hours to ensure complete polymerization. After precipitation and washing with methanol, the polyurethanes were isolated in medium to good yields (65-100 %) (Figures 1 and 2).



In order to learn more about the influence of the rigid dioxolane ring in the polymer backbone, we also prepared analogous polyurethanes from cyclohexane-1,4-dimethanol (see Figure 2).

Figure 2

### b) *Thermal analysis*

The results of DSC measurements are indicated in Figure 3. The table shows the relatively low melting temperature of the cyclohexane system as well as the dioxolane system. For the HDI-based systems two melting points were observed that may indicate the existence of two different crystalline forms. Upon rapid cooling in dry ice (quench), only the cyclohexane-HDI polymer displays an exotherm where the flexible chains return to a thermodynamically more stable crystalline structure [ $T_{\text{cryst}}(1)$ ]. Upon reheating, a glass transition temperature is observed [ $T_g(2)$ ] that is close to room temperature. Upon further heating, another exothermic wave indicates that more of the rigid chains are able to relax into a crystalline state [ $T_{\text{cryst}}(2)$ ] due to reduced viscosity of the polymer. When heating is continued, another melting curve is observed.

It is quite interesting that none of the dioxolane systems shows a significant exotherm for crystallization, not even when the molten polymer was cooled rapidly with dry ice (i.e. quenched). The rigidity of the cis-dioxolanediol ring may prevent the polymer chains from returning from a molten to a crystalline state, even though the polymer becomes crystalline when it is freshly prepared and precipitated from methanol. The cyclohexane-MDI polymer is also too rigid due to the aromatic units. As expected, the aromatic polymer backbones have a significantly higher  $T_g$  (2) than the flexible HDI systems.

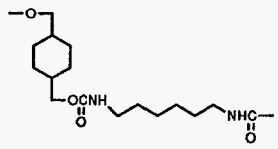
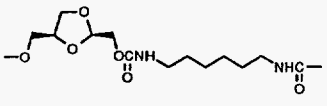
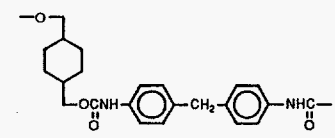
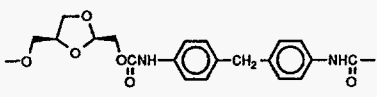
	$T_{\text{melt}}$ (1)	$T_{\text{cryst}}$ (1)	$T_g$ (2)	$T_{\text{cryst}}$ (2)
	167.7 178	108.3	29.8 (after quench)	95.5
	97.7 119.9	—	34.1 (after quench)	—
	164.6	—	136.4 (after $T_{\text{max}}=190$ )	—
	156.4	—	124.7 (after $T_{\text{max}}=225$ )	—

Figure 3

Thermogravimetric analysis (TGA) of these polyurethanes shows that the HDI systems (Fig. 4) start to decompose around 180-190°C, while the aromatic MDI polymers are a little more stable to about 220°C (Fig. 5). It is possible that the DSC experiments were influenced by a small degree of thermally induced depolymerization at the maximum scan temperature.



