12 PLANNING REPORT

NBS and Industrial Biotechnology:

Technical Developments and Future Measurement Needs

Thomas C. O'Brien, Ph.D.

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NBSIR 82-2549

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Acknowledgments

I wish to thank the many people and organizations who have contributed to this report, both in providing information, and in reviewing the report and suggesting corrections, modifications and additions. The author is especially appreciative of contributions to this report by Peter Heydemann, William Kirchhoff, Elaine Bunten-Mines, Dennis Reeder, and Kenneth Gordon.

The responsibility of any remaining errors or inaccuracies remains with the author. The author would appreciate having report omissions or oversights called to his attention so that they can be considered for future reports.

Executive Summary

Reports and articles have indicated that biotechnology will be one of the most significant industrial technologies in the next several decades. The major areas that will be impacted by this technology include chemicals, energy, health care, food processing, and agriculture.

Beause of the potential long-term industrial importance of biotechnology, the National Bureau of Standards began in the late 1970s a process to: (a) examine NBS's role and responsibilities related to industrial development and use of this technology, and (b) identify infrastructure support needed by future biotechnology based industries. Initial NBS Planning Office reports resulting from this examination established a policy framework that illustrates the opportunity for integrating traditional Bureau services and fundamental research capabilities in chemistry, physics and engineering with long-range industry non-proprietary (generic support) needs in biotechnology and in the applications of this technology. These reports also identified the commodity organic chemical industry as an early adopter of this technology, as well as an industry which would require NBS services.

This report describes those initial steps the Bureau could take to develop fundamental research capabilities and service-related initiatives to meet future industry needs for infrastructure support in biotechnology. This is accomplished by: (a) examining commodity organic chemical industry trends, (b) identifying research opportunities and barriers in biotechnology related to commercial production of products, and (c) evaluating existing NBS capabilities in relation to long-term industry needs.

Report Conclusions

- The commodity organic chemical industry is important to the U.S. economy and has maintained a favorable balance of trade. However, this industry will: (a) face increased international competition over the next several decades affecting its current trade position, (b) undergo a feedstock transition (coal, biomass) sometime in the future, and (c) undergo structural change as a result of overcapacity, pressure of economic forces, and technological innovations. The timing of these events will be dictated primarily by economic and political developments.

- The commodity organic chemical industry views its current investments in biotechnology as a means to keep options open on an important future technology. An industry output from this technology which will appear before the end of this decade will be higher value added organic chemicals. Application of biotechnology on an industrial scale for the production of "traditional" commodity organic chemicals is not expected much before the end of the century. Further, because of the difficulties that will be encountered in displacing commodity organic chemicals now being produced from petroleum feedstocks, the value added component which results from biotechnology may be a comparatively small $1 billion. However, biotechnology may have a significantly greater market impact in the production of "non-traditional" commodity organic chemicals such as biopolymers.

- Depending upon the economics and politics of producing organic chemicals via biotechnology, biomass could become an important feedstock for the production of organic chemicals. However, considerable research effort must take place to improve the productivity and economics of the biotechnological processes for biomass conversion that are under consideration. Critical to such an improvement for bioprocesses will be advances and innovations in biotechnology "tools", such as biocatalyst, recombinant DNA, cell culture, and fermentation technologies. Further, if biotechnology is to be applied on the industrial scale envisioned by organic chemical producers, innovation must also occur in bioprocess engineering technology areas, such as process monitoring and control, product separation and extraction, cell recycling, and process intensification.

- This report summarizes discussions with industry representatives on their perceptions of industry needs for infrastructure support in biotechnology as related to NBS's mission and capabilities. From these discussions, a number of measurement needs as well as fundamental science and evaluated data needs were identified. Concurrent with these discussions, an assessment of NBS capabilities in the physical, chemical and engineering sciences was undertaken to determine how well existing Bureau capabilities could address these long-term needs. In general, industry needs could be met by expanding and/or refocusing existing Bureau scientific capabilities. However, new staff capabilities in enzymology, molecular biology/biophysics, and microbial/cell biochemistry would be required in the next 2-5 years.

- Should NBS decide, in addition to its current competency development effort, to address long-term industry infrastructure support needs in biotechnology, this report provides a framework for possible short-term and long-term actions the Bureau could take in relation to its major activities which include Standard Reference Materials, evaluated data bases, measurement and analytic techniques, and non-proprietary (generic) research. In addition, this report stresses the need for: (a) a research climate at the Bureau that is receptive to the interdisciplinary cooperative and collaborative requirements of this technology; and (b) periodic evaluation of biotechnology in relation to NBS's mission and capabilities over the life cycle of this technology.

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1. REPORT OBJECTIVE

This is the third report* in a series of National Bureau of Standards (NBS) Planning Office reports on "NBS Contributions to Biotechnology." Its objective is to provide a description of the climate within which NBS can evaluate and begin to develop research initiatives and services to meet future industry needs in biotechnology. This will be accomplished by selecting a candidate industry--in this case, the organic chemical industry--and for that industry:

- o providing an overview of the industry, industry trends, and the technical and non-technical forces influencing the industry's strategies;
- o identifying research trends, opportunities and barriers in biotechnology related to the commercial production of products;
- o projecting industry long-range needs in biotechnology and their relationship to NBS' mission, as well as the future direction of NBS research and services; and
- o summarizing NBS' in-house research and service capabilities, the relationship of these capabilities to long-range, non-proprietary, generic industry infrastructure needs in biotechnology, and gaps in NBS capabilities.

This report will not link resource requirements to NBS long-range initiatives. Rather, this report will serve as a guide for NBS scientists as they conceptualize, justify and develop long-range research and service initiatives in biotechnology and for NBS managers as they evaluate research and service priorities and plan for the allocation of limited Bureau resources.

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*Bunten-Mines, E., Planning Report 1: NBS Contributions to Biotechnology. A Preliminary Report. National Bureau of Standards, Department of Commerce, April 21, 1980.
Coates, J. F., Planning Report 9: Implications of Biotechnology for the National Bureau of Standards. A Seminar Presentation. National Bureau of Standards, Department of Commerce, November 16, 1981.

2. INTRODUCTION

.1 NBS and Biotechnology

There are three basic roles of the National Bureau of Standards. (1) The primary role of the Bureau is to provide a basis for measurements throughout the United States by means of standards, evaluated data, and test methods. (2) NBS is also to provide technical assistance to the Federal Government by carrying out legislative assignments and by providing technical support to other Federal agencies. This would be accomplished by drawing upon skills and knowledge that arise from satisfying the first role. (3) The Bureau's final role is to solve technological problems which are related to national goals as recognized by the Government's leadership, and which are measurement and standards intensive and so related to NBS's primary role.

With the dramatic increase in scientific, industry, and public interest in biotechnology in the mid-late 1970s, NBS began a process to: (a) explore the long-range technical and nontechnical research and commercialization issues associated with this emerging and potentially significant industrial technology, (b) examine the Bureau's role and responsibilities related to industrial development and use of this technology, (c) identify infrastructure requirements needed for support of future biotechnology based industries and bio-industrial processes, and (d) identify NBS internal capabilities related to these needs (1-3). The Bureau's 1981 long range plan summarized the results of this policy analysis and projected that the commodity organic chemical industry, an industry facing significant competitive and technological challenges in the future, would be among the first to adopt biotechnological processes and to require NBS supporting services (2). As a result of this planning process, a policy framework was established that illustrated the compatibility between traditional NBS roles, Bureau capabilities in chemistry, physics and engineering, and some biotechnology industry needs for infrastructure support. However, policy guidance from this process also suggested that there was less of a rationale for justifying Bureau activities in areas of health care applications of biotechnology. The cummulative effect of this process and the subsequent policy guidance was to reduce concerns many NBS scientists had about the acceptability of initiating projects in biotechnology, and to call attention to incipient industry needs in this area.

.2 NBS Competency Development in Biotechnology

The Bureau's Center for Chemical Physics (CCP) proposed in their 1981 long-range plan a competency development initiative on enzyme catalysis, a technology essential for industrial biotechnological processes (4). In addition, the Center for Analytical Chemistry (CAC), in their 1981 Annual Report, proposed competency initiatives in immunoassay procedures, two-dimensional electrophoresis, and solid phase reagent development (5). Resources were subsequently allocated by the NBS Director to CCP and CAC in Fiscal Year 1982 for support of these in-house competency development initiatives. The purpose of this competency development effort was to begin to develop an in-house knowledge base in an area deemed pivotal to commercial developments in biotechnology. The development of this knowledge base has already begun in the CCP and CAC. In addition, scientific and general information exchanges have begun at the Bureau through the newly formed NBS Biotechnology Working Group. Concurrently, the Bureau began a dialogue with industry, technical and trade associations, and academia in order to determine what the future needs of biotechnology based industries will be, and how these needs may relate to NBS measurement, information, and industry infrastructure service roles and responsibilities. This dialogue with industry formally began in late 1981 and is discussed in more detail in Section 6 of this report. It is possible that these discussions with industry will lead to a core biotechnology research and service effort integrated with complementary expertise already at NBS in disciplines related to industrial applications of biotechnological processes. This report will provide (1) a basis for evaluating alternative initiatives for a focus of a core effort in biotechnology and (2) a framework for understanding some of the presently disaggregated research elements at the Bureau potentially related to industry needs in biotechnology.

The Bureau views its investments in biotechnology-related base capabilities as part of a long-range strategy. This strategy will allow the Bureau to anticipate and to provide in a timely manner traditional infrastructure services that will be responsive to biotechnology-based industry needs when in the latter part of this decade industrial processes employing biotechnologies start to move from developmental and pilot stages to commercial operations.

.3 Structure of the Report

The seven remaining sections of this report provide a basis for considering generic technological problems and issues faced by producers of organic chemicals in the implementation of biotechnological processes and how these problems and issues may relate to Bureau research, knowledge development and service activities.

Section 3 analyzes trends in the organic chemical industry, discusses industrial technological challenges and competitive pressures, and discusses the influence of biotechnology on industrial strategies.

Section 4 discusses the problems and opportunities associated with one potentially important area for biotechnology applications, e.g., use of an alternative feedstock (biomass) in the production of organic chemicals.

Section 5 uses the research and economic challenges of converting lignocellulose substrate to ethanol to identify generic barriers and problems that may be encountered in the scale-up of industrial biotechnological processes. Biotechnological process technologies, such as fermentation and enzyme catalysis are discussed. Also discussed are biochemical process engineering technologies, such as separation and extraction, process monitoring and control, and substrate pretreatment. Some comments are made on newer technological developments that may have potential applicability as biotechnology production technologies.

Section 6 provides a listing of industry contacts and information sources, and summarizes their perceptions of future biotechnology based industry needs that may be related to NBS' mission.

Section 7 summarizes current NBS scientific and technological capabilities that could complement a core biotechnology group at the Bureau. Projections are also made on areas where Bureau capabilities could be strengthened.

Section 8 presents comments by NBS laboratory scientists on possible Bureau roles and R & D opportunities related to biotechnology and on possible applications of Bureau expertise and services to industrial needs in biotechnology.

Section 9 discusses possible next steps the Bureau could initiate with respect to biotechnology, industry interactions, and resource allocations.

3. FUTURE TRENDS IN THE COMMODITY ORGANIC CHEMICAL INDUSTRY

The purpose of this report section is several fold:

- o to provide an overview of the commodity organic chemical industry;
- o to discuss hydrocarbon feedstock transitions within the industry and considerations associated with alternative feedstocks;
- o to illustrate the many internal and external pressures and forces influencing the competitiveness of the industry; and
- o to provide a framework for considering these forces and for guiding industry strategic planning.

.1 Industry Overview

.a Economic Importance. The U.S. chemical industry represents a significant and positive economic force domestically. It is the fourth largest U.S. manufacturing industry. Organic chemicals represent about 44 percent of the total value of chemical industry shipments (6), and are an important factor in international trade (7), as shown in Table 3.1. This favorable situation was fueled in part by the significant price increases from 1976-1981 in large volume, commodity organic chemicals (8). This situation could change dramatically, however, due to domestic and international economic climates, new competitors in world markets (particularly commodity organic chemicals), availability and price of raw materials, world capacity/demand relationships, and other factors.

.b Definition of Industry. Approximately one-third of the U.S. chemical industry is devoted to producing organic chemicals or petrochemicals. Table 3.2 provides a listing of the primary (commodity) chemicals and a partial listing of intermediate organic chemicals (SIC Codes 2865 and 2869). There are, in fact, about 100 intermediate organic chemicals of major industrial importance, several hundred more of lesser importance, and about 7000 different organic compounds in

Table 3.1

United States Trade Balance in Chemicals

A. U.S. Chemical Trade Balance
($ billions)

	1981	1980	1979	1978
exports	21.20	20.74	17.31	12.62
imports	9.60	8.58	7.49	6.43
balance	11.60	12.16	9.82	6.19
E/I*	2.21	2.42	2.31	1.96

B. U.S. Organic Chemical Trade Balance
($ billions)

	1981	1980	1979	1978
exports	5.87	5.70	5.06	3.37
imports	3.10	2.54	2.16	1.73
balance	2.77	3.16	2.90	1.64
E/I*	1.89	2.24	2.34	1.95

Source: Reference 7.

*E/I = Export/Import ratio

Table 3.2

Commodity Organic Chemicals (Petrochemicals) and Uses

Primary Petrochemicals	Petrochemical Intermediates	Petrochemical-dependent Products	Major End Uses
Unsaturates (Olefins)	Ethylene Oxide	Plastic Materials	Transportation/Automobiles
Ethylene	Ethylene Glycol		Housing/Construction
Propylene	Ethylene Dichloride	Synthetic Rubber	Home Furnishings & Furniture
Butylene	Vinyl Chloride		Apparel
Butadiene	Acrylonitrile	Synthetic Fibers	Personal and Medical Care
Acetylene	Cyclohexane		Packaging
			Industrial Machinery
Aromatics	Ethylbenzene	Surfactants	Electronics and Electrical
Benzene	Styrene Monomer		Agriculture
Toluene	Phenol	Medicinals & Pharmaceuticals	Toys/Sports
Xylenes (mixed)	Phthalic Anhydride		Detergents/Soaps
o-xylene	Terephthalic Acid	Nitrogenous Fertilizers	Paints
m-xylene			Adhesives/Sealants
p-xylene		Phosphatic Fertilizers	Fabricated Rubber Products
Naphthalene			
Methanol		Pesticides	

SOURCE: Reference 6.

chemical production. This table (Table 3.2) also illustrates the products and end uses of these organic chemicals. The dominant market for SIC Code 2865 and 2869 organic chemicals is polymers such as plastics, elastomers and fibers, representing about 60-65 percent of total U.S. commodity organic chemical consumption (9).

Table 3.3 provides general information on the hydrocarbon feedstocks (petroleum, natural gas, and coal) for primary (commodity or bulk) organic chemicals. These chemicals, such as ethylene, propylene, toluene, benzene, and the xylenes, serve as the key building blocks of many industrial organic chemicals. They are the foundation of the chemical industry, and rarely find direct consumer use. Ethylene also serves as the base chemical for about 50 percent of the largest volume industrial chemicals.

Table 3.4 provides production data of commodity (primary) as well as major intermediate organic chemicals, and general information on hydrocarbon feedstock requirements, number of manufacturers, and major product outlets. Comparing production information in this table with price information in Table 3.5 illustrates the sensitivity of primary (commodity) organic chemicals to the energy price increases of 1978-1980. These commodity chemicals are more sensitive to such energy price increases than intermediate organic chemicals or organic chemical-dependent products, since ethylene, toluene, propylene, etc. are made from hydrocarbon resources or raw materials that require hydrocarbon fuels (8). However, the problems associated with obtaining fuels and with producing chemicals are not closely related (12). For the period 1976-1981, primary organic chemicals had the greatest price increases relative to other chemicals (8).