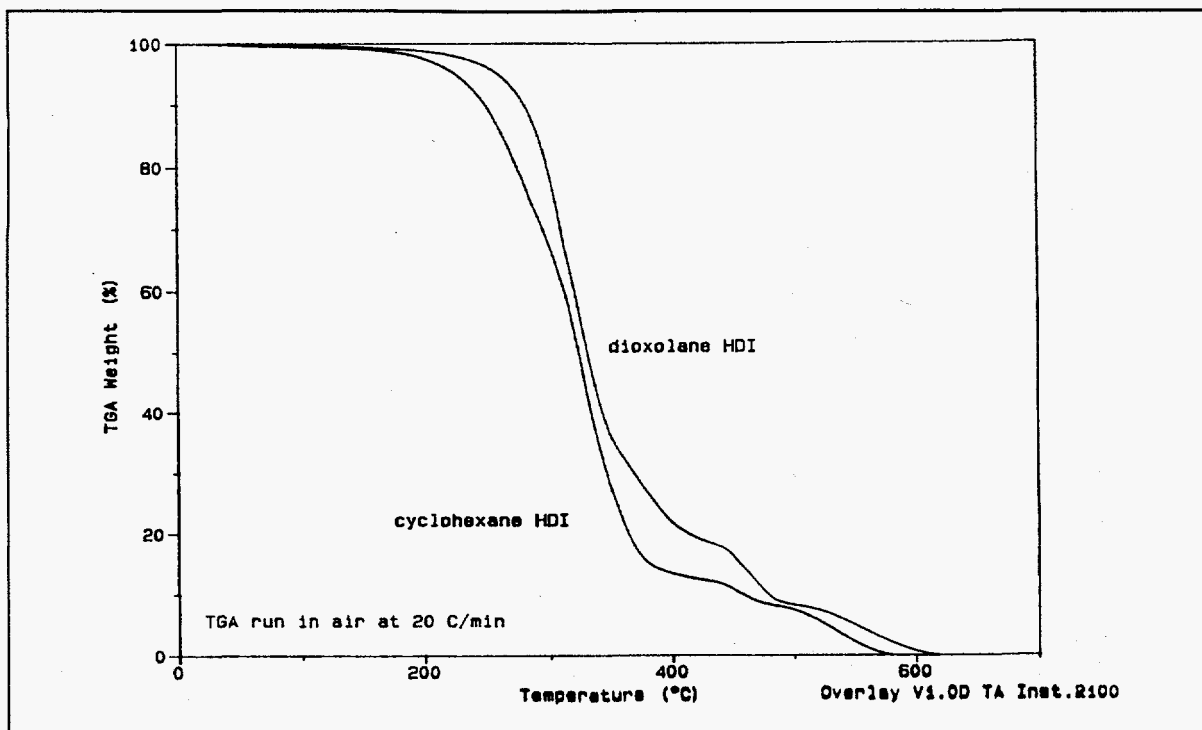


Figure 4

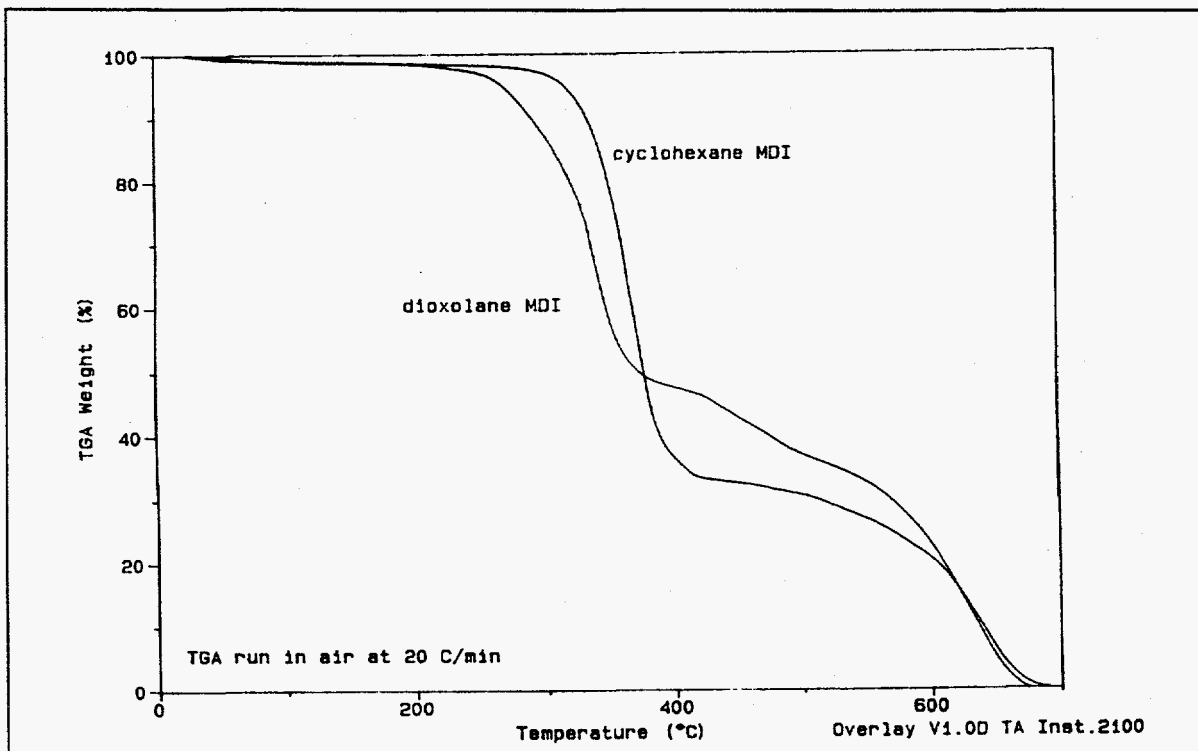


Figure 5

c) Preparation of polyurethane films

In spite of the low solubility of the polyurethanes we were able to cast brown-yellow, transparent films of both dioxolane polymers using hot pyridine as a solvent (Fig. 6). Upon evaporation under reduced pressure at 100°C, a somewhat flexible film was obtained from the dioxolane-HDI polymer. The MDI analogue was a much more brittle material, and therefore only the former was tested for its properties as a potential gas-separating material (Fig. 7). Unfortunately, not only did the dioxolane-HDI have very low selectivities for different gas mixtures, we also found out that the flexibility of this film material was mainly due to residual traces of pyridine. Further removal of these solvent traces decreased the mechanical strength of the film.

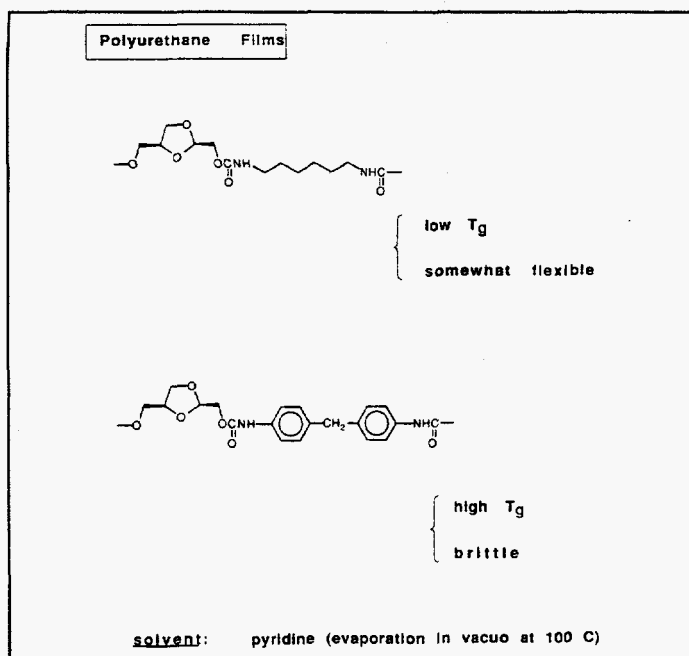


Figure 6

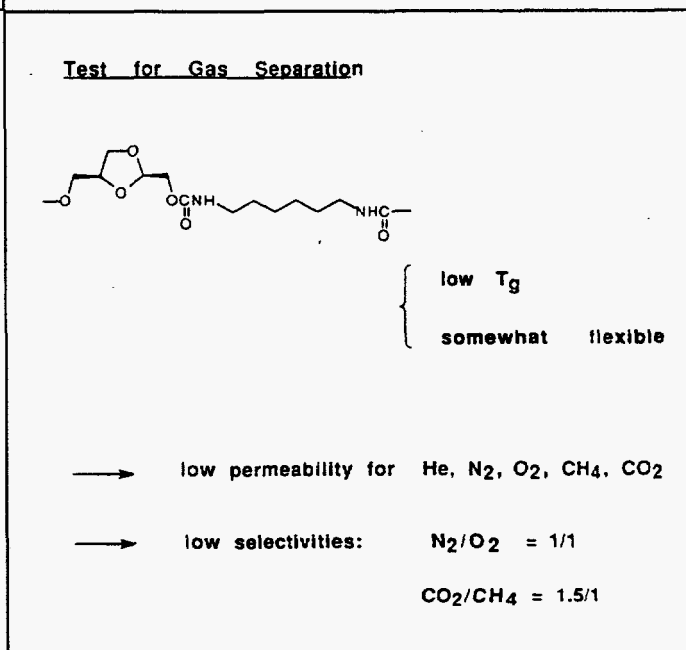


Figure 7

19. **PROJECT TITLE:** Solvent Effects on Clean Fractionation Severity

**PRINCIPAL INVESTIGATOR:** D. K. Johnson

**PROJECT SITE:** National Renewable Energy Laboratory

**DESCRIPTION:**

NREL researchers are developing solvent combinations for the clean fractionation of biomass by organosolv pulping routes that result in all polymeric components of the lignocellulose being obtained in high yield and with good selectivity. The three products provided by this route are a high purity cellulose pulp, hemicellulose and lignin. The hemicellulose and lignin portions are cleanly separated out of the pulping liquor. The cellulose pulp could be used for producing ethanol or could be upgraded to other higher value products. The lignin, containing virtually no carbohydrate, could be used as a precursor for pulping catalysts, phenolics, surfactants for enhanced oil recovery, and other high value products. A systematic study is being planned to examine how solvent composition and properties correlate with the extent of delignification and the characteristics of the cellulose, lignin and hemicellulose product fractions. It is known that the extent and rate of delignification of biomass is influenced by solvent strength, we intend to quantify these solvent effects by correlating solubility parameters, and hydrogen bonding capacity of solvent systems compatible with clean fractionation, with the various stages critical to effective delignification. The effectiveness of these solvent systems in swelling biomass will be measured as swelling is the first critical step in what is believed to be the process of delignification. For the lignin, depolymerization reactions as well as solubilization must occur before delignification takes place. The solvent is expected to have a large role in lignin solubilization. The solubility of lignin in various solvent systems will be measured to verify that the solvent systems of interest to clean fractionation fit with previously developed models relating solvent properties to lignin solubility. The effect of solvent parameters on the chemical and physical structure of the cellulose and lignin products will also be studied. A model of the effect of solvent parameters on organosolv fractionation will be developed based on the data obtained. By correlating solvent properties with swelling effectiveness, degree of delignification, removal of hemicelluloses, and the polymer characteristics of the lignin and cellulose products we expect to increase our understanding of how solvent composition effects fractionation selectivity, and determine what are the critical molecular level interactions involved. It is expected that the fractionation model will allow us to tune process conditions to give products for specific applications, i.e., pulp and paper, cellulose derivatives, glucose, quinones, phenolics, etc.

**1994 ACCOMPLISHMENTS:**

The effects of changing severity on the clean fractionation process have been quantified during the past year. Increasing severity increases lignin and xylan removal from wood with little loss of cellulose over the range studied. Results have also been compared with fractionation under normal ethanol organosolv conditions. Evidence for the enzyme accessibility of the pulps produced was obtained by simultaneous saccharification and fermentation. The apparent enzyme accessibilities of the cellulose were very high, but did not correlate with residual xylan and lignin levels observed or with changes in the crystalline to amorphous cellulose ratio.

Characterization of the lignins obtained at different severities was also undertaken. The molecular weights of the lignins tended to show slight decreases with increasing severity although scatter in the data decreased the significance of this trend. The ultra-violet spectral absorbances of the lignins increased with severity indicating higher levels of unsaturated conjugated functionalities in their structure.

Novel products formed from degradation of glucose and xylose were observed under organosolv and clean fractionation conditions. These previously unreported products only appear in the presence of organic solvent. The chemical structure of the products has not been positively identified, and so to date their rate of formation has only be estimated.

#### **1995 PLANNED ACTIVITIES:**

After completing research planned to run to the end of this year, a further years funding could be beneficially spent investigating the effect of fractionation conditions on the structure of the cellulose produced. It is not clear why enzyme accessibility increases so dramatically in some fractionated pulps when they contain only slightly lower amounts of lignin and xylan. Solid state NMR also does not show large differences in the structure of the pulps, crystallinities appear very similar. With additional funding we could examine whether there are molecular weight changes, porosity changes, or other structural changes that could be very important in determining the end-uses for the cellulose, in applications involving enzyme hydrolysis to sugars, dissolving grade pulps, and fiber applications.

Another area that needs to receive more attention in the clean fractionation process is what to do with the hemicellulose stream. This is an aqueous product stream that contains dissolved sugars from the hemicellulose fraction of the wood as well as other components of the hemicelluloses such as acetic and uronic acids. This stream also contains a small amount of lignin-derived material that will be phenolic in nature and also some furfurals, produced from degradation of sugars. The major component of this stream if it is derived from hardwoods or grasses is xylose or xylans, depending on the severity under which it is produced. The value of this stream would be greatly improved if the purity of the stream for these carbohydrates were increased. This could be achieved by a second solvent extraction to remove the phenolics, furfurals and acetic acid. Research in 1995 could be focussed on finding solvent extraction conditions that could achieve this purification. Subsequently ion-moderated partition chromatography could be looked at as a means of further separating the different carbohydrates in the mixture. The purified xylose stream could be used to make xylitol by reduction or lactic acid by fermentation. Lactic acid could be converted further to acrylic acid by thermal dehydration. Alternatively, without purification, the sugars in the hemicellulose stream could be converted almost quantitatively to furfural (five carbon sugars) and 5-hydroxymethyl furfural (six carbon sugars) by raising the temperature of the product stream for a short time. The furfurals could then be obtained by extraction and/or distillation from the other components in the hemicellulose stream. Research in 1995 could be focussed on optimizing the conditions for obtaining furfurals from the dissolved sugars in the hemicellulose stream.

## **ANNUAL TECHNICAL SUMMARY REPORT:**

The clean fractionation process is a form of organosolv pulping employing novel combinations of solvents that fractionate biomass feedstocks with high selectivity into three product streams derived from the three major components of biomass: cellulose, hemicellulose and lignin. The three product streams are separated through a simple manipulation of the solvents. The process is effective with both woody and herbaceous feedstocks.

There have been several studies of the effect of severity on biomass pulping, but these have mostly been conducted with aqueous or single solvent systems. Previously, no attempt has been made to study the effect of solvent composition on pulping severity. The goal of this study is to develop a correlation between solvent properties (hydrogen bonding capacity, dielectric constant, solubility parameter, etc.) and combined severity in multicomponent solvent systems compatible with clean fractionation pulping.

20. **PROJECT TITLE:** CO<sub>2</sub> Utilization and Conversion Study
- PRINCIPAL INVESTIGATOR:** K. Magrini-Bair
- PROJECT SITE:** National Renewable Energy Laboratory

### **DESCRIPTION:**

The focus of this project was to review the current scientific literature for processes that utilize CO<sub>2</sub> and determine what, if any, new types of chemistries could use CO<sub>2</sub> as a carbon source for the production of value-added chemicals and materials. Because CO<sub>2</sub> emissions to the atmosphere are increasing and because it represents a ubiquitous, if dilute, source of carbon we were interested in defining how it might be used by the chemical industry with respect to short term and long term feedstock availability. We found that CO<sub>2</sub> is currently used to make new materials with broad classes of reactions that include inorganic, organic, biochemical, and catalytic processes. Materials such as melamine-based polymers and polyesters, methanol and hydrocarbon fuels, paper whitener from precipitated calcium carbonates, and biodegradable plastics from bacteria and biodiesel fuel from microalgae are currently being made from CO<sub>2</sub>. CO<sub>2</sub> has been successfully used as a direct replacement for the toxic feedstock phosgene, which is a starting material for isocyanate and ultimately polyurethane production. Industry is currently testing this process at the pilot scale. Other new processes include directly incorporating CO<sub>2</sub> into polycarbonates under mild reaction conditions. The resulting polymer, made from a condensation reaction, has a higher molecular weight and better physical properties than material made with current chain polymerization processes. The next step will be to extend this reaction to polyesters, an important class of polymers. Thus, extending the scope of CO<sub>2</sub> usage is a viable research area. Review of the literature points out the need for identifying and developing new catalysts if CO<sub>2</sub> utilization is to be implemented. One area to begin searching for new catalysts is syngas conversion, which involves catalytic reaction of CO and H<sub>2</sub>. These catalysts, when modified, may work for CO<sub>2</sub> conversion as well. A review paper on CO<sub>2</sub> utilization was completed and submitted for publication in Chemistry and Industry.

The paper presents the latest available facts and figures on CO<sub>2</sub> and other greenhouse gas emissions to the atmosphere and discusses these figures with respect to world areas (Figure 1), types of emissions, and impact on global climate change. Data is also presented which shows that CO<sub>2</sub> is a ubiquitous, of dilute, carbon source for chemicals and fuels manufacture and that CO<sub>2</sub> utilization, though not able to function as a CO<sub>2</sub> mitigation strategy, can curb emissions by establishing a carbon recycle loop. Current industrial processes which use CO<sub>2</sub> as a feedstock are presented as well as new reactions and future markets for CO<sub>2</sub>-based products. The paper concludes that (1) CO<sub>2</sub> emissions to the atmosphere continues to be a growing problem with respect to global climate change; (2) the amount of CO<sub>2</sub> released yearly is such that a chemical and fuels industry based entirely on CO<sub>2</sub> will use less than 1% of the total emissions; however, the economic and environmental benefits of a CO<sub>2</sub>-based industry could be considerable; and (3) avoiding the use of toxic feedstocks, future CO<sub>2</sub> emissions, and hazardous byproducts are compelling environmental drivers. Already, CO<sub>2</sub> has been incorporated into pharmaceuticals, fuels, chemicals, and both known and new materials of new and/or better qualities.

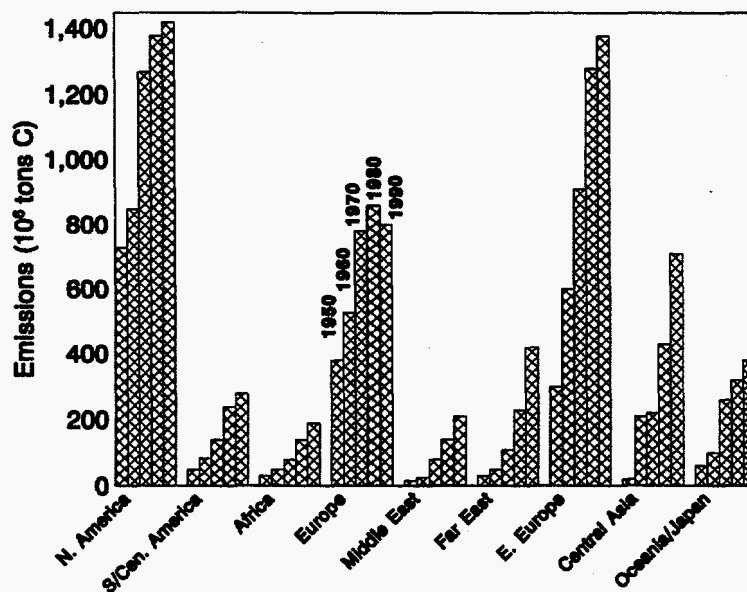


Figure 1

#### 1994 ACCOMPLISHMENTS:

A review paper on CO<sub>2</sub> utilization was completed and submitted for publication in Chemistry and Industry. This paper outlines the R&D needs for future efforts in CO<sub>2</sub> research. The paper presents the latest available facts and figures on CO<sub>2</sub> and other greenhouse gas emissions to the atmosphere and discusses these figures with respect to world areas (Figure 1), types of emissions, and impact on global climate change. Data is also presented which shows that CO<sub>2</sub> is a ubiquitous, of dilute, carbon source for chemicals and fuels manufacture and that CO<sub>2</sub> utilization, though not able to function as a CO<sub>2</sub> mitigation strategy, can curb emissions by establishing a carbon recycle loop. Current industrial processes which use CO<sub>2</sub> as a feedstock are presented as well as new reactions and future markets for CO<sub>2</sub>-based products. The paper concludes that (1) CO<sub>2</sub> emissions to the atmosphere continues to be a growing problem with respect to global climate change; (2)