Tables 3.6 and 3.7 list the major United States producers of organic (commodity) chemicals. These producers listed in Table 3.6 account for 80 percent of U.S. total capacity; 74 percent of this production capacity belongs to the petroleum companies, in part because of their process capabilities to produce a stream of C_1 to C_8 chemicals (6, 9). Petroleum companies are also consumers of about 40-45 percent of their organic chemical building materials (9). Tables 3.6 and 3.7 also illustrate the variety of recent research and

Table 3.3

Hydrocarbon Feedstocks for Production of Commodity Organic Chemicals

Primary Organic (Commodity) Chemical	Hydrocarbon Feedstock (1978 data)	
	Source	Approximate % of Chemical Product
methanol	synthesis gas	100 %
olefins	natural gas liquids (ethane, propane, butane)	43 %
	petroleum liquids (naphtha and gas oil)	57%
aromatics	petroleum liquids	97 %
	coal based (coal tars and light oils)	3 %

Source: Reference 9.

TABLE 3.4

Feedstocks and Outputs of and Outlets for Commodity Organic Chemicals

Major Organic Chemical	OUTPUT: U. S. Production In Pounds (billion) 1980	1979	1977	1975	1973	1971	Precursor or Feedstock Requirement	Number of Manufacturers (U.S. Plant Sites)	Examples of Major Outlets
ethylene[a,g]	27.54	29.90	25.17	20.50	22.33	18.45	(ethane, propane, butane,	23	polyethylene, ethylene derivatives
propylene[a,h]	13.71	14.20	13.33	8.71	9.88	6.89	naphthas, gas oil)	38	polypropylene, isopropyl alcohol
toluene[a]	11.27	11.86	7.3	5.1	6.9	6.4	naphthas, coal/light oil	31 (9)[c]	benzene, gas additive, solvents, polyurethane foams
benzene[a]	10.98	12.29	10.6	7.5	10.7	7.9	naphthas, toluene, coal/light oil	41 (10)[c]	styrene, phenol, cyclohexane
ethylene dichloride	9.97	11.79	11.00	7.98	9.29	7.56	ethylene	12	vinyl chloride monomers
ethylbenzene	7.61	8.45	8.31	4.82	5.69	4.98	benzene, ethylene	16	styrene
methanol[b,j]	7.00	7.37	6.45	5.18	7.06	4.95	hydrogen and CO_2, coal, fermgases	10	formaldehyde
styrene	6.90	7.48	6.87	4.67	5.98	4.68	benzene, ethylene	12	polystyrenes
vinyl chloride	6.46	6.39	5.99	4.20	5.35	4.34	ethylene dichloride	12	polyvinyl chloride, homo- and co-polymer resins
xylene[b]	6.40	6.89	6.15	4.6	5.6	4.4	naphthas, toluene, coal/light oil	16	p- and o-xylene, gas additive, solvent
terephthalic acid	5.92	6.16	5.41	4.61	2.56	3.57	p-xylene	5	polyester fibers
formaldehyde	5.77	5.97	6.05	4.56	6.42	4.52	methanol	18	resins
ethylene oxide	4.95	5.67	4.36	4.47	4.17	3.60	ethylene	13	ethylene glycol, surface active agents
ethylene glycol[e]	4.24	4.73	3.68	3.81	3.28	3.07	ethylene	13	antifreeze, polyester resins
p - xylene[b]	3.38	4.65	3.17	2.48	2.33	1.66	naphthas, toluene coal/light oil	11	synthetic fibers
butadiene[b,g]	2.89	3.58	3.26	2.60	3.64	3.34	butenes, butane, ethylene (by-products)	17	rubber
acetic acid[d]	2.82	3.27	2.57	2.20	2.43	1.96	acetaldehyde, methanol, butane, wood	10	vinyl and cellulosic acetate
phenol[k]	2.46	2.98	3.34	1.75	2.24	1.74	cumene, (benzene, chlorobenzene, toluene)	17	phenolic resins, caprolactam, bisphenol A
acetone[f]	2.12	2.65	2.22	1.64	1.99	1.54	isopropyl alcohol	16	methyl methacrylate, methyl isobutylketone, solvent, protective coatings
cyclohexane	1.97	2.43	2.27	1.73	2.12	1.75	benzene	8	nylon, caprolactam
vinyl acetate	1.92	1.98	1.59	1.29	1.50	0.93	ethylene, acetic acid	7	polyvinyl acetate lactices and resins, polyvinyl alcohol
isopropyl alcohol[e]	1.79	1.90	1.89	1.52	1.84	1.67	propylene	5	acetone, solvents
propylene oxide[e]	1.77	2.25	1.87	1.52	1.75	1.19	propylene	5	propylene glycol, polyester glycols for urethanes
acetic anhydride	1.47	1.51	1.50	1.46	--	--	acetaldehyde, acetic acid	4	cellulose esters
ethanol[b,d]	1.22	1.41	1.34	1.43	1.96	1.63	ethylene, sugar containing materials	12	detergent solubilizer, cosmetics, chem. intermediates, solvents
adipic acid	1.20	1.60	1.54	1.34	--	--	cyclohexane	5	nylon

[a] Key organic chemical (primary petrochemical) building blocks

[b] Other primary petrochemical building blocks

[c] Number in parenthesis represents those manufacturers (of total number) that use coal as hydrocarbon feedstock base

[d] Some current large scale production by biotechnological process (Synthetic Organic Chemicals, U.S. International Trade Commission, Washington, D.C., 1981)

[e] Possible targets of industrial bioprocess interest (References 47, 56)

[f] Significant pre-WWII industrial product produced by fermentation process (acetone-butanol)

[g] Could be produced from cellulose via ethanol (Reference 57)

[h] Could be produced by dehydration of isopropyl alcohol (Reference 56)

[i] As a fermentation product could be dimerized to form styrene (Reference 56)

[j] Biomass to methanol is an option for liquid fuel production (Reference 49)

[k] Can be produced as by-product of wood pulping process (Reference 53)

Sources: References 9-11.

Table 3.5

Sensitivity of Commodity Organic Chemicals to Energy Price Increases

Commodity Organic Chemical	Average Price Range of Organic Chemicals (cents)						Production Energy Requirements (Therms/ ton)*
Year:	1970	1974	1978	1979	1980	1981	
Toluene (¢/gal.)	22	58	61-70	99-135	115-140	123-145	556
Benzene (¢/gal.)	24	90-105	70-85	85-165	145-190	160-200	608
Xylenes (¢/lb.)	3.3	6.7	6.5-8.4	8.4-18	18-23	19-24	714
Ethylene (¢/lb.)	3.3	7.9	13.3-14	13.3-20	20-24.5	24-28	603
Propylene (¢/lb.)	2.3	--	9-9.8	9.8-16	17-20	18-22	584
Butadiene (¢/lb.)	9.5	9.5-18	20-22	20.5-26	32-34	33-34	666

Source: Reference 10.

*Chemistry and Industry, May 1, 1982, p. 285.

Table 3.6
Leading U.S. Commodity Organic Chemical (Petrochemical) Producers

	Primary Commodity Organic Chemicals (Petrochemicals)							Total Capacity (billions of pounds)	Biotechnology Investments				
	Methanol	Ethylene	Propylene	Butadiene	Benzene	Toluene	Xylenes		Health Care	Chemicals	Agriculture	Energy	Food Processing
Shell		X	X	X	X	X	X	9.60	X		X		
Exxon		X	X	X	X	X	X	8.07		X	X	X	
Standard Oil (Indiana)		X	X	X	X		X	7.70	X	X		X	
Atlantic Richfield		X	X	X	X	X	X	7.32			X		
Union Carbide		X	X	X	X	X	X	7.21					
Gulf		X	X		X	X	X	7.13		X			
Phillips		X	X	X	X	X	X	5.28	X	X	X	X	X
Dow		X	X	X	X	X		5.20	X	X	X		
Sun		X	X		X	X	X	4.19	X	X	X		
Texaco		X	X	X	X	X		3.53	X	X	X	X	
Monsanto	X	X	X	X	X	X	X	3.21	X	X	X		
Commonwealth					X	X	X	3.20					
Celanese	X	X	X					2.52		X			
Amerada Hess					X	X	X	2.50					
Du Pont	X	X	X	X				2.47	X	X	X		
Mobil		X	X	X	X	X		2.24					
Cities Service		X	X		X		X	1.95	X	X	X	X	
Ashland	X		X		X	X	X	1.92					
American Petrofina		X	X		X	X	X	1.79					

[a] Source: Reference 9.

[b] Sources: References 13-16.

Table 3.7
Leading U. S. Chemical Producers

1980 Rank	Company	Chemical sales 1980 ($ millions)	Industry Classification	Biotechnology Investments: In-house R&D	Biotechnology Investments: Equity Ownership; Other
1	Du Pont	$10,250	Basic chemicals	X	Cal. Inst. Tech., Harvard, Maryland, New England Nuclear Corp.
2	Dow Chemical	7,217	Basic chemicals	X	Collab. Genetics, Collab. Research
3	Exxon	6,936	Petroleum	X	Cold Spring Harbor
4	Union Carbide	5,650	Basic chemicals	-	-
5	Monsanto	5,453	Basic chemicals	X	Biogen, Genentech, Genex, Collagen, Harvard, Rockefeller, Washington University
6	Celanese	3,200	Basic chemicals	-	Yale
7	Shell Oil	3,089	Petroleum	-	Cetus, Celltech
8	W. R. Grace	2,733	Specialty chemicals	X	-
9	Gulf Oil	2,569	Petroleum	X	-
10	Occidental Petroleum	2,458	Petroleum	X	-
11	Allied Corp.	2,450	Basic chemicals	X	Biologicals, Calgene
12	Standard Oil (Ind.)	2,235	Petroleum	-	Cetus
13	Hercules	2,095	Basic chemicals	-	Adria Lab.
14	Atlantic Richfield	1,945	Petroleum	X	-
15	American Cyanamid	1,861	Basic chemicals	X	Molecular Genetics

[a] Rank based on 1980 sales.
[b] Source: Reference 17.
[c] Sources: References 13, 16, and 18.

other investments by these companies in biotechnology. Table 3.8 illustrates the diversity of major firms in the chemical industry and is a relative indicator of their commitment to this industry.

The following characteristics can be generally applied at present to the U.S. commodity organic chemical industry:

- o capital, energy, feedstock, and R&D/technology intensive;
- o established distributing and servicing networks;
- o global operations;
- o high plant and equipment investment in contrast to low labor content in cost of manufacture;
- o highly skilled work force;
- o high growth rate in past dependent on commodity chemicals (future growth potential may depend on specialty chemicals);
- o integrated plant complexes;
- o world scale capacity plants for commodity chemical production (current capacity, however, exceeds global demands);
- o chemical plant sites near sources of raw material (raw materials represent over 50 percent of manufacturing costs and industry outputs are highly dependent on their supply, availability, and price);
- o world's largest producer of organic commodity chemicals;
- o competitive position based on low price, ready availability, and high purity of commodity chemicals;
- o second only to West Germany in exports; and
- o signs of industry maturation and structural change on horizon.

.c Growth Projections and Economic Influences. The growth rate of primary (commodity) organic chemicals has been declining for the past three decades: 1950s--17 percent, 1960s--13 percent, and 1970s--4.6 percent (20). The growth projection for the 1980s is 4 percent, only slightly above the projected growth of the GNP (2.8 percent). But probably more revealing is the declining ability of the industry over the same time periods to rapidly penetrate end markets or create new end markets (20). This is an indicator of a maturing industry.

Table 3.8

Leading Chemical Industry Firms' Percentage of Total Firm Sales to Chemicals

Company	Chemical % of Total Sales	Company	Chemical % of Total Sales
DuPont	82	Esmark	6
Union Carbide	57	Gulf Oil	3
Monsanto	80	3M	9
Dow Chemical	69	Pennwalt	45
Exxon	6	Airco	39
Celanese	76	Pfizer	17
Allied Chemical	66	Tenneco	5
W.R. Grace	36	Dow Badische	100
Hercules	83	Kerr-McGee	25
Occidental Petroleum	28	Nalco Chemical	89
Eastman Kodak	20	American Hoechst	65
American Cyanamid	50	ElPaso Natural Gas	15
Shell Oil	15	Baychem	63
FMC Corp.	39	Union Oil (Cal.)	7
Phillips Petroleum	19	Uniroyal	8
PPG Industries	33	Cabot	56
Stauffer Chemical	85	GAF Corp.	20
Rohm and Haas	70	Atlantic Richfield	4
NL Industries	41	Borg-Warner	11
Standard Oil (Ind.)	7	Chemetron	47
Akzona	71	General Electric	1
Texaco	4	National Starch	79
Diamond Shamrock	60	Witco Chemical	47
Ethyl	55	Freeport Minerals	88
Cities Service	18	IFF	97
Olin	31	CF Industries	71
Standard Oil (Cal.)	5	Morton-Norwich	30
Goodyear	7	National Distillers	10
B. F. Goodrich	18	Northern Natural Gas	19
Mobil Oil	3	Williams Companies	21
Air Products	74	Arco Polymers	92
Firestone	9	Farmland Industries	13
BASF Wyandotte	71	Eli Lilly	14
Merck	23	Procter & Gamble	2
United States Steel	4	Dart Industries	12
Asnland Oil	12	Dow Corning	78
ICI America	86	Kewanee Oil	66
Lubrizol	99	Texasgulf	34
Continental Oil	6	Houston Natural Gas	33
Reichhold Chemicals	96	Commercial Solvents	90
CIBA-GEIGY	41	Emery Industries	90
IMC	37	Sun Oil	5
Borden	9	Kaiser Aluminum	14

Source: Reference 19.

Part of the problem in the poor growth prognosis for the U.S. commodity chemicals industry lies with the economic viability, vitality, and confidence of its major customers--automobiles, housing, textiles, and appliances. High interest rates, availability of capital, domestic and foreign economic downturns, increased production capacity, value of the U.S. dollar, active foreign government involvement in commodity organic chemicals, etc. will affect the rehabilitation of the chemical industry customers and, in turn, future growth prospects for the industry (8, 21, 22).

.d Capacity and Demand. Current capacity and future demand projections for U.S. organic commodity chemicals (based upon economic forecasts, projected chemical customer needs, new plants coming on-stream, new competitors, etc.) indicate that capacity will exceed demand by about 20-25 percent (10, 23, 24). In spite of this, U.S. market growth for organic chemicals is projected to be 1-2 percent over that for other industrialized countries (25). This will create additional competitive pressures on the U.S. commodity chemical industry in domestic markets, as well as on our chemical trade balance overall, and the trade balance for organic chemicals in particular (Tables 3.1, 3.9, 3.10).

.e Energy Requirements and Pressures. In spite of extensive chemical industry energy efficiency and conservation efforts in the 1970s, the chemical industry, and in particular the producers of commodity organic chemicals, is one of the most energy intensive and sensitive manufacturing industries. About 5.7 percent of U.S. petroleum and natural gas liquids are used by the chemical industry as hydrocarbon feedstocks for the production of primary organic commodity chemicals (ethylene, propylene, benzene, etc.), and an additional 1 percent is used as fuel in the manufacturing processes (23). In fact, chemical industry natural gas consumption represents about 10 percent of total U.S. consumption, e.g., 3 percent for feedstock and 7 percent for fuel (27).

A guiding principle of the commodity organic chemical industry has been conversion of low cost raw materials, such as petroleum and natural gas, into higher value added products. Geographic proximity of industry plants to such raw materials have been considered and will continue to be important in the strategic planning of firms in this industry (24).

Table 3.9

Example of Trade Balance Changes in Commodity Organic Chemicals

Organic Chemical (quantity & value in millions)	Exports				Imports			
	Quantity		Value		Quantity		Value	
	1981 total	Change from 1980	1981 Total	Change from 1980	1981 Total	Change from 1980	1981 Total	Change from 1980
benzene (gal.)	35.7	+202%	$51.8	+17.9%	132.8	+40%	$206.1	+41%
toluene (gal.)	34.3	-13%	43.9	-18%	68.8	-10%	86.5	+6%
p-xylene (gal.)	54.7	-17%	99.5	+ 1%	9.6	+42%	20.5	163%
styrene (lb.)	1,148.5	-5%	364.8	- 6%	28.0	-45%	9.0	-46%
ethylene glycol (lb.)	235.3	-5%	51.9	-13%	67.9	+282%	16.6	+231%
vinyl chloride (lb.)	835.5	-4%	142.7	-16%	112.1	-19%	15.0	-28%

Source: Reference 26.