the amount of CO<sub>2</sub> released yearly is such that a chemical and fuels industry based entirely on CO<sub>2</sub> will use less than 1% of the total emissions; however, the economic and environmental benefits of a CO<sub>2</sub>-based industry could be considerable; and (3) avoiding the use of toxic feedstocks, future CO<sub>2</sub> emissions, and hazardous byproducts are compelling environmental drivers. Already, CO<sub>2</sub> has been incorporated into pharmaceuticals, fuels, chemicals, and both known and new materials of new and/or better qualities.

**1995 PLANNED ACTIVITIES:**

Conduct the appropriate literature searches based on existing studies and attempt to find opportunities for potential R&D that expand the range and application of CO<sub>2</sub> as a feedstock for chemicals production. This is contingent on availability of resources.

## 4.5 Novel Process Development

21. **PROJECT:** Over Production of Lignocellulosic Enzymes of *Coriolus Versicolor* by Genetic Engineering Methodology

**PRINCIPAL INVESTIGATOR:** A. K. Williams

**PROJECT SITE:** Howard University

### DESCRIPTION:

The project seeks to understand the biological and chemical processes involved in the synthesis and secretion of the enzyme polyphenol oxidase (PPO) by the hyphae, the basic unit of the filamentous fungus, Coriolus versicolor. These studies are performed to determine rational strategies for the over production and enhanced secretion of PPO, both **with** and **without** recombinant DNA techniques.

### 1994 ACCOMPLISHMENTS:

During the past year (09/01/93 to 08/31/94), we devoted efforts to the over production of lignocellulosic enzymes of Coriolus Versicolor by genetic engineering methodology. Specifically, we scaled-up the 'batch' culturing of C. versicolor in defined medium. Subsequent to culturing assessment, the following tasks were fulfilled: (i) successfully cloned and expressed the PPO gene in E. Coli cells; (ii) initiated the mass production of PPO from genetic engineered gene as well as cultural manipulations; (iii) demonstrated the use of the PPO system as a 'model' to over produce other lignocellulosic enzymes (e.g., C1 cellulase, etc.); and (iv) generated one minority Master's degree student (Felicia c. Goins) via DOE-NREL subcontract support.

Collectively, the mass production of C. versicolor PPO could serve as means for the biological processing of lignocellulose to facilitate industrial utilization of lignocellulosic biomass.

### 1995 PLANNED ACTIVITIES:

1. To 'mass' produce PPO from Coriolus versicolor via genetic engineering methodology and/or cultural manipulations;
2. To characterize the catalytic properties and activities of the PPO complex;
3. To utilize PPO as a biocatalyst in wood bioprocessing (e.g., lignocellulose utilization); and
4. To initiate the development of a course curriculum impacting upon the environment with heavy emphasis pertaining to Biotechnology, Manufacturing, and Bioprocessing in keeping with renewable energy resources.



## ANNUAL TECHNICAL SUMMARY REPORT:

During this period (09/01/93 to 08/31/94), we devoted efforts to the over production of lignocellulosic enzymes of Coriolus versicolor by genetic engineering methodology.

Coriolus versicolor, a white-rot basidiomycete, elaborates cellulolytic and ligninolytic enzymes in situ and in vitro. These enzymes can degrade cellulose and lignin, the main organic polymers of wood. Besides its presence within wood, lignocellulose often constitutes an unwanted component in the paper-pulp industry and, also, renders agricultural commodities less digestible to ruminants.

In this study, we scaled-up the 'batch' culturing of C. versicolor in defined liquid medium. Subsequent to the culturing protocols, we completed the cloning and partial expression of the PPO gene of C. versicolor. Then, vectors harboring the cloned PPO gene were subjected to mutagenesis as well as cultural manipulations to achieve over production of PPO. In addition to the PPO gene, we directed attention to the cloning of another lignocellulosic gene i.e., C1 cellulase gene. That is, several methods were employed to generate high **quality** and **yield** of mRNA from C. versicolor hyphae. A Guanidium Thiocyanate/Cesium Chloride Gradient method appeared superior for extracting RNA from mycelial tissue at day 6 (see Table 1). Bulk RNA was purified and fractionated into poly (A)+ and poly (A)- species via oligo (dt)-cellulose chromatography. One poly (A)+ fraction directed the synthesis of a 30kDa protein (e.g., C1 cellulase and strongly supported cDNA synthesis. Immunoblots were utilized to confirm the presence of the C1 cellulase gene **underscore** the utility of the PPO system as a 'working model' for the mass production of lignocellulosic enzymes.

### Project Output:

In reference to scientific output, four manuscripts have been prepared for submission to Molecular Biology and Biotechnology Journals.

22.    **PROJECT TITLE:**                               Separations by Reversible Chemical Association
- PRINCIPAL INVESTIGATOR:**    C. J. King
- PROJECT SITE:**                               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.

#### **1994 ACCOMPLISHMENTS:**

1. Identification of Amine-based Extractants and Sorbents Having Optimal Basicity for Conversion of Dilute Acetic Acid at pH = 6 to Calcium Magnesium Acetate (CMA). The favored extractant and sorbent are Amberlite LA-2 and Amberlite IRA-35, respectively. This accomplishment establishes a viable approach for making CMA from dilute acetic acid, without recovery and concentration of the acetic acid, and also establishes agents that retain good uptake at fermentation pH, while also yielding acetic acid to aqueous suspensions of slaked dolomitic lime, producing CMA, the environmentally benign road de-icer.
2. Investigation of Reversible Reaction with Aldehydes as a Means for Efficient Recovery of Glycols from Dilute Aqueous Solution, and Establishment of Reaction with Acetaldehyde and Subsequent Extraction as the Preferred Approach for Propylene Glycol. The reversible reaction with acetaldehyde forms 2,4-dimethyl-1,3-dioxolane (24DMD), which is much less hydrophilic than the glycol and which is therefore much more readily extractable. Energy costs are estimated to be about 42% of those for three-effect evaporation of all the water from a dilute (1.5% w/w) propylene glycol solution.
3. Measurement of Equilibria for Extraction of Propylene Glycol with an Organoboronate Extractant and Preliminary Success of CO<sub>2</sub> Displacement as an Effective Means of Regeneration. These results show that propylene glycol is reversibly extractable with a mixed Aliquat 336-phenylboronate ion-pair extractant, primarily or exclusively by 1:1 complexation. Carbon dioxide has been shown to be absorbed by the loaded extractant, displacing free phenylboronic acid and thereby releasing the extracted glycol for back-extraction into concentrated aqueous solution. The carbon dioxide can be stripped, to at least a substantial extent, readying the extractant for reuse.
4. Finding and Confirming that Activated Carbons Have Substantial Reversible Capacity for Uptake of Sugars and Glycols. This result is promising for recovery methods based upon adsorption with carbon and/or functionalized polymeric sorbents.

#### **1995 PLANNED ACTIVITIES:**

1. Regeneration of Basic Extractants and Sorbents. Two methods will be explored for regeneration of extractants and sorbents that are sufficiently basic so that they take up carboxylic acids at fermentation pH (pH about 6). The first of these is a change of diluent composition, obtained by distillation and probably accompanied by a temperature swing as well. The second is back-extraction with a basic leachant other than aqueous trimethylamine

(TMA). TMA has already been found to be effective, but has the drawbacks of odor and a tendency to discolor lactic acid systems.

2. **Regeneration of Organoboronate Extractants with Carbon Dioxide.** Carbon dioxide will be investigated further as an agent for regeneration of organoboronate extractants, which are effective for removal and recovery of glycols, glycerol, sugars and related substances from aqueous solution. We shall investigate whether loss of capacity upon repeated use of the extractant is due to incomplete removal of CO<sub>2</sub>, thermal degradation, or some other cause. With this knowledge, we may be able to improve the retention of capacity. Retention of 30-40% of the original capacity, as already demonstrated, may itself be satisfactory.
3. **Boronate-based Polymeric Sorbents or Ion Exchangers.** We will investigate the use of boronate functionalities in a solid form for recovery of glycols. The two avenues to be explored are pairing boronate anions (e. g., phenylboronate) with a quaternary ammonium anion exchanger and mounting boronate groups chemically onto a polymeric sorbent.
4. **Selectivities in Amine Extraction and Sorption of Carboxylic Acids.** Two selectivities are of interest to us -- the selectivity among carboxylic acids (i. e., multi-acid equilibria) and the selectivity between carboxylic acids and substrate sugars. Generally the selectivity between carboxylic acids and sugars should be as high as possible. Dissociation extraction (extraction with a stoichiometric deficiency of the extractant) may be an effective means for fractionation among multiple acids.
5. **Adsorption of Glycols and Sugars by Activated Carbons.** The differences in adsorption capacities among different carbons will be related to differences in surface area and chemical composition. Reversibility of adsorption will be investigated in more detail. With this information, it should be possible to identify the types of chemical interactions that are effective for reversible uptake of multi-OH compounds by carbons and to identify possible alternative sorbents.

## **ANNUAL TECHNICAL SUMMARY REPORT:**

### Recovery of Carboxylic Acids

Separations of carboxylic acids, glycols and other OH-bearing compounds from aqueous solution are critically important for economical production of these chemicals from biomass by fermentation, for recovery from aqueous waste streams, and for production by petrochemical and other routes. Separations using reversible chemical complexation with reactive extractants or adsorbents are effective for these purposes, because these agents are selective and take up dilute solutes with high capacities.

In earlier research on this project we defined equilibria and methods of regeneration for recovery of carboxylic acids by means of amine extractants and related basic polymeric sorbents. We have established that extractants and sorbents of optimal basicity can recover carboxylic acids from aqueous solutions at  $\text{pH} > \text{pK}_a$  of the carboxylic acid (a condition common in fermentation operations), while being regenerable by back extraction with aqueous trimethylamine (TMA),

followed by thermal decomposition of the resultant trimethylammonium carboxylate to provide product acid and recycle TMA. We also examined phenol- and boronate-based extractants for recovery of alcohols and glycols.

#### Recovery of Acetic Acid as Calcium Magnesium Acetate (CMA)

Calcium magnesium acetate (CMA) is an important, environmentally benign road de-icer that can be made by fermentation to form a dilute acetic acid solution, followed by uptake of the acetic acid by a basic extractant or sorbent, followed in turn by back-extraction or leaching of the acetic acid with an aqueous suspension of mixed calcium/magnesium hydroxides (slaked dolomitic lime). We have carried out research to identify extraction and sorption agents of optimal basicity.

Triocetylphosphine oxide and secondary, tertiary and quaternary amine extractants and solid sorbents with tertiary or quaternary amine functionalities were investigated as agents for production of CMA from dilute aqueous acetate solutions with pH in the range 5.5 to 6.5, typical of fermentation processes. This pH range is well above the  $pK_a$  of acetic acid, which is 4.76. The pH and temperature dependencies of the capacities of these extractants and sorbents and extent of regeneration by aqueous suspensions of dolomitic lime were determined and interpreted. Among extractants, in 1-octanol diluent, Amberlite LA-2 (Rohm & Haas Corp.), a secondary amine, gave the best combined performance for extraction and regeneration. Among the solid sorbents, Amberlite IRA-35 (Rohm & Haas Corp.), with tertiary amine functionalities, gave the best performance. Both should give effective and economical processes for recovery of fermentation acetic acid as CMA.

Triocetylphosphine oxide has been recommended for this purpose in two previous studies. However, it does not retain capacity at pH 5.5 and above, and is thus not effective at fermentation pH.

#### Recovery of Glycols, Sugars and Related Multi-OH Compounds

Reversible Reaction with Aldehydes. Glycols and related compounds are extremely difficult to remove from water, because they are highly polar and therefore hydrophilic, as well as low in volatility and not crystallizable. Aldehydes react reversibly with glycols to form dioxolanes. Dioxolanes are cyclic ethers and are therefore much less polar and hydrophobic than are glycols. We have explored the use of reversible reaction with aldehydes as a means of recovery of glycols from aqueous solution.

We carried out experiments to evaluate the recovery of propylene glycol (1,2-propanediol) from dilute aqueous solutions via reactive distillation with formaldehyde to form 4-methyl-1,3-dioxolane (4MD), or with acetaldehyde to form 2,4-dimethyl-1,3-dioxolane (24DMD). The equilibrium and kinetics of the reaction with formaldehyde were studied in systems catalyzed by Amberlite IRA-120 (Rohm & Haas Corp.) ion-exchange resin. The equilibrium constant varied from 5.9 to 8.7 in the temperature range 25-85°C, with no apparent trend with respect to temperature. Kinetically, the reaction was found to be first order in the concentrations of formaldehyde, glycol and catalyst, with an activation energy of 102 kJ/mol. For the reaction with acetaldehyde the equilibrium constant decreased from 18.1 at 40°C to 8.5 at 83°C. The reaction rate was faster

than for formaldehyde. The volatilities of 4MD and 24DMD with respect to water were found to be 100 and 33, respectively.

In the formaldehyde process, excess formaldehyde is needed for a high degree of recovery of propylene glycol. However, this residual formaldehyde is difficult to remove and recover from the aqueous solution. In the acetaldehyde reactive distillation process unfavorable equilibrium and the high volatility of acetaldehyde require an excessive flow of acetaldehyde and produce incomplete recovery of propylene glycol.

A process that combines reaction of acetaldehyde with propylene glycol and extraction of the resultant 24DMD is more attractive. The energy requirement of such a process was computed, by simulation, to be 42% of the energy required for removal of the water from a 1.5% (w/w) solution by a triple-effect evaporation process.

Reversible Complexation with Organoboronates. Organoboronate anions reversibly form cyclic complexes with *cis*-vicinal diols. Complexation with organoboronates can therefore potentially be used to recover and fractionate selectively among glycols, glycerol, sugars and related compounds.

The partition coefficient of propylene glycol from water into 2-ethylhexanol was measured with greater precision than heretofore. The result ( $0.075 \pm 0.002$ ) enabled recalculation of previous data on extraction of 1,2-propanediol, fructose, glycerol and sorbitol from water with 3-nitrophenylboronate in 2-ethylhexanol diluent. The organoboronate is used in an organic ion pair with Aliquat 336 (Rohm & Haas Corp.), a quaternary ammonium compound. New and more precise data were obtained for extraction of 1,2-propanediol with the Aliquat 336-phenylboronate ion pair. (Phenylboronic acid is more available and should be less costly than is 3-nitrophenyl boronic acid.)