Table 3.10

Commodity Organic Chemical Projections

% Share of World Trade	Exporters (%) 1979	1985	1990		Importers (%) 1979	1985	1990
Ethylene dichloride							
U.S.	90%	29%	10%	Japan	73%	36%	58%
Western Europe	9	20	10	Asia-Pacific	26	36	6
Canada	1	28	44	Com. Countries	-	28	36
Middle East	-	23	28	Latin America	1	-	-
Latin America	-	-	8				
Styrene							
U.S.	78%	16%	-	Western Europe	29%	37%	28%
Canada	22	80	71	Latin America	25	12	10
Communist Countries	-	4	2	Asia Pacific	20	26	22
Middle East	-	-	27	Com. Countries	-	-	-
				Japan	9	20	19
				Middle East	6	5	7
				U.S.	-	-	14

Source: Reference 27.

In spite of current perceptions of an "oil glut" (28-30), oil is a wasting resource (31-34). The era of low price and ready availability of these hydrocarbon feedstocks is growing to a close. Although deregulation of oil has increased new drilling as well as oil prices, production of oil from existing facilities will decline from current levels and total oil production will probably peak around the year 2000 (35, 36). Also, natural gas reserve additions, usually associated with oil discoveries, have been less than production for over a decade (35, 37). With projected decontrol by 1985, natural gas price increases have encouraged greater exploration with some encouraging results; but documented reserves of natural gas have not been sufficient to reverse the decline in the reserve/production ratio (37). In addition near term prospects for decontrol of natural gas by the 1985 timetable are not promising, and eventual decontrol greater than 50 percent is unlikely (27, 38).

To a considerable extent, the pace at which the curtain is drawn on the petroleum era will depend upon the oil production and policies of and the economic and political stability of Saudi Arabia. Other key uncertainties include: (a) U.S. and global economic growth, (b) energy conservation, (c) oil production and stability of other OPEC nation, and (d) energy contributions from nuclear, and coal, and from other sources--tar sands, shale oil, solar, biomass, hydroelectric, wave power, etc. (35).

In spite of these projections, estimates have been made by some that 75-80 percent of U.S. organic chemical production will still be based on petroleum and natural gas feedstocks in the year 2000 (8). Table 3.11 illustrates the variety of strategies (e.g., securing hydrocarbon feedstock positions, forward integration into higher value added products) that chemical companies are pursuing in response to hydrocarbon feedstock price and availability, as well as future competitiveness pressures. Table 3.12 also reflects the increasing presence of petroleum companies in the production of organic commodity chemicals and the location of their production facilities at the sources of lowest price and of available hydrocarbon raw materials (41). Petroleum companies control 20 percent of U.S. coal production and 25 percent of U.S. coal reserves (35, 42).

Table 3.11

Feedstock Security Positions of Some Leading Organic Chemical Firms

Chemical Company	Hydrocarbon Base
DuPont	merger with Conoco, expanded oil/gas exploration effort through recent acquisition (Terrapet)
Dow	owns oil/gas properties*
Union Carbide	purchases feedstock supplies
Monsanto	oil/gas exploration revenues indirectly support some of liquid feedstock needs
W. R. Grace	oil/gas exploration revenues indirectly support feedstock needs; has coal resources
Allied Corporation	oil/gas exploration revenues indirectly support some feedstock needs, plans expanded energy exploration effort through subsidiary (Union Texas Petroleum)
American Cyananid	purchases feedstock supplies
Celanese	purchases feedstock supplies
PPG Industries	purchases most feedstock supplies

Source: Reference 39.

*Recently sold domestic oil and gas reserves to Apache. Still owns Freeport, Texas crude oil processing refinery (Chemical Week, May 26, 1982, pp. 34-38).

Table 3.12

Location of Chemical Firms' Production Facilities at Source of Feedstocks

A. Saudia Arabia (SABIC)[a]

COMPANY[b]	PRODUCT
Celanese	methanol
Dow	ethylene, polyethylene
Exxon	polyethylene
Shell	ethylene, ethanol, ethylene dichloride, styrene, benzene

SOURCE: Reference 22.

[a] SABIC = Saudi Basic Industries Corporation
[b] Production facilities at Al Jubail.

B. Canada

COMPANY[a]	PRODUCT
Celanese	methanol
Union Carbide	ethylene glycol
DuPont	polyethylene
Exxon	benzene, styrene
Shell	benzene, styrene, polyethylene
Dow	polyethylene

SOURCE: Reference 40.

[a] Production facilities in Western Provinces.

In spite of these company activities, a chemical industry hydrocarbon feedstock transition will take place in the future. This transition will involve a shift from oil and natural gas feedstocks to coal and to renewable hydrocarbon feedstocks. Economics will dictate the timing of this transition. Technology and technological innovation will be essential components in shifts made. The transition to alternative hydrocarbon feedstocks will greatly influence strategic decisions being made now, and stimulate evolution and structural change in the chemical industry.

.2 Feedstock Alternatives

The raw material hydrocarbon feedstock bases for organic chemicals will be undergoing changes over the next few decades. Although projections indicate that the transition to alternative feedstock bases will not be dramatic or complete, the organic chemical industry in the future will depend upon a different mix of feedstocks and on new feedstock conversion processes (43).

Petroleum is currently the primary feedstock of the organic chemical industry. In spite of the current availability of this hydrocarbon feedstock, there are long-term chemical industry concerns regarding its price and availability. Since any organic chemical can be made by either chemical or biological synthesis, two alternatives to petroleum and shale oil include coal and biomass.

The basic technologies supporting the conversion of either coal (44, 45) or biomass (46) have been known for several years. However, to improve the economics of these conversion technologies, significant investments in R&D and new and innovative processes are required over the next several years.

In addition to capital investments in existing plant and equipment, basic factors, such as: (a) technical feasibility, (b) process economics (cost of the raw materials, efficiency of the process, cost of product recovery, cost of recovery and value of by-products, cost of waste disposal and/or of conversion of waste to useful products), (c) cost of process development, (d) market size and future growth potential, and (e) present and future competition will govern

the choice of hydrocarbon feedstock, of target organic chemicals, and of synthesis routes (47). Table 3.13 provides an overview of some of the economic considerations involved in determining what alternative feedstock to use and when to convert to an alternative hydrocarbon feedstock.

The projected time table for the commercial production of organic chemicals via coal conversion is about 1990 (20, 50). However, recent developments in coal conversion technology in the United States by Eastman Kodak (acetic anhydride), by Gulf-SRC-II (commodity olefins, BTX), and by Mobil-zeolite catalysis (methanol, ethylene) (44, 52, 60), and in West Germany (45) suggest that the timetable may be advanced to the mid-to-late 1980s.

Although use of corn starch as a chemical feedstock in the production of ethanol is becoming increasingly competitive at commodity levels with ethanol produced via chemical synthetic routes (61, 62), commercial production of commodity organic chemicals from biomass via biotechnological processes is not expected until the mid-to-late 1990s (51). In spite of this, the chemical industry's emphasis on conserving energy, on improving process efficiency, on reducing hazardous wastes and less caustic reaction processes, and, where applicable, on moving to production of higher value added products favors use of fermentation and other biotechnology tools (e.g., enzymes, plant and animal cell cultures) in the production of organic chemicals in the long-term. Although biochemical engineering process scale-up barriers (such as heat removal, mixing, sterilization, instrumentation and controls, and environmental/nutritional influences on the stability of genetically engineered microorganisms) are present (63, 64), supplementation of traditional mutation and selection techniques with gene amplification, fusion and recombinent DNA techniques (46, 51, 65-67) and improvements and innovations in bioprocesses (63, 68, 69) will eventually shift the economic momentum currently favoring conventional chemical synthetic processing approaches to bioprocesses for production of higher value added chemicals and some commodity chemicals.

Biotechnology, via chemoautotrophic microorganisms, can also provide a range of organic compounds from hydrogen and carbon dioxide which are available from coal following reaction with steam (70).

Table 3.13

Some Considerations Involved in Selecting Alternative Raw Material Feedstocks

Economic Considerations (Alternative Raw Material Process)	Coal	Biomass
1. Flexibility	o inflexibility of fossil mass composition o physical and chemical properties influence process selection	o greater tailoring of crops to end use demands (silviculture, aquaculture, sugar-starch crops) o production can be maintained in many environments; land use alternatives o breeding and genetic manipulation options; optimization of composition o adaptability of bioprocesses/biocatalysts to use of low cost substrates
2. Renewability	o non-renewable - but U.S. has about 27% of earth's reserves. 150-440 billion tons projected to be recoverable; current production at 0.8 - 1.1 billion tons	o renewable o annual U.S. agriculture and forestry biomass (million metric tons): - primary - 1540 (crops-corn, grains) - secondary - 975 (forest/crop residues) - tertiary - 550 (wastes) o secondary/tertiary biomass will be major feedstocks in long-term because of food vs. chemical/energy end use conflicts with primary biomass
3. Conversion Technology	o basic liquification (gasification, hydroliquification, pyrolysis, solvent extraction) technologies known and used for several years o considerable investment by petroleum companies in coal R&D technology (synfuel energy industry developments could have significant spill-over effects to organic chemicals) o important innovations (catalysis, in combining processes) and improved understanding of reaction conditions critical to improving conversion process economics	o basic bioprocess and fermentation technologies known and used for several years o active R&D in cellulosic biomass conversion o improvements/innovations in process technology (cellulose conversion, combining unit process steps) will contribute substantially to favorable biomass process economics o not a competitive energy alternative

Table 3.13 continued

Alternative Raw Material Process Economic Considerations	Coal	Biomass
4. Conversion Efficiency	o 60-65%	o 35%
5. Substitution for Petrochemicals	o capabilities for producing cost competitive commodity organic chemicals, for example, ethylene, propylene, toluene, butadiene, the xylenes	o acetic, citric and lactic acids, and ethanol, butanol and sorbitol already produced via biotechnological processes; food processors also becoming producers of commodity organic chemicals. o large scale of petrochemical facilities and projections for commercial coal liquification facilities suggest that substitution for large volume organic commodity chemicals via bioprocesses will be difficult; substitution of organic chemicals, for example, olefins, currently made through multiple chemical synthetic steps offers some targets of opportunity via improved production sophistication (refer to Table 3.14). o chemical and/or bio-conversion of wood/-wood by-products (for example, by wood - wood pulp industry) could provide cost competitive range of primary and intermediate organic chemicals and more refined products.
6. Impacts of Existing Organic Chemical Markets	o projections for scale of coal liquification plants and for volume outputs if directed primarily to production of commodity organic chemicals--ethylene, propylene, BTX--would have significant effect on traditional markets for these hydrocarbons o several coal conversion processes at pilot plant/demonstration phase o commercial impacts projected to begin around 1990, although recent developments could accelerate this projected timetable	o corn derived organic chemicals (ethanol) already have had major market impacts, economics of conversion of primary biomass to some alcohols and aliphatics well established; economics of conversion of ethanol to ethylene not established yet. o sugar/starch plants based chemical industry has potential to supplement some bulk chemicals (ethylene, butadiene) by early to mid-1990s. o specificity and process specification will encourage production of higher value added organic products. o projected timetable for substitutions -

Table 3 (Continued)

Material Process Economic Considerations	Coal	Biomass
7. Economies of Scale	o large scale o an estimate of commercial plant at coal rates of 30,000 tons/day with 30% of energy output producing 1.1 billion lbs/year of ethylene	o economics of transporation of feedstock to process site will encourage smaller scale, more localized process facilities o scale smaller with outputs directed to specialty and fine chemicals
8. Sensitivity to Petroleum Costs	o production economics highly sensitive to oil costs (oil/coal price ratio), particularly with major coal liquification process outputs being synfuels and stream of C_2-C_6 primary chemicals	o less sensitive to petroleum and energy costs, particularly when outputs are higher value added intermediate, fine and specialty organic chemicals o oil glut, delays in natural gas deregulation, comparative return on investments with coal conversion technologies will push back substitution timetables for biomass as a feedstock
9. Feedstock Cost as Percentage of Process Costs	o feedstock costs (30-35%) are decisive parameter (the higher the value of coal feedstock, the greater the likelihood that greater percentages of process stream outputs will be directed to production of commodity aliphatics and aromatics) o other major costs include mining and waste disposal and environmental regulatory demand costs	o critical to the economics of the process is the efficiency of conversion of all biomass (cellulosic) raw materials o raw material is 50-75% of process costs, and depends on such factors as: - type of cellulose material - climate, location of plant/source of material - density of material, transporation, storage - current availability - alternate demand profiles
10. Impact on Fossil Reserves	o reduce dependence on foreign petroleum and natural gas o ample future U.S. reserves of this non-renewable resource	o reduce dependence on foreign petroleum and natural gas o relatively small pressure on hydrocarbon raw material reserves
11. Workforce	o more highly skilled; use more energy intensive process technology	o unskilled and semi-skilled; use of simpler, potentially more energy efficient technologies (however, more highly skilled workforce required for production of high value added organic chemicals)

Table 13.3 continued

Alternative Raw Material Process Economic Considerations	Coal	Biomass
12. Producing Facilities	o large, localized processing units	o smaller, geographically disperse processing units
13. By-products	o generally toxic and present environmental problems o magnitude is substantial (ash)	o generally can be converted to beneficial end products (municiple waste to methane; whey to foods/feeds; pulp wastes to organic chemicals; corn stover to ethanol)
14. Environmental Considerations	o mining--acid drainage, land use and reclamation, worker safety/health, water demands o emissions--sulfur dioxide, nitrogen oxide, acid rain, carbon dioxide, trace elements chemical	o conservation of non-renewable resources o land demands--soil degradation, soil sedimentation and water runoff, impacts on stream fauna and flora o volume and load of fermentation discharges o ground point source pollution

Sources: References: 8, 12, 20,, 35, 44, 48-59.

In spite of deficiencies in data on the economics (a) of biotechnological processes and (b) of starting materials in the production of organic chemicals, some examples of possible targets of opportunity (specialty and commodity organic chemicals) are listed in Table 3.14. Although several of these chemicals represent higher priced/value added chemicals, possible future increases in their volume of sales could shift these currently high priced, low volume chemicals to low cost/high volume commodity chemicals. Critical to improving investment decisions on (a) industrial scale production and (b) the economics of a bioprocess to produce a particular product is a fundamental understanding of cell metabolic pathways. Information on pathway throughput capacity; on pathway intermediates; on pathway enzymes, their stability, the amount of enzymes produced and enzymatic reaction rates; on substrate/product relationships; and on the significance of the metabolic pathway will be essential for industrial investment decisions on bioprocesses (72).

.3 International Competitive Trends

Chemicals, particularly primary and intermediate organic chemicals, are important contributors to the U.S. export performance (Table 3.1) and chemical industry profits, and an increasing proportion of domestic chemical production has been exported (7, 23, 73). However, a number of factors will tend to moderate the rate of growth of exports of U.S. commodity organic chemicals and increase the rate of growth of organic chemical imports.

Some U.S. export moderating factors include:

- o continued near-term reliance on petroleum and natural gas as hydrocarbon feedstocks and declining feedstock price advantage;
- o lower demand for organic chemical-dependent products by chemical industry customers, such as automobile manufacturer, building construction, fibers, plastics industries;
- o domestic and foreign economic pressures and currency valuations; and
- o protectionist barriers.