Data for the effect of temperature on extraction equilibria showed that a temperature-swing process would not be effective for regeneration of the organoboronate extractant. We have instead explored a novel approach wherein carbon dioxide is added to the loaded organoboronate extractant, converting Aliquat 336 to the bicarbonate form and releasing phenylboronic acid. The  $\text{CO}_2$  is then stripped out at higher temperature, restoring the Aliquat 336-phenylboronate ion pair for reuse. We have found that  $\text{CO}_2$  is readily absorbed and effectively displaces propylene glycol from the loaded extractant. Stripping of  $\text{CO}_2$  at 110-120°C restores about 30 to 40% of the original capacity upon repeated regeneration and reuse of the extractant. We are presently investigating whether this reduced capacity reflects thermal degradation or incomplete removal of  $\text{CO}_2$ .

Adsorption with Carbons. Certain activated carbons exhibit surprisingly high capacities for uptake of sugars and glycols. We have measured uptakes of glucose from water by a wide variety of carbons, finding substantial capacities (0.2 g/g carbon or higher) in many cases and uptakes that are largely or completely reversible. Since glucose departs negatively from ideality (activity coefficients  $< 1$ ) in water, this uptake must result from specific interactions with the carbon surface.

These results are promising in two ways. First, a well chosen activated carbon can be an effective agent for recovery of sugars and glycols from aqueous solution. Second, with knowledge of the specific chemistry at play in the interactions of these compounds with carbon, it may be possible to identify or create synthetic polymeric sorbents that will be effective for removal and recovery of these compounds.

23. **PROJECT TITLE:** The Electrocatalytic Hydrogenation of Edible Oils

**PRINCIPAL INVESTIGATOR:** P. Pintauro

**PROJECT SITE:** Tulane University

**DESCRIPTION:**

A novel method of hydrogenating polyunsaturated fatty acids found in vegetable oils to monounsaturated acids low in the trans isomer product is being explored. The selectivity of the electrochemical route is superior to that of heterogeneous catalysts at high temperature, since it allows the production of the nutritionally preferred cis (oleic acid) isomer, which is not the preferred thermodynamic product at higher temperatures (the trans isomer is more stable). The electrochemical selectivity could be extended to other classes of reductions in which the stereoselectivity is necessary.

**1994 ACCOMPLISHMENTS:**

1. A semi-empirical porous electrode Raney nickel flow reactor model has been developed, which can predict the dependence of soybean oil hydrogenation current efficiency on applied current and electrolyte oil content.
2. The porous electrode reactor FORTRAN code has been linked to a commercially available computer design software package (PRO/II<sup>TM</sup> by Simulation Sciences, Inc.) for heat and mass balance calculations on an entire soybean oil electro-hydrogenation plant.
3. An equipment size and cost analysis of an oil electro-hydrogenation plant (6.5 MM lbs/yr capacity) was performed, based on a tentative plant flowsheet, the porous electrode reactor model, and the results of the PRO/II heat and mass balance calculations.

**1995 PLANNED ACTIVITIES:**

DOE/AICD funding for this project will conclude prior to the end of 1994. The electrocatalytic oil hydrogenation work will continue for an additional two years, with U.S. Department of Agriculture funding (National Research Initiative Competitive Grants Program), as a collaborative research project involving Tulane University and the USDA's Midwest Regional Research Laboratories in Peoria, Illinois.

## ANNUAL TECHNICAL SUMMARY REPORT:

A first attempt was made to design and cost an oil electro-hydrogenation plant, with an annual production of  $6.5 \times 10^6$  lbs. of partially (brush) hydrogenated soybean oil. A plant flowsheet containing the electro-hydrogenation reactors and the oil purification unit operations equipment downstream from the reactors has been devised. The design and operating conditions of the commercial-scale reactors were based on laboratory experiments, where a single anode/cathode element radial flow reactor was used. In the oil hydrogenation plant, a bank of three electrocatalytic Raney nickel powder reactors will be employed. Each reactor module consists of 200 anode/cathode tubes, 200 cm in length enclosed in a common shell (1.0 meter in diameter). The downstream purification equipment is to remove solvent and supporting electrolyte from the oil product and recycle this material back to the hydrogenation reactors. Heat and mass balance calculations were carried out on all unit operations in the oil hydrogenation plant using PRO/II<sup>TM</sup> software (Simulation Sciences, Inc.). The FORTRAN code of a porous electrode model for the Raney nickel powder electrocatalytic hydrogenation reactor was incorporated into PRO/II as a user defined subroutine. When the reactor feed composition (oil content and water/t-butanol solvent composition), the percent reduction in double bonds to be achieved during electro-hydrogenation, and the applied current density are specified, the porous electrode code computes the oil hydrogenation current efficiency, the total number of tubular elements needed in the reactor network, the total number of reactor modules, the reactor's heat duty, and the optimum Raney nickel powder bed thickness.

The results from the PRO/II heat and mass balance code were used to size and cost the oil hydrogenation plant's unit operations equipment. The capital and operating costs of the oil hydrogenation plant were determined for reactors operating at  $15 \text{ mA/cm}^2$  with a reaction medium of 10 wt.vol% soybean oil in a water/t-butanol solvent containing tetraethylammonium p-toluenesulfonate as the supporting electrolyte. In most cases, the cost of the various units in the plant were obtained from equipment vendors. The total production cost for the electrochemical hydrogenation process was found to be \$0.0265 per pound of soybean oil (based on a 5 year straight line depreciation of all installed equipment). This cost is approximately four-times greater than the cost of a turn-key high temperature, chemical catalytic brush hydrogenation plant, where  $\text{H}_2$  is supplied in gaseous form. When the cost of the raw soybean oil reactant was included in the economic analysis, the percent increase in total product cost by converting from a chemical to an electrochemical processing route amounted to only 8.3%. The selling price of a low trans isomer electrocatalytic hydro-oil may be higher than oil products in the market today. In this regard, the electrochemical process for oil hydrogenation appears to be a promising alternative to existing high temperature and pressure chemical catalytic schemes. Work is continuing on lowering the production costs of the electro-hydrogenation process, as will be discussed in the following paragraph.

The major cost in the electrocatalytic oil hydrogenation scheme was found to be the Raney nickel flow reactors. In an effort to lower production costs, modifications in the design of the radial flow reactor have been proposed. With no change in oil hydrogenation current efficiency at  $15 \text{ mA/cm}^2$ , it appears that a 20-30% reduction in production costs could be realized by the following reactor design changes: (i) A tube-side feed in the reactor (as opposed to shell-side feed) with the flow in the outward radial direction and an expanded anode grid placed outside the concentric porous

ceramic tubes which trap the Raney nickel powder, (ii) a decrease in the anode/cathode element diameter from 5.6 cm to 4.0 cm, which will double the number of tubular elements in a 1.0 m diameter shell and lower the number of reactor modules needed to hydrogenate a given amount of oil, and (iii) a decrease in the anode/cathode spacing (reducing the thickness of the outer porous ceramic tube), which will decrease the voltage drop in the reactor and lower the electric power costs for oil hydrogenation. Over the next year (with USDA funding), experiments will be carried out to test the proposed modifications in reactor design. Additional experiments will be performed to eliminate all non-food grade chemicals in the reaction medium and to evaluate the quality, stability, and flavor/odor of the electro-hydrogenated oil.