Table 3.14

Some Possible Targets of Opportunity for Production of Organic Chemicals via Biotechnological Processes

Category	Chemical	Production (x1000 lb.)	Sales Quantity (X 1000 lb.)	Price ($/lb.)
Amino Acids	glutamic acid	650,000[f]	NA	1.80
	lysine	129,000[f]	NA	2.10
	methionine	210,000[f]	NA	1.20
Vitamins[a]	(total)	42,577	26,535	9.04
	vitamin E	7,254	5,287	16.23
				0.
Aliphatics[a]	acetic acid (synthetic)	2,976,772	514,995	0.18
	acrylic acid	611,172	68,966	0.40
	adipic acid[d]	1,500,000	NA	0.57
	ethanol (synthetic)	1,450,769	1,159,446	0.27
	ethylene glycol	4,385,731	3,008,147	0.27
	ethylene oxide	5,220,400	530,986	0.34
	glycerol	140,578	137,784	0.57
	propylene glycol	487,526	426,762	0.37
	propionic acid	102,975	69,753	0.23
Aromatics[a]	aniline	659,421	116,223	0.35
	benzoic acid	73,414	32,220	0.40
	phthalic anhydride	818,247	418,519	0.34
Alginates[b]	(e.g., sodium alginate)	NA	NA	2.00
Enzymes (for industrial bioprocesses)	--	22,000[g]	NA	20.00 to 700.00
Peptides[c]	(e.g., aspartame)[e]	40	NA	110.00
Polymers (for fibers)[a]	--	6,583,357	454,338	0.58
Polymers (water soluble)[a]	--	316,016	269,720	1.38
Plastic and resin materials[a]	--	11,753,214	9,606,419	0.66
Rubber[a]	--	2,798,951	2,249,397	0.27

NA = not available

[a] U.S. production only. (Source: Synthetic Organic Chemicals, U.S. Production and Sales, 1980.)

[b] 1982 prices are about 30 % higher than 1980 prices. 1982 production is about 12 % lower than 1980 production. Source: Reference 72.

[c] Source: References 51, 71. Peptides could be used as a nutrient medium component (synthetic) for cell culture on a laboratory and industrial scale, and as biological regulatory substances.

[d] Source for U.S. production information--Chemical Products Synopsis, Mannsville Chemical Products, August 1981.

[e] Aspartame, a synthetic sweetener, consists of the amino acids aspartate and phenylalanine.

[f] Represents global production. Japan, primarily through Anjinomoto Co., Ltd., produces about 70% of the world volume of amino acids. Glutamate, methionine and lysine currently represent over 99% of the world production of amino acids. (Source: References 51, 71, 72).

[g] Represents global production. Microbial amylases (94%) and proteases (5%) represent 99% of current global commercial production of bulk enzymes. Major companies in bulk enzyme production are foreign owned (for example, Novo, Gist-Brocades, Miles-Bayer AG). A U.S. commercial bulk enzyme producer, Rohm and Haas (enzyme production facilities), has been acquired recently by Corning Glass Works. Production figures are underestimated since firms such as CPC, Clinton, Staley produce starch hydrolyzing enzymes for captive use. (Source: References 51, 71).

Some U.S. import enhancing factors include:

- o emergence of Canada, Saudi Arabia, and Mexico as major commodity chemical manufacturers;

- o chemical industry industrialization plans and export strategies of developing countries such as Brazil and Saudi Arabia; and

- o industry nationalization and state/government ownership and direction of industry and industry economic incentives in some countries.

These and other factors, individually or collectively, will over the next decade and one-half present significant challenges to the international industrial competitiveness of U.S. organic chemical producers, and may cause U.S. producers to lose their position in world commodity chemical markets.*

The following will highlight some examples of the pressures from industrialized and developing countries that will influence the U.S. commodity organic chemical industry competitiveness over the next several years.

a. Trade Liberalization. The European Economic Community (EEC) is the world leader in both chemical exports and imports (7). Nearer term competitive pressures from the United States and Canada as well as intra-EEC exports may force structural changes in the European commodity organic chemical industry because of overcapacity, weakened economies, lower profit margins, and projected pressures from producers in Saudi Arabia and other countries in the Middle East. In spite of the Tokyo Round of Multilateral Trade Negotiations (MTN), nearer-term responses to these mounting pressures may include actions counter to trade liberalization. These may include delays in implementing multilateral trade agreements, shifts to bilateral trade initiatives, import relief through protectionist policies, joint industry ventures, countertrade agreements, mergers, plant closures, government ownership of chemical industries, and government directed competitive practices and industrial policies (22-25). The outcome

*One projection indicated that U.S. net chemical trade will decline at an average annual rate of 15 percent between 1980-1985 (Chemical and Engineering News, May 24, 1982, pp. 21-22).

from these pressures affecting the European organic chemical industry and from their subsequent responses to these pressures will probably be considerably fewer producers in the commodity chemical marketplace and an industrial movement to higher value added organic chemical products. European chemical industry interest in biotechnology as one alternative route to production of higher value added products is illustrated (a) in Table 3.15, (b) by West German industry and government substantial investments in this area since the early 1970s*, (c) by Elf Acquitaine's investment in Engenics (16), (d) by the French Government's increased investments in biotechnology and efforts to orchestrate a national public-private sector initiative in this area (74)**, (e) by the United Kingdom's Agriculture Research Council hybridoma research center (75), and (f) by British Technology Group investments in Speywood Laboratories and in Celltech (76, 77).***

Japan's commodity organic chemical industry, without local access to raw material hydrocarbon feedstocks, may be facing pressures even stronger than those being experienced by European chemical manufacturers, including substantial competitive pressures from the United States and Canada. Industry and government responses to these pressures have focused on protectionist measures and industry cartelization (21, 22). However, like the European industry, chemical industry structural changes will be required in the long-term, probably resulting in fewer companies and with a movement toward production of higher value added products. Japan, however, plans to utilize its industrial experience in fermentation technology (for example, Anjinomoto Company Ltd. and MITI's Fermentation Research Institute) to leverage its industrial movement to high technologies, such as biotechnology, and to higher value added products.

*It is estimated that the West German government invested about $60-65 million in biotechnology R&D between 1972-1978, including funding responsibility for the Gesellschaft fur Biotechnologische fur Forschung (Nature 283: 124, 1980).

**The French government's funding in biotechnology is expected to be about $35 million in 1982 (Chemical Week, March 10, 1982, p. 36).

***By the year 2000, the European biotechnology market is estimated to be $16-17 billion (Information Research Ltd., November 1981). Also, of the 125 firms currently in fermentation production, nearly one-half are European (L. Hepner & Assoc., London, February 24-25, 1982).

Table 3.15

Leading European-based Chemical Companies

Company	1979 Sales ($ million)	Home Country	Biotechnology Investments: In-house	Biotechnology Investments: Equity ownership; other
BASF	16,014	W.G.	X	Heidelberg Univ.
Hoechst	15,474	W.G.	X	Mass-Gen. Hosp., Hoechst-Kawagoe
Bayer	14,953	W.G.	X	Miles
ICI	11,757	U.K.	X	Cell Tech
Rhone Poulenc	7,719	Fr.	X	Transgene, Genetica, Institute Merieux
Montedison	7,592	Italy	X	Adria Lab.
DSM	6,711	Neth.		
Akzo	6,324	Neth.		
Ciba-Geigy	5,557	Switz.	X	DNAX Res.; Alza
Henkel	3,886	W.G.		
Solvay	3,873	Belg.		Biochem

Sources: References 13, 16, 18, 73.

This long-range government-industry strategy was emphasized in the report, MITI's Vision of the 1980's (78, 79).* Examples of Japanese implementation of this strategy with respect to biotechnology include the following:

- o formation of a chemical industry consortium in biotechnology (Mitsubishi, Sumimoto, Mitsui, Asahi, and Kyowa);

- o formation of a 14 company biotechnology research organization in technology areas key to the successful implementation of industrial strategies in biotechnology and in areas that will complement existing industrial experience in fermentation technology (Table 3.16); and

- o Japanese firms' active interest in investment in and establishment of cooperative technology transfer and other agreements with U.S. biotechnology firms (Table 3.17; 80-86).

This approach certainly reinforces trends to reduce their dependence on high priced hydrocarbon feedstocks and to embody greater value and higher technology in organic chemical products.

Developing countries such as Mexico and Brazil were not included in the 1979 MTN agreements and codes, and, under the Generalized System of Preferences (GSP), qualify for tariff-free access to U.S. chemical markets (22, 23). The rationale behind GSPs is to allow developing countries to establish an industrial base. Although this approach is reasonable, it is extremely difficult to determine when a developing country has developed a base for an industry and when GSPs are no longer required. Two of the objectives of Brazil's Proalcool program for production of ethanol from sugar cane are to reduce dependence on petroleum imports and to improve foreign currency exchange. Of the program's 1985 goal of 10.7 billion liters, less than 30 percent (3.1 billion liters) would be used for blending with gasoline (87, 88). With only a small percentage

*Japan's industry is currently spending about $200-220 million per year on biotechnology R&D. MITI is predicting that the value of Japan's biotechnology industry will be $21-34 billion by the year 2000. It is estimated that the biotechnology contribution of Japan's chemical/fermentation industry will be about 75 percent of these year 2000 projections.

Table 3.16

Japanese Biotechnology Research Organization
10 Year Biotechnology Research Program

Area of Research Emphasis	Government Funding (millions)	Companies
rDNA applications	$ 43	Sumitomo Chemical Mitsui Toatsu Chemicals Mitsubishi - Kasei Institute of Life Sciences
Bioreactor development	$ 43	Kao Soap Daicel Chemical Industries Denki Kagaku Kogyo Mitsui Petrochemical Industries Mitsubishi Gas Chemical Mitsubishi Chemical
Mass cell culture technology	$17-22	Asahi Chemical Industry Ajinomoto Kyawa Hakko Kogyo Takeda Chemical Industries Toyo Jozo

Sources: References 81, 82.

Table 3.17

Examples of Japanese Investment in and Collaboration with U.S. Firms

Japanese Firm	U. S. Firm	Investment or Biotechnology Area of Interest
Mitsui Toatsu Chemicals	Genex	urokinase; genetic engineering technology
Takeda Ahiza	Genex	enzymes
Green Cross	Bristol-Myers	interferon
	Collaborative Research	equity; urokinase
	Genex	human serum albumin
	Interferon Sciences	interferon
Mochida	G. D. Searle	interferon
Nissho Iwai Corp.	Cetus	equity
Kinto K.K. (trading company)	Enzo Biochem	develop R & D projects with Japanese pharmaceutical and chemical investors; establish subsidiary firms
Japanese Financial Institutions (unnamed)	Genentech	equity
Mitsubishi Chemical Industries	Genentech	human serum albumin
	Hybritech	monoclonal antibodies
Yamanouchi	Schering-Plough	interferon
Toya Menka Kaisha, Ltd.	Biosearch Lab.	polynucleotide synthesizer
Mitsui	Gentronix/EMV Assoc.	biological-molecular electronic circuits
Fujizoka Pharm.	Biotech Res. Lab.	monoclonal antibodies

of the balance being used by the domestic chemical industry, a significant volume of competitively priced ethanol could be released for export to industrialized nations. In addition, Brazil's low technology-based turnkey plant operations for ethanol production could be exported to other developing countries (87, 89). Brazil may also develop other low technology bioprocess approaches for world scale production of organic chemicals such as acetic acid.

When Mexico's government-owned Pemex petrochemical complexes produce a sufficient volume of commodity organic chemicals to satisfy its growing domestic market and when a sufficient industrial infrastructure is developed, Mexico will, because of its raw material feedstock base, become a significant commodity chemical exporter (21, 22).

b. Import Pressures from Neighboring Countries. The United States is the world's largest (and still growing) market for industrial chemicals.

As indicated above, products from Mexico's commodity organic chemical industry may be competitive in the future in United States markets, although U.S. organic chemical imports from Mexico presently represent only 2-2.5 percent of U.S. imports (73). Mexican government intervention in the market place, for example, currency devaluation, and the development of an industrial infrastructure will in large measure determine Mexico's future organic chemical industry export successes and the timing of these successes.

Despite the economic climate and current falling demand, Canada, and the world scale production facilities coming on line in the Western provinces, will provide substantial competitive pressures on the U.S. commodity organic chemical industry in the near term (24, 40, 90). Already Canada, the major source of U.S. chemical imports, exports about 24 percent of its petrochemicals to the United States (73). A number of factors will enable Canada to double its share of world production, particularly of commodity organic chemicals such as ethylene, benzene, polyethylene, styrene, etc., by the mid-to late 1980s and make significant inroads into U.S. domestic industrial markets. Some of these factors include: Canadian Government intervention to fix natural gas prices below comparative oil prices, the current magnitude of and plans to expand capacity, the value of Canadian currency, and the increasing petroleum costs of its major trading partners (United States, Japan: 21, 22, 40, 73, 90).

c. Middle East (Saudi Arabia). As Saudi Arabia is a critical determining factor in the future price and availability of petroleum, so it may also become a significant competitive factor in the global production and trade of commodity organic chemicals. Table 3.18 provides some primary organic chemical post-1985 production plan estimates for SABIC operations. The overall goals and targets for Saudi Arabia exports are listed in Table 3.19.

In addition to the availability of a hydrocarbon feedstock, Saudi Arabia projects that the construction of modern, flexible chemical manufacturing plants will help it to gain a competitive edge in international markets over industrialized countries with out-of-date production facilities. Because of construction problems and other industry infrastructure considerations and barriers, whether the timetable for Saudi Arabia plant commercialization is achievable and the projected scale of production is realizable is the subject of much discussion. However, in spite of these difficulties, Saudi Arabia's global market share of commodity organic chemicals will increase, but it may take longer to reach its target share than it originally projected (22, 74).

These represent some of the major pressures facing U.S. producers of commodity organic chemicals over the next several years. The magnitude and integration of the domestic chemical market and U.S. chemical industry management and technological know-how will help U.S. manufacturers to weather some of these international competitive pressures from new commodity chemical producers. One alternative, but not exclusive approach, to coping with these competitive forces and feedstock disadvantages would be for the U. S. organic chemical industry to use its technological edge to produce higher value added, high technology products.

Table 3.18

SABIC* Commodity Organic Chemical Production Projections

Chemical Complexes	Commodity Organic Chemical	Projected (mid-late 1980s) Annual Production Capacity (million lbs)
SABIC +		
National Methanol (Celanese, Texas Eastern)	methanol	1400
Saudi Methanol (Japanese Consortium)	methanol	1300
Petrokemya (Dow)	ethylene	1100
Eastern Petrochemical (Mitsubishi)	ethylene glycol	660
	polyethylene	286
Kemya (Exxon)	polyethylene	572
Sadaf (Shell)	ethylene	1440
	ethanol	618
	ethylene dichloride	1000
	styrene	649
Yanbu (Mobil)	ethylene	990

Table 3.19

SABIC Commodity Organic Chemical Export Targets

Target Country	Percentage of Saudi Arabia Exports Absorbed (goal)
United States	20%
Japan	20%
Europe	22%
Middle East and African Countries	38%

*SABIC = Saudi Basic Industries Corporation

Source: Reference 22.

.4 R&D, Technological Innovative and Industry Profitability

The chemical industry is an R&D intensive industry. A high percentage (97 percent) of its R&D is supported directly by private sector chemical firms (91). Table 3.20 illustrates trends in chemical industry R&D spending. It is estimated that petrochemical (organic chemical) R&D currently represents about two-thirds of all non-drug R&D expenditures (6). Firms with 25,000 or more employees and with annual R&D expenditures greater than $100 million represent about 50 percent of the total industry R&D spending (91).

The data presented in Table 3.20 show that the annual growth rate in industry R&D spending for the past decade was about 10 percent. However, estimates of total industry R&D spending from 1980-82 suggest a renewed commitment to investments in R&D. For example, the annual growth rate from 1979-1980 was 14 percent, from 1980-1981 was 16 percent, and from 1981-1982 is expected to be 17 percent. When this rate of increase is adjusted to projected rates of inflation, there is a real growth (or a real expansion of R&D activity) of about 7-10 percent (92), as compared with a real growth rate of 4.5-5 percent in the previous decade. Also R&D as a percentage of sales, at a low of 2.5 percent in 1979, is projected to be at 3 percent in 1982, similar to the 1973 level (92).

There are a number of factors that will maintain and enhance trends in industry R&D investment levels. Examples of these factors include:

- o new entrants to and increased international competition;
- o world overcapacity pressures in commodity organic chemicals;
- o regulatory cost requirements;
- o price and availability of hydrocarbon feedstocks;
- o Economic Recovery Act of 1981;
- o need for innovations in process technology to increase efficiency of existing and of new operations;
- o use of alternative feedstocks;
- o need for automation to optimize plant operations and management and for flexibility in new plant design to meet market demands;

Table 3.20

Chemical Industry R&D

$ Millions	1982[a]	1981[b]	1980[c]	1979	1978	1977	1976	1975	1974	1973	1972	1971	1970
Chemicals & allied products	$6,220	$5,320	$4,600	$4,035	$3,584	$3,256	$3,017	$2,727	$2,450	$2,116	$1,932	$1,832	$1,773
Industrial chemicals	-	-	2,300	2,026	1,835	1,685	1,524	1,391	1,299	1,119	1,031	1,009	1,031
Drugs	-	-	1,670	1,441	1,270	1,154	1,091	981	807	698	607	549	485
Other chemicals	-	-	630	568	479	417	401	354	344	299	294	274	257

[a] Chemical and Engineering News estimate (94).
[b] Chemical Manufacturers Association estimate (23).
[c] Estimate based on Chemical and Engineering News projections (92).

Sources: References 93, 94.

- o possibilities of new processes and products via biotechnology (Tables 3.6 and 3.7); and
- o promise of discovering or of acquiring higher value added speciality products such as pharmaceuticals (23, 25, 92, 95-97).

Investment in and ultimate successes in research and technology development are but one element in determining the economic success of an innovative product or process and the level of industry profitability. Recent experiences in the chemical industry bring home the fact that possession of state-of-the-art technology is little consolation in the face of global overcapacity (95). The probability of the success of a product or process resulting from a firm's R&D depends on three separate but interdependent factors: 1. the probability of technical success; 2. the probability of commercialization; and 3. the probability of economic success (98).

The research and technology development efforts of major organic chemical industry producers can be categorized as dynamic rather than static. These firms have but one goal--to avoid industrial stagnation and decline by rolling-over investments (99). Proponents of the "long wave" theory suggest that current industrial upheavals and the technological ferment, also characteristic of the current status of the commodity organic chemical industry, may indicate the end of a "long wave" (100-103). Here there is a struggle between established industries and the industrial identification of those technologies that will become the bases for the establishment of new, future industries. In addition to technological innovation, a major force influencing the initiation of each "long wave" in the past appeared to be energy (fuel wood, coal, petroleum and natural gas). In the future, technological innovations linked to energy efficiency may be the driving force in the next "long wave". Two key technologies with important long-term industrial and economic implication, and that also meet this energy efficiency criterion are microelectronics and biotechnology. The identification of important future technologies and the timing of an industry's move to these technologies as an integral part of its plans for future growth are critical factors in the future viability and profitability of a firm (104). Present investment in R&D on a variety of fronts, such as appears to be the pattern of major organic chemical firms, will create the options necessary to facilitate future industry decisions to move to

new technologies. Decisions on company selection of specific new technologies and on the timing of company investments in technologies selected will require a closer integration and responsiveness of current industrial R&D efforts to future industry business profitability and success requirements (95).

.5 Industry Strategies

In spite of the inherent technical structure that defines functions within the organic chemical industry, there is considerable heterogeneity in individual company motivations and stakes in this industry (19). Thus, no single industry strategy would be applicable in addressing the many forces and challenges influencing the survival of producers of organic chemicals over the next two decades. Table 3.21 outlines some of the forces that are guiding industry strategic planning. Tables 3.22 - 3.24 list some of the factors that will influence industry strategic decisions.

In addition to the dynamic influences of economic and political forces, major challenges that will affect the strategic plans and directions of commodity organic chemical industry include the following:

Feedstock and Energy

- o feedstock security
- o feedstock availability at competitive prices
- o feedstock flexibility
- o alternative hydrocarbon feedstocks and synthesis processes
- o process efficiency

Growth and Differentiation

- o R&D investments in process innovations vs. development of new and innovative products
- o high technology, higher value added specialty and fine chemicals vs. commodity chemicals

Table 3.21

Overview of Some Forces Influencing Strategies of the Commodity Organic Chemical Industry

Industry Strengths, Weaknesses, and Other Considerations	+	Industry Competitive Forces	+	External Influences
o technology		o competitors		o social
o manpower		o suppliers		o political
o raw materials		o buyers		o economic
o products		o markets		o technical
o manufacturing/ distribution		o new entrants		o Governmental/ regulatory
o foreign trade				
o margins/profits				
o internal competition				
o structural adapt- ability				

Sources: References 105-107.

Table 3.22

U. S. Commodity Organic Chemical Industry
Strengths, Weaknesses, and Other Considerations

Technology
- o increased real dollar expenditures for R&D; technology leadership position
- o emphasis on process research (e.g., process energy efficiency; position alternative feedstocks)
- o broad range of R&D as window on future technologies and on new markets
- o proprietary knowledge base

Manpower/Management
- o organizational ability
- o process engineering and other high skill position requirements high

Raw Materials
- o increased emphasis on geographic positions relative to feedstock sources
- o priority placed on feedstock security as a fuel, chemical feedstock and/or revenue source
- o need for development of alternative supplies of hydrocarbons for chemical feedstocks (synfuels, biomass)
- o increasing presence of petrochemical companies

Products
- o need for product differentiation--find specialty product niches; new and higher value added products (by acquisition, R&D)
- o greater need for successfully exploiting patent protection
- o need for continued reliance on maintaining complete product lines
- o maintaining quality of products and reliability of supplies to markets

Manufacturing/Distribution
- o over capacity, lower demand, lower productivity
- o world scale and highly integrated plant complexes
- o good supporting industry service infrastructure
- o proximity of manufacturing operations to energy/chemical feedstocks
- o capital requirements for plant/equipment expansion and modernization

Foreign Trade
- o increased global trade competition
- o decreasing rate of growth of exports and increasing rate of growth of imports
- o European recession plus strong dollar equals curtailed demands for U.S. exports

Margins/Profits
- o slower growth, profitability projections
- o maturing industry, declining rate of penetration of USGNP
- o increase is costs of oil and natural gas
- o declining share of world productive capacity
- o recent rapid increase in value of petrochemicals more from price than quantity increases.

Internal Competition
- o serious price competitions forcing industry structural change--shakeouts

Structural Adaptability
- o flexibility to use alternative feedstocks for fuel and chemical feedstock needs; capital requirement demands for flexibility
- o manufacturing plant obsolescence and capital invested in existing plants and processes
- o pressures between low growth projects and need to develop innovative and/or retrofit existing facilities in plant design
- o need to build management of plant into new designs

Table 3.23

Industry Competitive Forces

Competitors
- o increased price competition from industrialized countries
- o growing competitiveness of developing countries; exemptions from GSPs
- o middle east petrochemical capabilities for share of global capacity
- o dynamic technologies for production of commodity chemicals and impacts on global economics and competitiveness
- o foreign competitor investment in plants in United States and impact on U.S. markets
- o increasing presence of Canada as major exporter to United States of commodity chemicals causing reduced U.S. competitive advantages in domestic and some loss of position in world markets
- o excess capacity, declining demand for certain U.S. commodity chemicals and increasing capacity of Canada, Mexico and Saudi Arabia
- o trading partner willingness to live up to MTN agreements
- o plant obsolescence in Europe
- o prospects for reduced number of chemical companies

Suppliers
- o decontrol of U.S. crude oil
- o feedstock availability and competitive pricing of feedstocks
- o increasing probability of petroleum feedstock shortages
- o rising feedstock and fuel prices
- o pace and extent of natural gas deregulation
- o feedstock shifts
- o expanded oil and natural gas recovery efforts
- o declining domestic supplies and increased costs of oil and natural gas
- o coal as an increasingly important feedstock (fuel and chemicals)
- o nation/state control of operations and of exports

Buyers
- o economic health of major chemical customers--housing, autos, plastics, fibers, paint, rubber

Markets
- o United States has large and integrated domestic market
- o United States has growing market and is target for foreign competition

New Entrants
- o new sources of higher value added chemicals expected and impacts on smaller chemical firms
- o price competitiveness of substitute materials vs. organic chemical end products
- o food processors (corn to ethanol), forestry companies (wood to organic chemicals) and other new competitive forces in domestic markets

Table 3.24

External Influences

Social
- o aging workforces (industrialized countries)
- o worker entitlement demands
- o unemployment, workforce disruptions, economic conversion needs
- o workforce education/training/safety
- o public perceptions of chemical industry

Political
- o delays in deregulation of natural gas and real extent of deregulation
- o foreign country investment policies
- o protectionism/reciprocity/import license policies/cartel formation
- o responses to increased trade pressures on Europe because of imports (from United States, Asia) of manufactured good with high chemical content.

Economic
- o emergence of internationl trade in U.S. economic plans
- o debt positions of countries and trade efforts/strategies to service debts
- o disurptions in balance of payments and global money flows; defaults in world credit markets
- o high interest rates, availability of capital
- o national economic downturns and slowed consumer demands for some of chemical industry outputs (autos, housing, clothing)
- o high underlying inflation in some countries
- o currency manipulations to improve export positions
- o government deficits
- o linkages between European and U.S. economies and slow growth projections
- o value of dollar vs. other currencies
- o global interdependence considerations

Technical
- o increased pace of technology transfer and of technological change
- o patent protection and longevity of patents in foreign countries

Government/Regulatory
- o foreign government industry nationalization, national industrial policies, nation/state support of uncompetitive industries
- o chemical industry priority for available feedstocks and government allocation priorities
- o regulations and their costs (air quality standards, Clean Air and Water Acts, National Contingency Plan, environment/health/cancer policies, TSCA and pre-manufacturing notices, Resource Conservation and Recovery Act, FDA and/or USDA requirements)
- o shifts from multilateral to bilateral trade negotiations
- o growing foreign government/state owned and directed competition (new chemical producers with access to low cost feedstocks)
- o foreign national/state policies on price and supply of energy feedstocks, subsidized worker training, preferential access to capital and to restrictions on quantities of imports
- o pace and costs of country/state development of industry service infrastructure
- o government subsidies

Marketing

- o capacity vs. proprietary base
- o market share increase vs. competitive advantage increase for market share niche

Biotechnology has and will play an important role in the investment decisions of firms and in the long-term future of the commodity organic chemical industry (97). However, substantial impacts of this technology on the commercial products and processes of this industry will probably not begin to take place until 1995-2000.* In the interim, industry investments in biotechnology will provide organic chemical firms with a window on a potentially significant technology, as well as expand their range of strategic options.**

*The value added component of commodity organic chemicals (SIC Codes 2865, 2869) in 1980, based on a total shipment value of $54.2 billion, was $22.6 billion (1981 U.S. Industrial Outlook, Bureau of Industrial Economics, U.S. Department of Commerce, Washington, D.C., January 1981). It is estimated that by the year 2000 biotechnology could contribute $1 billion (William Chang, Corning Glass Works, personal communication, May 25, 1982; Daniel Wang, MIT, seminar, January 22, 1982) to $7-10 billion (Chemical Week, September 30, 1981, pp. 36-41; Industry Week, September 7, 1981, pp. 67-70; T. A. Sheets Co., Cleveland, February 24-25, 1982) in constant 1980 dollars to the value added component of commodity organic chemicals. The conservative estimate ($1 billion) may be more realistic, since biotechnological feedstocks and processes for the production of commodity organic chemicals will not easily displace existing petrochemical feedstocks and production processes. Both estimates, however, are focused primarily on projections for traditional commodity organic chemicals, and do not necessarily take into consideration expansion of uses for existing organic chemicals or development of new, "non-traditional" commodity organic chemicals such as biopolymers (Inside R&D, May 12, 1982, p. 4; Biotechnology Newswatch, April 5, 1982, pp. 1-2). Market impacts for these new, "non-traditional" commodity organic chemicals are unknown but could adjust upward significantly the above estimates.

**Conservatively, about $500 million has been privately and publically invested in new biotechnology companies to date. Most of these investments have been made in the last two years. Of the amount invested in new firms, about one-half has come from venture capital sources (Chemical and Engineering News, March 29, 1982, pp. 10-15; Genetic Engineering and the Engineer, National Academy of Engineering, National Academy Press, Washington, D.C., 1982). Figures are not available for established chemical, pharmaceutical, agricultural, etc. firms in-house investments in biotechnology. A projection on the value of the U.S.'s biotechnology industry is $64 billion by the year 2000 (Genetic Eng. News, May/June 1982, p. 5).

Projected initial impacts of biotechnology on this industry will be most likely in the higher value added specialty chemicals. This may fit closely with the strategies of those firms lacking a secure feedstock base. Also, some of these specialty chemicals may, in turn, become the commodity chemicals of the next century.

In addition to products, biotechnology will also influence process developments and selection (63), and industry structural changes via new entrants and use of alternative (biomass) hydrocarbon substrates (53, 57, 61).

Although it may not be evident in the near term, changes now in motion both internal and external to the industry will significantly affect both the form and substance of the industry by the close of the century.

4. BIOMASS FEEDSTOCKS FOR ORGANIC CHEMICALS

.1 Introduction

The decade of the 1970s demonstrated clearly our national industrial vulnerability to foreign sources of petroleum. Increasing economic, industrial and political concerns about the continued availability of petroleum and natural gas at competitive prices has stimulated efforts to seek alternative sources of hydrocarbon raw materials, such as coal and biomass, for both energy and chemical uses.

Biomass can potentially be used by the organic chemical industry in certain circumstances as a renewable energy source. Non-renewable petroleum reserves can then be extended for their more exclusive use as primary organic chemical feedstock and the economic viability of capital intensive organic chemical process facilities tied to petroleum feedstocks can be prolonged. The environmental and energy economics and concerns associated with this approach have been discussed elsewhere and will not be discussed in this report (49, 55, 108).

In this report, biomass is considered in the context of an alternative hydrocarbon feedstock for the production of commodity organic chemicals. In the previous section of this report, Table 3.13 illustrated the many technical, environmental and other considerations involved in determining the economic viability of using biomass as a feedstock or substrate in the production of organic chemicals. It should be emphasized that, even if successful approaches are developed for converting biomass to organic chemicals (for example, cellulosic materials to ethanol), industry must continuously weigh and compare the return on investment (ROI) expected from such an approach versus alternative approaches. These comparisons will include evaluation of ROIs from existing processes, capital investment in existing processes, processes using alternative feedstocks (for example, coal), and other projects or programs competing for a firm's limited resources.

.2 Supply

Biomass is composed primarily of lignocellulose materials, and includes forestry residues, agriculture residues, and municipal/agricultural/industrial waste residues. Table 4.1 illustrates the forms of biomass in the U.S. and the relative amounts produced per annum. As one proceeds from primary to secondary to tertiary biomass, both the quantity produced per annum and the biomass product value decrease. Currently there is large scale production in the U.S. of ethanol from cereal crops (primary biomass) such as corn and wheat. However, in the future, woody plants and underutilized secondary and tertiary biomass may be the substrates of choice for fermentation processes for three major reasons. First, neither the technology required for their full conversion nor their economic potential has been fully realized (58). Second, use of crop plants for chemicals may conflict with global food needs. Third, nature has, particularly in woody plants, provided a mechanism for the abundant production of lignocellulose and for concentrating it. In addition and in contrast to other products of photosyntheses (such as grass, algae, and crop plants), woody plants can be collected on a year-round basis and can be stored for long periods of time (109).

.3 Biomass Chemical Components

Biomass, for example, lignocellulose from woody plants, is complex and consists of three major organic chemical components: cellulose, hemicellulose, and lignin (Table 4.2). Cellulose (approximately 40-45 percent of the dry weight of wood) is a high molecular weight, crystalline, relatively insoluble polymer consisting of B-1-4, linked D-glucose (hexose; C_6) units. The composition of hemicellulose (about 20-35 percent of the dry weight of wood) varies considerably with the type of biomass (for example, hardwoods as compared to softwoods are relatively xylan rich), and consists of hexose (C_6) and pentose (C_5) non-crystalline, relatively insoluble polymers, such as D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose, and D-glucuronic acid (110). Table 4.3 provides information on the percentage composition of hexosans and pentosans in primary and secondary biomass sources.