24. **PROJECT TITLE:** Engineering Analysis of Electrochemical Conversion of Methane to C<sub>2</sub> Hydrocarbons and Synthesis Gas

**PRINCIPAL INVESTIGATOR:** D. Eng and M. Stoukides

**PROJECT SITE:** Tufts University

**DESCRIPTION:**

This project is a follow-on to research previously conducted for DOE under the Office of Industrial Technologies Advanced Industrial Concepts Division (AICD). It involved R&D for the electrochemical conversion of methane to C<sub>2</sub> hydrocarbons or synthesis gas. Technical advances were made during the initial R&D study that indicated favorable processes for upgrading the methane. A study was undertaken to assess the technical and economic feasibility of this process. Technical hurdles were to be identified that outline the potential research areas that would permit these processes to be further developed and demonstrated to industry. Efforts include a) the evaluation of the need and impact of upgrading methane, b) an engineering analysis of the proposed processes, c) an assessment of the direction and specifications for the research at Tufts to allow sustainable commercial interest, and d) an evaluation of novel derivative technologies and cross-benefits for other technologies.

**1994 ACCOMPLISHMENTS:**

An interim report was completed. Findings included usages of methane and natural gas, incentives and rationales for scale-up processes, progress in solid electrolyte fuel cell technology, and engineering parameters. A literature search and a qualitative engineering conceptualization have been completed.

**1995 PLANNED ACTIVITIES:**

Completion of the final analysis and submission of the final report to DOE/NREL. Details of the work reported in the interim report will be completed. In addition, a qualitative analysis of economics and scaling factors for potential processes will be completed.



#### 4.6 Systems Analysis and Process Control

25. **PROJECT TITLE:** Bioengineering Simulation Technology (BEST) Development.

**PRINCIPAL INVESTIGATORS:** R. Wooley, G. Phillipidis & R. Landucci

**PROJECT SITE:** National Renewable Energy Laboratory

#### **DESCRIPTION:**

The development of the necessary mathematical algorithms to simulate bioprocesses, which is carried out in part through a subcontract, to encode the algorithms into commercially available ASPEN/SP-386. Once developed and validated, the bioprocess simulator can be used to rapidly and accurately determine the technical and economic feasibility of existing and proposed bioprocesses, or evaluate the impact of proposed bioprocess improvements and R&D accomplishments. These analyses can be done on a consistent basis to allow comparison of alternative processes and the assessment of progress from different R&D projects. It can also be used to establish the most cost effective research goals and, thereby, minimize the time to commercialization and maximize the impact of the research investment.

#### **1994 ACCOMPLISHMENTS:**

Delivery of unit operation models developed jointly between NREL and Simulation Sciences (SimSci) have been further delayed due to the turn-over of key personnel at SimSci. The additional batch and CSTR unit operations with rigorous mixing, aeration and heat transfer calculations will hopefully be delivered in the 1st quarter FY95. Delivery of these additions along with all source code and documentation will complete the current contract with Simulation Sciences for the bioprocessing unit operation additions to the chemical simulator ASPEN/SP.

#### **1995 PLANNED ACTIVITIES:**

Work was completed 1994. No additional work proposed.

26. **PROJECT TITLE:** Biological and Chemical Process Integration and Assessment Computer Models

**PRINCIPAL INVESTIGATOR:** J. D. Ingham

**PROJECT SITE:** Jet Propulsion Laboratory

#### **DESCRIPTION:**

This project will develop a user-friendly computer model (BCPI). The model will provide economic analysis, energy requirements, and the potential of biochemical processing in existing

or proposed chemical and engineering applications. The package, together with supporting data, can be used on the personal computer, and as an effective teaching aid.

#### **1994 ACCOMPLISHMENTS:**

- A general model for biochemical and chemical process assessments has been formulated. For each process, information is entered in a data file with a defined format, followed by nearly automatic computer applications of the model to obtain a series of assessments where different values of numerous independent variables can be selected and used.
- Methods have been established to readily convert process models to recent or earlier versions of Basic, to run directly in DOS and Window environments, and to use EXCEL or Lotus Works for graphics displays.
- Reports on the 50 commodity chemicals produced in the largest amounts in the US have been completed. These were prepared to assist in the determination of priorities for research areas that should result in maximum improvements in energy efficiency and in greater utilization of renewable energy resources for the industrial production of chemicals.

#### **1995 PLANNED ACTIVITIES:**

No funding is available for this work; however, process model development may be continued. Specific plans will be defined before the end of the current year.

#### **ANNUAL TECHNICAL SUMMARY REPORT:**

In the planning and implementation of research to improve energy efficiency, it is often necessary to determine if new proposed advances in biochemical or chemical technology would be expected to result in decreased energy consumption and potential cost advantages in the production of industrial chemicals. Computer models have been developed to assess proposed process concepts and anticipated energy-economic benefits.

The objective is to develop process models and use them to determine the energy requirements and economics of biological and chemical processes and process improvements for the production of industrial chemicals. The goal is to support the DOE Advanced Industrial Concepts Division Catalysis/Biocatalysis Program, by contributing to the technology base for chemical and biocatalyzed processes for large-scale production of industrial chemicals from renewable resources. Advantages of these models (in comparison with alternative models) are that they can be adapted and used with any IBM-type computer, with no expanded or extended memory, are totally user-friendly, and can be readily modified for multiple assessments of many types of biological or chemical processes. There is no limit to the database or subprogram size because the entire model can be operated from any number of disks and drives. The reason for this advantage is that only one subprogram is processed and in the computer memory at a time, with the values of variables passed from one subprogram to the next. It is more convenient (but not really necessary) to use a computer that operates at higher speed, e.g., >16 MHz, because of the minor time delays required to consecutively load each subprogram. Eventually, if the application is so

extensive that more than one diskette is required, the model display will provide prompts to insert the next disk, or a hard disk can be used to provide uninterrupted program operations.

Commercial computer-aided design (CAD) programs, such as ASPEN, can be used for chemical process assessments; however, their primary purpose is to develop detailed process designs for specific chemical plants. To complete a study estimate of plant costs with moderate predictive accuracy, can cost thousands of dollars. Except for recent work on the BEST simulator (based on ASPEN) at NREL, CAD programs have not been satisfactorily adapted for bioprocess assessments. In any case, operational CAD programs are not normally available at moderate cost, are not very user-friendly, and require special computer capabilities. In addition, as CAD simulators have been improved, computer requirements have been extended to accommodate larger databases and increased complexity, and this trend is expected to continue. The current software discussed here has been developed to minimize or circumvent factors that tend to limit applications for rapid, reliable energy and economic assessments of various process concepts, particularly for timely research applications.

A series of process reaction types (for example, specific fermentation processes and chemical processes such as hydrogenation, dehydration, esterification) are included in the models for selection from menu lists, and the selected type is modified as appropriate for an assessment. For a specific reaction of each type, default values for numerous parameters and variables, such as stoichiometric factors, properties, rates, concentrations, materials costs, and economic values, are displayed and can be changed during the assessment run, or in the program, to create a new assessment. Mass and some energy balances are provided, and costs are computed on the basis of a factored estimate. Known processes are included and verified to ensure that energy and cost estimate protocols are reliable. After any base-case estimate, each additional assessment (where numerous process variations can be incorporated) may be completed within a few minutes. Since only the subprograms for each specific assessment are put into the computer memory (and are later automatically deleted), expanded memory or other special computer capabilities are not needed. Current models consist of interactive menu-driven subprograms that run consecutively. Very short process-specific subprograms and the primary data file are automatically preselected when the process name is selected from the first menu. The completed models contain information on equipment types that may be automatically scaled according to process flow rates, with appropriate cost factors for materials, year of construction, and specific process requirements. The purpose of the model is to provide rapid, reliable assessments of new processes to evaluate potential energy or economic advantages.