Table 4.1

Forms of Biomass in the United States

Biomass substrate	Annual Amount (million metric tons)
Primary biomass	
Crops:	720
Corn	195
Wheat	85
Hay	150
Soybeans	105
Wood	286
Secondary biomass	
Crop residues	430
Cereal straw	141
Stover-cobs	145
Forest residues	260
Tertiary biomass	
Feedlot manures	237
Municiple waste	150
Forest product waste	100
Industrial wastes	45
Sewage solids	15

Reference 58.

Table 4.2

Chemical Composition of Some Biomass Feedstocks

Biomass Substrate/Feedstock	Chemical Components (%) Cellulose	Hemicellulose	Lignin
hardwoods	45	30	20
softwoods	42	27	28
wheat straw	30	27	11
corn stover	38	26	19
hemlock bark	26	16	15
feedlot manures	17	19	7
municiple solid waste	61	-	NA
domestic sewage sludge (dry)	35	6	NA

References 53, 58, 111, 112.

NA = Not Available

Table 4.3

Composition of Hexosans, Pentosans and Other Biomass Components

Biomass Substrate/Feedstock	Percentage Composition			
	Hexosans	Pentosans	Lignin	Pectin/Starch
Primary				
Woods				
Softwoods				
White Fir	58	9	28	-
Douglas Fir	57	4	27	-
Redwoods	44	6	34	-
Hardwoods				
Oak	45	20	25	-
Sycamore	46	14	22	-
Sweet gum	45	19	19	-
Crops				
Corn/Wheat	4	-	2	85
Secondary				
Agriculture Residues				
Cereal Straw				
Wheat	39	19	14	-
Rice	39	17	10	-
Barley	40	19	14	-
Corn Stover	36	16	15	-
Corn Cobs	36	28	10	-
Bagasse	41	20	20	-
Seed Hulls				
Rice	36	15	14	-
Oat	34	30	14	-

Sources: References 110, 113.

The third major chemical component, lignin (about 15-30 percent of the dry weight of normal wood), is a high molecular weight, relatively insoluble, non-polysaccharide cross-lined polymer of sinapyl, coniferyl, and p-coumaryl alcohols. Softwood lignins can be distinguished from hardwood lignins by the predominence of guaiacyl units. These chemical units contain more reactive sites and contribute to a greater degree of cross-linking (53). Thus, softwood lignocellulose complexes are more difficult to degrade into individual chemical components.

.4 Technology and Chemicals from Woody Plants

The basic technology for the conversion of wood biomass to commodity organic industrial chemicals is known (12, 57, 114). However, the economics for using this technology and substrate as a feedstock have not, until recently, been favorable because of the price and availability of competing petroleum feedstocks. In spite of this, some chemicals from wood or wood pulp are made in large quantities (Table 4.4).

The forestry industry and pulp and paper manufacturers have been quite cognizant of the importance of efficient use of forestry by-products on their process economics, and have made substantial R&D investments in this area in the past. This is illustrated by Koppers', Mead's, and Canada's MacLaren Power and Paper's equity investments in Engenics and by Weyerhauser's contractual relationship with Cetus. These firms view biotechnology not only as an attractive alternative for improving the quality of forest stock more efficiently and economically but also as a potential vehicle for improving forestry product and by-product processing and as a vehicle for their becoming a greater market force in the production of organic chemicals. Also Canada, in addition to its strong petrochemical and natural resource base, has a vast lignocellulose natural and renewable resource base. Canada's biotechnology development plan has given high priority for the application of biotechnology to cellulose and to forestry residue waste utilization (115-117).*

*An example of Canadian application of biotechnology to forestry waste use is the University of Waterloo/Envirocon process for converting pulp mill sludge waste to protein or gasohol (Canadian Research, April 1982, p. 8).

Table 4.4

A. Organic Chemical By-products Formed in U.S. Pulping Processes

Kraft pulping, tons X 10^3		Sulfite pulping, tons X 10^3	
Thiolignin	8,800	Lignin sulfonic acid	1,920
Fatty and resin acids	405	Hexoses	550
Formic acid	600	Pentoses	87
Acetic acid	840	Acetic acid	140
Acids derived from carbohydrates	4,500	Formaldehyde and small amounts of ethyl alcohol, furfural, and acetone	18
All other organics (phenols, etc.)	650		

Source: Reference 53.

B. Silvichemicals of Commerce

Cellulose	Dimethyl sulfide
Cellulose esters	Dimethyl sulfoxide
Cellulose ethers	Kraft lignin
Rayons	Lignin sulfonates
Wood resin	Ethyl alcohol
Turpentine	Vanillin
Pine oil	Yeast
Tall oil	Charcoal
Tall oil resin	Bark products
Tall oil fatty acids	Hemicellulose extracts

Source: Reference 57.

In addition to an improved economic climate for use of wood biomass as a feedstock in the production of organic chemicals, improvements are also needed in basic process conversion technologies and conditions. Selective adaptation of these technologies and conditions to a variety of wood biomass substrates are needed to achieve optimum separation and subsequent conversion of major chemical components. Table 4.5 illustrates the variety of industrially useful chemicals and products that potentially can be derived from wood biomass. Using current R & D activity as an indication of technological and commercial developments, a near-term commodity organic chemical target of opportunity will be ethanol via (a) hydrolysis and saccharification of lignocellulose and (b) fermentation of C_5 and C_6 sugars. In the longer-term, a better understanding of the complex structure of lignin and improvements in process technologies associated with its modification offer the potential for providing an important and renewable source of monoaromatic chemicals and higher quality and value added products such as surface coatings, adhesives and foams.

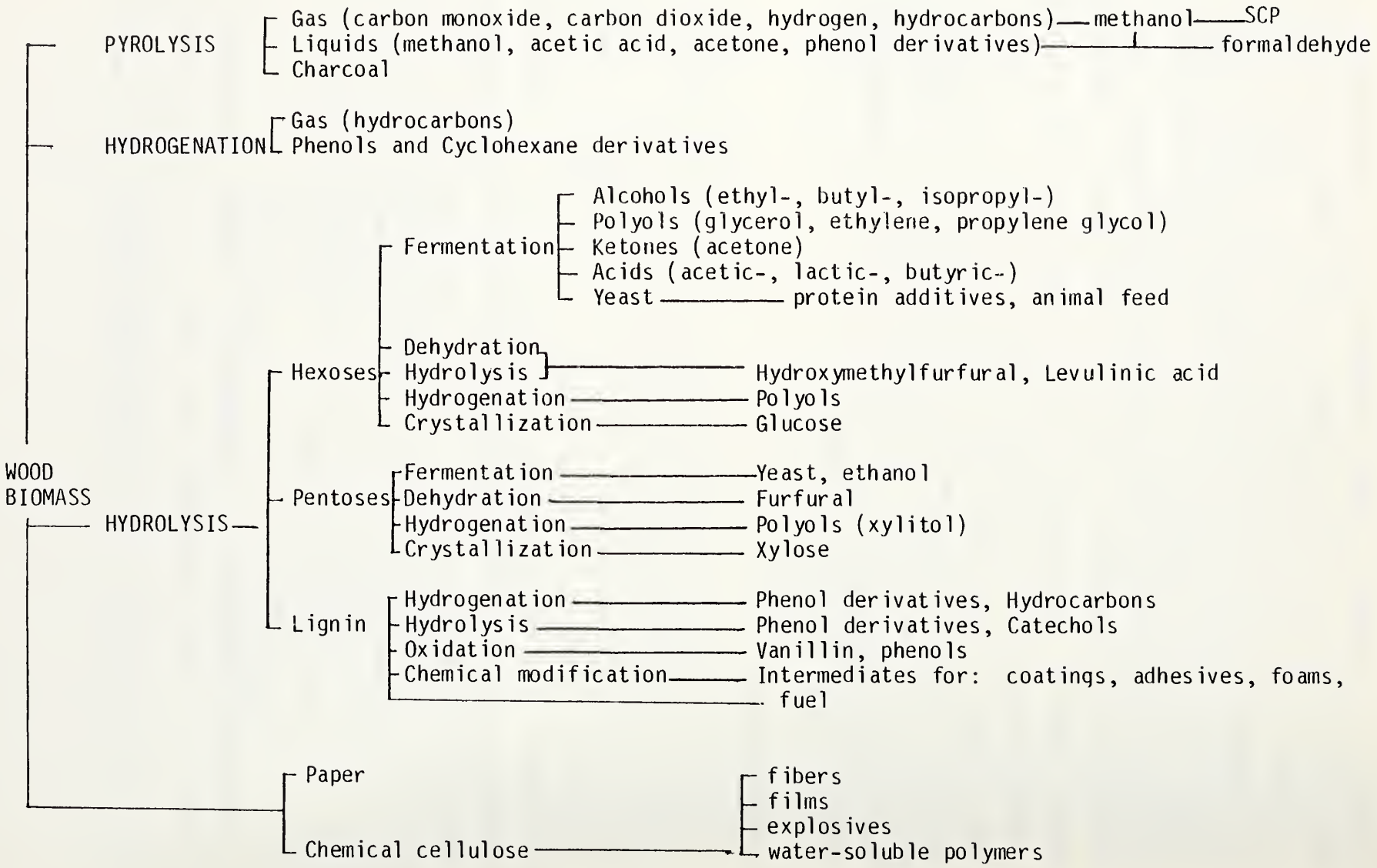
.5 Waste Residues

Wastes from industry, agriculture and municipalities have been defined broadly as resources misplaced in time, location or need (118). Characteristically, these waste residues have a high cellulose content, are generally of low commercial value, and, in many instances, are major sources of pollution. There appears to be renewed interest in the possibilities of applying biotechnologies for upgrading these residues to more valuable organic chemicals. For example, the economics are becoming favorable for the anaerobic digestion of agriculture or sewage waste residues or organic solid waste residues from landfills to methane (119-124), and for the industrial use of an immobilized enzyme (lactose) to convert dairy industry waste (whey) to commercially useful syrups and protein concentrates (125).

Based upon experiences in the pharmaceutical industry, fermentation by-products are a major wastewater source (118). The dairy food processing industry is also a major contributor to biodegradable waste (126). With projections for expanded industrial use of fermentation process technologies for

TABLE 4.5

Use Possibilities for Wood Biomass



Sources: References 53, 108, 109.

feedstock conversions and waste residue utilizations, development of new, less empirical, and more sophisticated bioprocess approaches to the conversion or disposal of waste residues will be essential from both an environmental and economic perspective. From an economic perspective, bioprocess economics will become more favorable with (a) improved approaches to recycling chemical by-products from bioprocesses, (b) production of commercially useful chemical products from biomass waste residues, and (c) an expanded market for feed supplements and nutritional components derived from biomass waste residues.

.6 Chemicals from Plants

One possible alternative to synthesizing chemicals from biomass is to obtain industrially useful chemicals from plants directly. Currently there is global interest in finding chemical derivatives from both aquatic and terrestrial plants.

One approach being investigated is the development of aquaculture management technologies for the production of algal biomass (127). In the past, considerable emphasis was placed on the high nutritional value of algal biomass, with protein up to 70% of the dry weight in Spirulina species, a blue-green algae (128). Commercial application of algal cultures for food production have generally not been successful because of high capital costs, poor yields, product quality control problems, and lack of market outlets. Use of large scale algal ponds for liquid waste treatment have been more successful (129). There is much current interest in the use of ponds and coastal waters for the cultivation of algal biomass as an energy source, for example, methane biogas (127, 129, 130).

The global harvest of marine algae is illustrated in Table 4.6. Major commercial products of marine algae processing are shown in Table 4.7: polysaccharide polymers agar, algin, and carrageenan (108). With increasing industrial interest in biotechnology and in immobilized biocatalytic processing systems, both alginate and carrageenan are receiving consideration as polymer support and entrapment matrices for biocatalysts (131-133).

Table 4.6

Global Harvest of Marine Alge
(metric tons)

Year	Brown Algae	Red Algae	Green Algae
1971	483,600	375,500	1,100
1972	506,500	322,800	700
1973	586,900	450,300	900
1974	696,567	525,654	2,237
1975	633,182	422,424	2,487

Source: Reference 108.

Table 4.7

Sources and Uses of Algal Products

Algal Product	Algal Source	Uses/Properties	Industry
Agar	red	suspending, thickening, stabilizing solutions	bakery, confectionary
Algin	brown	gelling, thickening, suspending, emulsifying, and water-holding properties	paper and pulp, pharmaceutical, food processing
Carrageenan	red	thickening, stabilizing, gelling agent; texturing binder; gel base to control release of substances	food, health care

Source: Reference 108.

In addition to possible use of algal biomass as a source of vitamins and as an organic fertilizer, algal biomass with high concentrations of polysaccharide can be easily converted via anaerobic fermentation to produce organic acids which can subsequently be converted to olefins, acetic acid, and liquid hydrocarbon fuels (134). Other research advances in this area include reports of the economically competitive production of glycerol from algae; of future prospects for production of plant growth hormones, pharmaceuticals and specialty chemicals; of the use of immobilized algal cells for specific bioconversions; of the use of carrageenan or other saccharides in vaccine detoxification; and of the possibilities for transforming algae via DNA from other species (135-139).

Another area of active research interest involves hydrocarbon-producing crops as sources of biologically synthesized organic chemicals. Species from Euphorbiaceae and Asclepiaeceae plant families have received much attention because of their possible cultivation on "petroleum plantations" as energy sources and as sources of latex-isoprenoid hydrocarbons (140, 141). Oilseed plants (sunflower, linseed, rapeseed, buffalo gourd, Chinese tallow tree) can potentially provide a renewable, domestic resource of natural glycerin and of fatty acids of varying chain length containing epoxy, hydroxy, and keto, as well as other functional groups, in addition to being a possible source of the nutritionally important amino acids lysine, cystine, and methionine (49, 142-145). The chemical constituency of peat also indicates some prospects for its alternate use as a natural resource chemical feedstock. Peat biomass extraction processes can yield a variety of potentially useful chemicals such as peat wax, asphalts, peat coke, alkaloids, tannins, resins, fulvic acid, humic acid, carbohydrates, amino acids, and B-vitamins (146). To improve the commercial prospects of these and other domestic agriculture-based, biomass alternative chemical feedstocks, however, a number of technological, economic, and environmental barriers need to be overcome, for example:

- selected plants need to provide high yields per acre on marginal lands in a particular climate or to serve as an acceptable alternative cash crops in agricultural crop rotation management schemes;

- o chemicals produced from such plants must have a higher value than other plant product uses such as fuel; and

- o efficient and cost sensitive extraction technologies need to be developed for separating valuable chemicals from other plant constituents.

In addition, natural fats and oils from domestic renewable resources will have to compete in the future with similar imports of these natural products from Southeast Asia and the Philippines coconut and palm oil plantings, as well as tallow from domestic sources (145).

These considerations also apply to the production of ethanol from high carbohydrate content root crops, such as the Jerusalem artichoke, cassava, yams and fodder beets (48, 147-149).

On a smaller scale, plant cell culture biotechnology including the use of immobilized plant cells has the potential to produce a number of specialty, low volume, high value added chemicals that can impact on a number of industries (Table 4.8; 150-153). Technologies such as plant and protoplast cell culture, protoplast fusion/somatic hybridization, protoplast-liposome interactions, and genetic engineering, used individually or in combination with conventional plant breeding technologies, offer the promise in the long-term of revolutionizing the production of biomass crops with desired characteristics, such as:

- o genetically uniform and predictable characteristics;
- o tolerance to adverse environments; and
- o ability to produce high concentrations of biologically synthesized chemicals or to produce plant constituents tailored to chemical feedstock needs.

In the interim, considerable R&D is required to realize this promise (151, 154, 155).

Table 4.8

Potential Range of Chemical Products from Plant Cell Cultures

Alkaloids
Allergens
Amino acids
Anthraquinones
Antileukemic agents
Antimicrobial agents
Antitumour agents
Antiviral agents
Aromas
Benzoquinones and related compounds
Carbohydrates
Cardiac glycosides
Chalcones
Dianthrones
Enzymes
Enzyme inhibitors
Flavanoids, flavones
Flavours (including sweeteners)
Furanocoumarins
Hormones
Insecticides
Latex
Lipids
Napthoquinones
Nucleic acids
Nucleotides
Oils
Opiates
Organic acids
Proteins
Peptides
Perfumes
Phenols
Pigments
Plant growth regulators
Polysaccharides and derivatives
Steroids and derivatives
Sugars
Tannins
Terpenes and Terpenoids
Vitamins

Source: Reference 151.

.7 Barriers to Research and Commercial Progress

Impediments to biomass research progress and to commercialization successes using biomass (lignocellulose) as a substrate that are relevant to NBS's mission include: (a) lack of standard biomass samples for comparison of experimental (domestic and international) findings, (b) lack of standardized and reproducible biomass (lignocellulose) assay methods, and (c) in general, lack of information for characterizing lignocellulose and its major chemical components (156). These barriers and possible approaches for overcoming them will be explored in more detail in subsequent sections of this report.

5. CONVERSION OF CELLULOSIC BIOMASS TO ETHANOL

.1 Overview and General Process and Economic Considerations

The process of converting lignocellulose biomass to ethanol via hydrolysis and fermentation technologies was selected for more in-depth discussion in this report because it serves as an example of an area: (a) where R&D interest is intense and industrial and economic potential is high, (b) where solutions to scale-up and commercialization problems and barriers may have generic applicability to production of other organic chemicals using different substrates and or bioprocesses, and (c) where NBS can possibly provide a range of measurement, information and other infrastructure services to facilitate commercial applications in biotechnology.

The desire to replace petrochemicals with biomass (such as lignocellulose) as a feedstock or substrate for the industrial production of organic chemicals such as ethanol has been stimulated by fluctuations in price and availability of petroleum feedstocks over the past decade, and by projections on the amounts of under-utilized lignocellulose that may be available as a substrate for bioconversions.

The primary constituents of lignocellulose biomass are cellulose, hemicellulose, and lignin (refer to Section 4). The major physicochemical characteristic of lignocellulose substrate that limits its direct fementation to ethanol is its complex and polymeric nature (157). Hexose and pentose polymers of cellulose and hemicellulose by themselves can be enzymatically converted to organic chemicals, but their embedment in the lignin matrix inhibits the activity of microbial cellulases by steric hindrance. In addition, the presence of lignin in certain concentrations may be toxic to microrganisms. Thus, chemical, mechanical, and/or enzymatic hydrolysis pretreatment of lignocellulose is a prerequisite for the conversion to simpler sugars.

Depending on the nutritional environment, the atmospheric conditions of the reaction vessel, and the microogranism(s) selected, these sugars can, following pretreatment, be converted by fermentation to a number of high volume, low margin acids, alcohols, and gases that could serve as organic chemical feed-

stocks (58, 113, 158; Table 5.1). For example, lactic acid produced in this manner has applications in both organic chemical commodity and speciality markets as a component of new plastic materials and of controlled release matrices for pharmaceutical agents and pesticides (159). In addition, the large scale of fermentations required for commodity organic chemical production will produce process reaction by-products that will necessitate the development of efficient recovery processes and of market outlets to improve process economics (e.g., enzymes; vitamins; substrates for methane production; residues for fertilizers or energy sources; and protein concentrates). Improvements in process economics will also depend upon developments in continuous industrial processes including technologies for process water, nutrient medium, and cell recycle (160).

Table 5.1

Products of Fermentation and Potential Organic Chemical Feedstocks

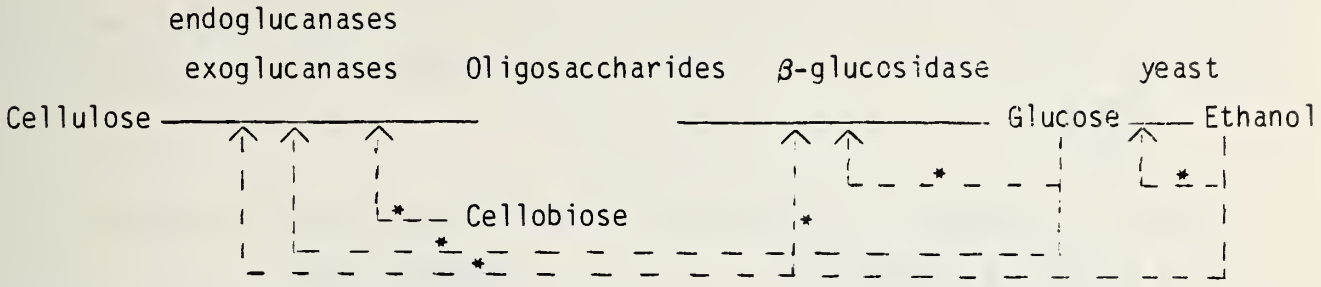
Organic acids	Alcohols-ketones	Esters	Gases
Propionic	Ethanol	Ethyl acetate	H_2
Acetic	Acetone	Ethyl butyrate	CH_4
Caproic	Butanol	Poly-3-hydroxbutyrate	CO_2
Lactic	Isopropanol		
Succinic	Glycerol		
Butyric	Methanol		
Formic	2, 3-butanediol		
Acrylic			

Sources: References 58 and 158.

Thus, the basic steps in the conversion of lignocellulose biomass to ethanol can be represented as follows:

- o feedstock collection, handling and pretreatment for biomass size reduction and for dissociation of lignin and cellulose;
- o saccharification of more complex sugars to simple sugars using the cellulase system;
- o fermentation of simple sugars (C_6 and C_5) to ethanol; and
- o ethanol separation and recovery.

A simplistic model of the bioconversion of cellulose to ethanol is illustrated below:



*Indicates product inhibition

There is much in the historical literature on the technologies associated with bioconversion of cellulosic materials to ethanol. However, improvements in the industrial economics of this and other fermentation processes in comparison with chemical synthetic processes will occur only with significant advances or improvements in many areas such as:

- o handling large amounts of fermentable materials:
- o direct enzymatic conversion of cellulosic materials;
- o production of cellulase and other process biocatalysts;
- o product tolerance and higher substrate and product concentrations;
- o product separation and recovery from reaction by-products;
- o productivity of fermentation reactions;
- o understanding interactions between various unit processes and an overall process; and
- o closer integration of the biological system with process steps (161, 162).

Several of these areas will depend upon improvements in bioprocess engineering technology. Strategies for improvements in this technology, referred to as process intensification, are concerned with:

- o increasing the volumetric rates of reaction;
- o improving yield coefficient ratios;
- o increasing the number of reactions in a single reaction vessel;
- o reducing the number of reaction and recovery steps;
- o reducing the number of separate, dedicated reaction vessels; and
- o improving recycle engineering technology and reactor design (160, 163).

Table 5.2 identifies several areas where technological innovations could lead to significant process intensification and improved process economics.

Table 5.2

Areas for Development Leading to Process Intensification Gains

Area requiring improvement	Increased volumetric rates of reaction	Reduced reactor size	Improved separation processes (cell/product/liquid)	Reduced number of vessels in the total process	Enhanced design capability	Enhanced design flexibility
Increased biomass concentration	X	X	X	X		
Predetermined biomass concentration					X	X
Biomass concentration independent of process throughput	X	X		X	X	X
Predetermined biomass size and shape					X	X
Improved biomass recovery			X	X	X	X
Improved yield coefficients	X	X	X			
Multiple reactions in a single reactor due to local variations in environment and/or species	X	X		X		X
Retention by the fluid phase of dilute, suspension free aqueous properties, particularly viscosity	X	X			X	X

Source: Reference 163.

It should be noted, however, that the major component influencing any shift to biomass based processes for the production of organic chemicals will be the price of the biomass raw material relative to the price of petroleum or natural gas hydrocarbon feedstocks. This is illustrated in Table 5.3 by categorizing the costs of ethanol production using molasses as the hydrocarbon feedstock.*

Table 5.3

Production Cost Categories in the Bioconversion of Molasses to Ethanol

Cost Category	% of total production cost
raw material (molasses)	68%
fixed costs including labor	14%
electricity + steam	10%
materials	5%
water	3%

Source: Reference 158.

Although the costs of both petrochemical and renewable biomass will increase in the future, the rate of increase for biomass will probably be less in the long term. When the costs of these two feedstocks begin to intersect, greater emphasis will be placed on seeking technological innovations to improve the efficiency of the process technology. Process efficiences will be a major factor in determining what bioengineering processess will be used and when it will be economical to make them operational in industrial biotechnological processes.

.2 Pretreatment of Cellulosic Biomass

In order to enhance the conversion of lignocellulose raw material to simple sugars and chemicals, active research and technology development on a variety of chemical, physical and mechanical processes are in progress. Table 5.4 illustrates a number of pretreatment process approaches (used individually or in combination) that are attempting to expose the molecular bonds of major lignocellulose constitutents (cellulose, hemicellulose, and lignin) to enzymatic

*Although molasses is a feedstock for ethanol production, the economics of its conversion are marginal. Hydrolyzed starch and cellulosic materials are more likely biomass feedstock candidates for the production of organic chemicals.

Table 5.4
Chemical and Physical Lignocellulose Pretreatment Approaches

Lignocellulosic Feedstock (substrate)	Pretreatment: Chemical	Pretreatment: Mechanical/Physical	Process	Reference
saw dust		grinding and heating	Gulf - U. of Arkansas	111
crop residues	o Sulfuric acid 0.1%-1% (95-120°C) 2-4% (90°C) (hemicellulose hydrolysis) o cadoxin solvent (lignin & cellulose separation)		Tsao	157
wood chips		steam explosion: 240-300°C 500-1000 psi	Iotech	164
saw dust	sulfuric acid 1-1.5%	o extruder screw o 230°C, 500 psi	NYU	164
wood chips, pulp mill wastes	ethanol/water (200°C) - lignin: ethanol phase - hemicelluiose: water phase - cellulose: undissolved	hammer mill	General Electric	165, 166
wood chips	o solvent delignification o dilute acid, low temperature prehydrolysis o dilute acid, high temperature hydrolysis	steam explosion	Georgia Tech - Stake Technology	165
wood logs	o hot water o 0.6% sulfuric acid (140-180°C)	wood debarking	Inventa	167
agriculture celluiosic waste material	sodium hydroxide delignification		Trivedi and Rao	168
wood chips	hydrogen fluoride	wood chip drying	Michigan State University	165, 166, 169, 170
wood pulp for papermaking	sulfuric acid 0.1-1% (190°C)	gamma irradiation	David et al.	171
aspen wood chips	2%-10% chlorite	steam explosion 250°C, 560 psi	Saddler et al.	*
agriculture/forest residues	phenol (100°C)		Battelle-Geneva	**

*Biotechnol. & Bioeng. 24: 1389-1402, 1982.
**Chemical & Eng. News, May 31, 1982, p. 38.

have progressed on pretreatment alternatives, a number of problems have become evident, for example, loss of carbohydrate material, corrosion of equipment, undersirable side reactions, and high capital costs.

Some advantages and disadvantages of alternate pretreatment approaches are summarized in Table 5.5. If pretreatment of cellulosic material will be required in the future, and if pretreatment methods are to be cost-effective, many research and development hurdles must be overcome. For example, there is a critical need to identify and to select optimum conditions and pretreatments for cellulosic materials from specific sources and to relate these choices to process economic, efficiency, energy use, and scale-up considerations. Ideally, one would like to circumvent these problems by developing biological systems (microbial and/or enzymatic) that would allow the direct conversion of cellulosic materials to hexoses and pentoses and then to ethanol. This approach would avoid or at least reduce the need for the currently used and proposed capital intensive, destructive, and relatively inefficient pretreatment processes. The physical approaches are particularly costly and time consuming.

.3 Biological Conversion

For the efficient and economical biological conversion of renewable lignocellulosic material, extracellular enzymes from microorganisms capable of degrading cellulosic biopolymers to mono- and disaccharides will need to assume an increasing importance in biotechnological processes. Before economically feasible process technologies are developed, several complicated and interrelated problems need to be addressed, for example:

- o thermo- and chemical stability of enzymes;
- o enzyme(s) production variables;
- o multiplicity of enzymes and substrates complicating enzyme assays;
- o optimization of activity and rates of reaction of individual enzymes in a multi-enzyme system;
- o multiplicity in terms of origin of lignocellulose substrates and varying degrees of reactivity of such substrates to enzymatic hydrolysis;
- o low rates of hydrolysis of lignocellulosic materials;

Table 5.5

Approaches to Pretreatment of Cellulosic Materials
to Enhance Enzyme Susceptibility

Type	Advantages	Disadvantages
Physical		
Hammermill	Less Costly	Ineffective
Colloid mill	Improves handling	
Ball mill	Effective	Energy-intensive
Two-roll mill	High bulk density	Expensive
Steam explosion	Effective	Low bulk density
220-240°C, 600 lb/in.^{2}	Less costly	Ineffective for softwoods
Alkali swelling NaOH	Moderately effective	Low bulk density
	Less costly	Must wash or neutralize
Delignification		
Chemical pulping	Moderately effective	Expensive
Biological	Conserve enzyme and reactor space	Low bulk density
		Pollution
Dissolve, reprecipitate		
Cadoxen (toxic)		Low bulk density
Hydrazine (toxic)		Expensive
Ferric tartrate NaOH	Effective	Difficult handling
Cuprammonium		Solvent recovery
Viscose		Side reactions
Concentrated acid		Pollution, lignin degradation

Source: Reference 172

- o changing patterns of substrate susceptibility to enzymatic hydrolysis with time;
- o nature of substrate handling and pretreatment;
- o product inhibition of enzyme activity;
- o enzyme inactivation by heat, pH, mineral ions, proteases, etc.;
- o identification of microorganisms that can enzymatically attack specific lignocellulose substrates such as wood, bagasse, straw, etc.;
- o molecular level understanding of the metabolic pathways and the efficiency and stability of these pathways used in the decomposition of cellulosic materials; and
- o identification of important process enzymes and approaches to regulating their activity at the gene and transcription levels and to increasing their productivity in biotechnological processes.

The following sections provide a description of some areas of active R&D in the bioconversion of lignocellulose residues to ethanol. These sections also provide a sampling of the many problems that still need to be addressed before economically feasible commercial products can be developed.

.a Cellulose Degradation. Considerable research work on the enzymatic hydrolysis of cellulose has been done with two fungi, namely, the white-rot fungi S. pulverulentum and Trichoderma reesei (173). The three primary hydrolytic enzymes of the cellulase complex system of these microbes are:

(a) endo-β-1, 4-glucanases (act randomly on cellulose);

(b) exo-1, 4-β-glucanase (splits off cellobiose or glucose units from the nonreducing end of the cellulose); and

(c) β-glucosidase or cellobiase (hydrolyzes cellobiose and cellodextrins to glucose and cellobionic acid to glucose and gluconolactone; 173).