### Summary of Activities

It had been assumed that either advanced BASIC or GWBASIC, or a version of QBASIC (Quick BASIC) with equivalent capabilities, would be readily available. Unfortunately, that is not the case. It is much simpler to start a new subprogram in GWBASIC with all variables automatically passed to each succeeding subprogram than in QBASIC versions currently available with DOS. Furthermore, program pause commands in QBASIC have been changed. As a result hundreds of modifications to the initial model were necessary and were completed. The end product was improved to provide for operation under any reasonable environment, such as DOS, WINDOWS, or EXCEL versions. A further improvement is that cumulative data files are produced as an

optional selection. These can be used to graphically plot the results of the effects of variables on process costs, energy consumption, or any relevant dependent variable.

For each process, information is assembled and entered in a data file with a defined format. Then generalized computer programs are used to model the process and prepare the assessments. The two main subprograms are essentially long, complex subroutines, that are the same for all processes. The assessments are less involved than for some earlier models, but are as reliable and require less memory. The current programs can be more readily converted to any version of Basic, or Quick Basic, and to operating system executable programs, or to an entirely new environment, such as Excel or Lotus Works. Both of these spreadsheet programs have been used to prepare plots of assessment results; e.g., where the effect of variations for ethylene cost on the selling price of vinyl acetate has been demonstrated. Another example was a series of assessments for hydrogen production (conventional process from methane). Plots can be produced easily for relationships among many process variables.

The new computer method for process assessments has been improved to include a new routine to derive process energy requirements. Except for obvious improvements that may be required to accommodate many other processes, the generalized model is complete. Input files for about 12 processes have been prepared, and corresponding assessments and comparisons have been completed.

27. **PROJECT TITLE:** Impact of Catalysis on the Production of the Top 50 U.S. Commodity Chemicals

**PRINCIPAL INVESTIGATOR:** Lee Tonkovich

**PROJECT SITE:** Pacific Northwest Laboratory

**DESCRIPTION:**

Pacific Northwest Laboratory (PNL) supports the DOE Advanced Industrial Concepts Division (AICD) in systems analysis for the Biological and Chemical Technology Research (BCTR) Program. Meeting the diverse requirements of BCTR requires systems analysis activities to target the industry R&D needs with the potential for greatest impact.

**1994 ACCOMPLISHMENTS:**

Completed project investigating impact of catalysis on energy consumption

- Filed report # PNL-9432 (Impact of Catalysis on the Production of the Top 50 U.S. Commodity Chemicals)

This study assesses the impact of catalysis on process energy utilization, and the associated energy savings resulting from catalytically-induced higher yields. The maximum feedstock and process energy savings during the production of the top 50 commodity chemicals is 0.47 quads/year. The energy savings resulting from process yield improvements ranging from 1% to 25% is also quantified.

**1995 PLANNED ACTIVITIES:**

Review panel requested quantification of the potential impact of catalytic improvements on the production energy of the top 50 chemicals.

28. **PROJECT TITLE:** Top 50 Commodity Chemical Study

**PRINCIPAL INVESTIGATOR:** Ed Lipinsky

**PROJECT SITE:** Pacific Northwest Laboratory

**DESCRIPTION:**

AICD's mission is to develop and undertake a balanced program of high-risk, long-term, directed interdisciplinary research and development that will improve energy efficiency, reduce waste generation, and enhance fuel flexibility in the industrial sector. PNL support the DOE AICD in systems analysis for the BCTR program. Meeting the diverse requirements of BCTR requires system analysis activities to target industry R&D needs with the potential for the greatest impact. PNL seeks justification of a BCTR goal to conduct research that results in saving 3.5 quads/yr of energy by 2030 using a combined strategy of feedstock substitution (CO<sub>2</sub> or renewables and process energy savings (via catalyst design, advanced separations, and reaction engineering). It is anticipated that the greatest amount of energy can be saved by improving production technology from the commodity chemicals industry, although other process industries are possible targets as well.

**1994 ACCOMPLISHMENTS:**

In 1994, Pacific Northwest Laboratory undertook an effort to characterize the Top 50 U. S. Commodity Chemicals as identified by Chemical and Engineering News that annually has the highest reported production volumes. The purpose of this task was to describe industrial chemicals that are significant to the future of the U. S. and to identify R&D needs and opportunities based on new or novel approaches to improve U. S. industrial competitiveness in these markets. This effort ranked these important chemicals based on 1992 production volumes, energy, intensity, estimated energy consumption, price, revenue, capital intensity and environmental considerations. Based upon these scores and the results of a companion report "Impact of Catalysis on the Production of the Top 50 U. S. Commodity Chemicals," (Tonkovich) opportunities were identified which focused on non-incremental changes in process technology and more environmentally benign alternatives.

This study resulted in the following two reports: Brief Characterization of the Top 50 U. S. Commodity Chemicals and Initiatives for Top 50 Commodity Chemicals. The key conclusions for each of these reports were discussed with other PIs and the Guidance and Evaluation Panel at the Golden, Colorado PI meeting.

**1995 PLANNED ACTIVITIES:**

Characterize the Second 50 Commodity Chemicals

PNL will identify, gather data, analyze, and report the results of the second 50 U. S. Commodity Chemicals in a similar format to that developed for the Top 50 U. S. Commodity Chemicals. The report will characterize these chemicals on the basis of volume, energy intensity, total quads

consumed, price, revenue, capital intensity, impact of catalysis, and environmental scores. These "Fact sheets" will form the basis of development of program plans to work with U. S. Industries.

#### Refine the Top 50 Commodity Chemical Study

Based upon input from industry and subsequent review, refine the methodology and numbers reported in the "Brief Characterization of the Top 50 U. S. Commodity Chemicals." Based upon the R&D drivers in industry, where BCTR offers unique capabilities to improve energy efficiency and/or decrease environmental impacts, to further develop.

#### Develop Initiatives

Assist BCTR in the development of initiatives to address the high priority needs of the chemical industry based upon energy and environmental considerations. These initiatives will be reviewed with the industry guidance panel to gain support and concurrence on the selected topic areas.

## 5.0 PROGRAM OUTPUT

### 5.1 Publications in Professional Journals, Books and Symposium Chapters, and DOE Technical Publications (alphabetical by institution).

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#### Idaho National Engineering Laboratory

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### **Jet Propulsion Laboratory**

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### University of Pittsburgh

D.N. Beratan, J.J. Regan and J.N. Onuchic, "Electron Transfer Pathways in Proteins," Chemistry and Industry, submitted, 1994.

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## **5.2 Patent Awards and Filings**

### **Awards**

#### **California Institute of Technology**

U. S. Patent 5,316,935. "Subtilisin Variants Suitable for Hydrolysis and Synthesis in Organic Media" (1994).

#### **Oak Ridge National Laboratory**

Scott, Charles D. and Brian H. Davison, "Attrition Reactor System," U.S. Patent 5,248,484 (September 28, 1993).

### **Filings**

#### **Idaho National Engineering Laboratory**

Biomediated Continuous Release Phosphate Fertilizer

Process For Manufacture of Encapsulated Biomediated Slow Release Phosphate Fertilizer

#### **Jet Propulsion Laboratory**

Kern - Sialic Lewis Antigen Analog JPL Case No. 18707A - CIT Case No. 2118A

#### **University of California, Berkeley**

C. J. King & L. J. Poole, "Carboxylic Acid Sorption Regeneration Process".



### **5.3 Special Awards and Presentations**

#### **California Institute of Technology**

"Engineering Enzymes for the Chemical and Pharmaceutical Industries: Getting the Right Biocatalyst," Annual Lecture, Advanced Centre for Biochemical Engineering, University College London, April 20, 1994.

#### **University of California at Berkeley**

C. J. King, "Solvent Extraction in the Process Industries: Quo Vadis", Principal Conference Plenary Lecture (invited), International Solvent Extraction Conference (ISEC'93), York, England, September 9, 1993.

#### **License agreement:**

#### **California Institute of Technology**

Caltech and Eli Lilly signed a license agreement for technology to develop variants of p-nitrobenzyl esterase with improved performance in organic solvents.