A recent review by Ryu and Mandels summarizes research progress with the cellulase system and the need for research progress in the enzymatic hydrolysis of cellulose in priority areas such as:

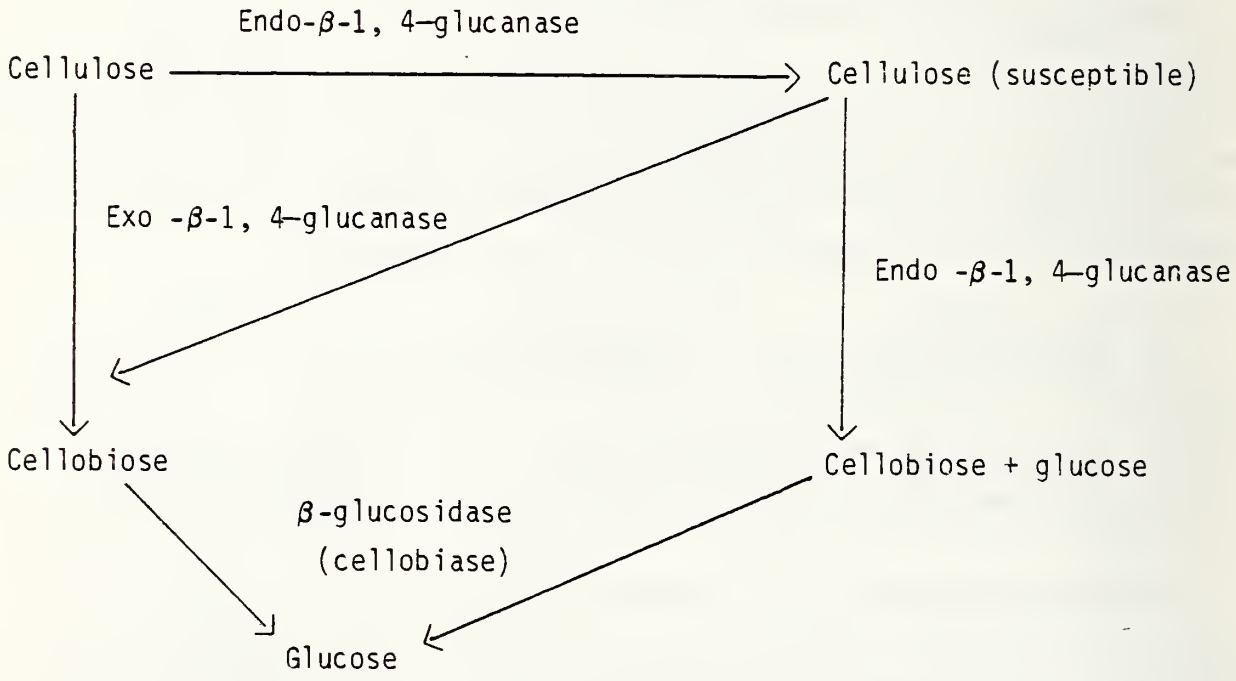
(a) substrate characteristics, properties and multiplicity;

(b) multi-enzyme system kinetics, characterization and interactions;

(c) enzyme purification and characterization; and

(d) enzyme inactivation and process design improvements (172).

The sequential and/or simultaneous mode of action of the cellulase system on cellulose is illustrated below:



.b Hemicellulose Conversion. In addition to dilute acid treatment, a variety of endo-mannanases, -mannosidases, and -xylanases exist that can release C_6 and C_5 monomeric sugars from the water soluble dextrin, hemicellulose. These enzymes are also produced by microorganisms (Trichoderma, Aspergillus, Penicillum) that produce cellulases. A major problem in comparing and in extrapolating from experimental findings where hemicellulose is used as a substrate is that the variety of hemicellulose conversion approaches being used also produce a variety of reaction products.

.c Lignin Conversion. In several of the R&D, pilot plant phase and commercial processes used in or proposed for in the conversion of lignocellulose to chemical products, lignin has been separated from the cellulose and hemicellulose components and later used as an energy source to improve process economics. Less is known about the opportunities for bioconversion of lignin. White-rot fungi appear to be one of few microbial species capable of enzymatically degrading the lignin component of lignocellulose through phenol oxidases. Metabolic products of this catalytic reaction include vanillic acid and quinones (173). Recently, research findings on the enzymatic activity of the fungus Cyathus stercoreus in the breakdown of lignin also appear promising (174). Additional research on physicochemical aspects and kinetics of delignification on a molecular level also appear warranted (175).

It would appear that a systematic review and evaluation of the enzymatic activities, properties and pathways of other wood degrading fungi may reveal microorganisms and microbial enzyme systems that can more efficiently hydrolyze lignocellulose chemical polymers to simpler monomers under temperatures required for higher reaction rates necessary for industrial processes.

.d Saccharification and Fermentation: Thermostability. Two major problems encountered in the conversion of cellulase polymers to simple monomers are the thermostability of cellulase enzymes and low hydrolysis rates of cellulosic materials. Thus, a significant process parameter in saccharifications is temperature. With increasing operating process temperatures, the rate of enzymatic activity may increase 2-5 fold until the enzymes are thermally inactivated. However, at these higher temperatures, the potential for growth of culture contaminants also decreases.

Microbial enzymes show considerable variability regarding their thermostability. The activity of the enzymes of thermophilic (45°C+) microorganisms is more stable then those from mesophilic (37°C-45°C) microorganisms (176).

Table 5.6 gives some examples of the kinds of R&D activity in progress to improve the thermostability and the reaction rates of the cellulase process system.

Table 5.6

R&D to Improve Thermostability and Reaction Rates

Microbial Strain(s)	Optimum Temperature Reaction Range (experimental)	Reference
Trichoderma reesei	45-50°C	111, 157
Sporotrichum thermophile	55-60°C	177
Thermomonospora sp.	55-60°C	176, 178
Thermomonospora sp. + Clostridium thermocellum	60-65°C	165, 166
Cellulomonas sp. + T. reesei	37-45°C	179
Fusarium sp.	50°C	180
Thermobacteroides saccharalyticum	55°C	181

An alternative to the above and to using the popular cellulolytic fungus, T. reesei, is to isolate cellulase genes from other themostable, cellulolytic fungi and to clone these genes into yeasts. Such an approach is being pursued at Stanford Research International with Thielavia terrestris, a more heat resistant fungus with a higher temperature optimum (70°C) than T. reesei (182, 183).

A related approach is being pursued at USDA where the cellulolytic fungus Penicillium funiculosum is viewed as an alternative, less complex source of genes than T. reesei for a cellulase system (184). Attempts will be made to isolate the three cellulase genes from P. funiculosum and to transfer them to a microorganism that could participate in combined saccharification-fermentation.

In addition to genetic manipulation approaches for improving process thermostability and reaction rates, recent experience with the microorganism Caldariella acidophilia, a thermophile with a growth temperature optimium of 87°C, suggests the possibility of using polyurethane foams to immobilize whole, thermophilic microbial cells for industrial biocatalytic reactions (185).

.e Product Inhibition: Saccharification. Saccharification end products include not only glucose and cellobiose but also other sugars such as xylose. Cellulases are inhibited competitively by both glucose and cellobiose and the extent of inhibition increases with increasing resistance of cellulose to hydrolysis (refer to figure in Section 5.1; 172). Cellobiase enzyme is also inhibited by glucose. Refer to the system model illustrated in Section 5.1 for additional details.

Since glucose does not accumulate to any great extent in a saccharification and fermentation process, cellobiase concentration and activity have important influences on an overall cellulase system activity. Traditional mutation and selection techniques to improve the cellobiase levels in T. reesei have not been too successful to date. However, cultures of Aspergillus phoenicis have shown promise as additive sources of cellobiase to T. reesi enzyme levels (172). Also, some yeast strains can convert both cellobiose and xylose (186).

.f Product Inhibition: Ethanol. With reference to the model system illustrated in Section 5.1, conventional fermentation processes or combined saccharification-fermentation processes for the production of ethanol are limited by the inhibiting effect of ethanol. This inhibition decreases both rate of ethanol production and the volume of microbial cell mass when the final ethanol concentration reaches 10-15 percent V/V. In order to reduce or eliminate ethanol inhibition, ethanol must be removed from the fermentation broth as it is being formed and/or fermenting microbial strains more resistant to ethanol must be developed.

Traditional distillation approaches are too energy intensive for practical consideration in future industrial biotechnological process engineering approaches. Biochemical/microbiological solutions to the problem of ethanol tolerance in yeast may be complicated by the relationship between ethanol tolerance and altered lipid composition in yeast plasma membranes (162). Further research is needed in this area.

A number of alternative technical approaches are currently being pursued in order to reduce ethanol and product inhibition in bioconversions. Some of these

approaches include vacuum extraction, cell floculation, two-phase bioconversions, immobilization of microorganisms, etc. (Table 5.7).

Continued efforts are also needed to establish the kinetic relationships of growth, substrate use, and both sugar and ethanol formation during saccharification and fermentation in order to better understand process variables and to improve/optimize process design and control.

.g Conversion of Pentose Sugars to Ethanol. The technology and industrial process parameters for the conversion of glucose (C_6) to ethanol using Saccharomyces cerevisiae yeast strains and the bacterium Zymomonas mobilis are generally well understood. Yeasts, however, are generally capable of metabolizing only a small range of carbohydrates to ethanol.

Rosenberg has recently reviewed research on the conversion of pentose sugars to ethanol (110). For improving the process economics of converting biomass residues to ethanol, conversion of xylose (C_5), which makes up about one-third of the potentially fermentable sugars in cellulosic biomass, is essential. Although some recent progress has been noted, additional research efforts are needed (174, 204).

Identification of a yeast that can simultaneously convert C_6 and C_5 sugars and/or can metabolically work in concert with other microbial strains would be prefered because of the wealth of industrial experience with yeast, as well as the rapidly accumulating knowledge base about yeast genetics and the application of rDNA techniques to yeasts. Such an approach is being pursued at USDA to transfer into S. cerevisiae the two genes of P. tannophilus required for producing the enzymes used in converting xylose (C_5) to xylitol and then from xylitol to ethanol. S. cerevisiae could then potentially convert both C_6 and C_5 sugars in the fermentation process step (184). Another approach is to transfer into yeast the one gene from E. coli that expresses the enzyme xylose isomerase. This enzyme converts xylose to a ketone sugar that can be fermented by S. cerevisiae (184).

Table 5.8 illustrates some of the current research activity on the conversion of C_5 sugars/xylose to ethanol.

Table 5.7

Examples of Technical Approaches to Reducing Ethanol/Product Inhibitions

Approach	Organism	Reference
Mixed culture (different substrate-product inhibition characteristics)	S. cerevisiae NCYC 87 + NRRL-Y-11, 572 (sake yeast)	187
Aqueous two-phase bioconversion	T. reesei + S. cerevisiae	188, 189
Continuous vacuum extraction and cell recycle	Zymomonas mobilis	190, 191
Ultrafiltration (capillary cross flow microfiltration)	Z. mobilis	192
Multistream ethanol feed in multistage tower fermenter	Candida utilis	193
Fermentation coupled to liquid-liquid extraction	S. cerevisiae (immobilized cells)	194
Settler--cell floculation and recycle	Z. mobilis	195
Drying ethanol using organic cellulosic residues	---	196
Thermostable enzymes from thermophilic organisms	Bacillis stearothermophilis	197
Immobilization:		
o calcium alginate	S. cerevisiae	131, 198, 199
o calcium alginate	Z. mobilis	200
o ion exchange resins	S. cerevisiae	201
o borosilicate-glass fibers; polystyrene beads	Z. mobilis	202, 203
o coentrapment of Sepharose-immobilized β-glucosidase in calcium alginate (+yeast) spheres	Talaromyces emersonii	*
rDNA (engineer microorganism with rapid enzymatic activity + micro-organism resistant to ethanol toxicity)	---	174

*Biotechnol. & Bioeng. 24: 1461-1463, 1982.

Table 5.8

Approaches to Improved Conversion of C_5 Sugars/Xylose to Ethanol

Substrate	Organism	Culture Environment	Reference
wood chip hemicellulose hydrolysate	Candida sp. XF 217	aerobic/anaerobic	205, 206
wheat straw hemicellulose	Pachysolen tannophilis	aerobic	204, 207, 208
xylose	Candida tropicalis	aerobic	209
D-xylulose	Schizosaccharomyces pombe	aerobic/anaerobic	210

.h Simultaneous Saccharification-Fermentation. To improve bioconversion process economics by reducing capital costs and production costs of ethanol, a number of technological approaches are in progress to achieve process intensification. One approach involves either simultaneous saccharification-fermentation or the combining of saccharificiation and fermentation process steps. In this biological system both cellulose and hemicellulose would be hydrolyzed to C_6 and C_5 monomers followed by the direct conversion of these sugars to ethanol. Since ethanol has less inhibitory effect on the hydrolysis of cellulose than cellobiose, the saccharification process would be improved. However, since there are different optimum reaction temperatures for enzymes in the two processes and different microorganism are required for C_5 and C_6 conversion, a coupled system may be difficult to optimize (172).

In spite of the limitations, Table 5.9 gives examples of attempts to couple these two bioconversion process steps.

Table 5.9

Technical Approaches to Simultaneous Saccharification and Fermentation

Substrate	Saccharification	+	Fermentation	Reference
saw dust	*T. reesei*		*S. cerevisiae*, *S. carlsbergenis*, *Candida brassicae**	111
cellulosic materials	*Clostridium thermocellum* *Acetivibrio cellulyticus*		*Z. mobilis**	211
wood chips, pulp mill wastes	*Themomonospora* sp.		*Clostridium thermocellum**	162
soda floc, corn stover	*Clostridium thermocellum* *C. thermosaccharolyticum*	or	*C. thermohydrosulfuricum*	58, 162, 174
cellulose	*Thievlavia terrestris*		(anaerobe - *Z. mobilis*)*	182
ball-milled pulp, paper, cellulose powder	cellulolytic mesophile		*Clostridium saccharolyticum* sp.	212
straw	*Trichoderma viride*		*Saccharomyces uvarum* *Pachysolen tannophilus*	213

*Conversion of C_6 sugars only.

Using rDNA techniques, scientists at Helicon are working on the isolation of genes from *Escherichia adecarboxylata* for the conversion of cellobiose to ethanol via glucose. Their work includes attempts to get expression of these genes in an *E. coli* system and on the characterization of the enzyme system (184). The ultimate goal of this research would be to express the genes in *Z. mobilis*.

.4 Technologies Important for Bio-industrial Processes

As indicated in previous sections of this report, developments in certain technologies will be essential to the commercialization and market success of products developed via biotechnological processes. Technologies that will be critical to the industrial and, ultimately, commercial success of organic chemical products synthesized by biological routes include biocatalyst, separation and purification, process monitoring and control, and rDNA technologies. The following discussion will illustrate examples of research and developments in these technologies and their potential application.

.a Biocatalysts. A catalyst increases the rate of a chemical reaction but does not effect reaction equilibrium and is itself usually unchanged by the reaction. It does this by lowering the activation energy necessary for a reaction to proceed. Catalysts are used throughout the chemical industry. A few examples of catalysts used in industrial and technologicially important processes include those used in the manufacture of sulfuric acid and ammonia and the "catalytic converters" used to convert carbon monoxide to carbon dioxide and nitrogen oxides to nitrogen and oxygen in automobiles. Enzymes are proteins which act as catalysts (biocatalysts). Some enzymes facilitate very complex reactions or series of reactions. By selection of suitable enzymes, one can obtain very specific reactions to transform large complicated bio-organic macromolecules such as cellulose to simple organic feedstock molecules (e.g., ethanol, acetic acid, etc.). In effect the choice of a specific enzyme promotes the reaction path from among the many possible reactions. Examples of their use are the conversion of whey to glucose and galactose and the hydrolysis of cellulosic materials to ethanol.

The ecological backgrounds and biochemical diversity of microorganisms should make them important sources of a wide variety of biocatalysts (enzymes) for biochemical conversion of raw materials to higher value added organic chemicals. In spite of this potential, only about 20 of the approximately 2500 known enzymes currently have commercial significance. The current annual market value* of these 20 enzymes is about $125-150 million and they are used in the pharmaceutical, food processing, and chemical industries (214). Many

*The projected market value of bulk enzymes is $500 million by 1986 (Chemical Week, May 26, 1982, pp. 44-47).

extracellular enzymes could become articles of commerce if they could be produced in sufficient quantities to make biochemical conversion processes more economical than chemical synthesis (215). However, commercial production of additional enzymes will require continued development of microbial growth and isolation procedures, developments in applied genetics, improved/real time process control, and implementation of continuous process operations. Commercial development of industrially useful enzymes is a long and capital intensive process. Some of the approaches that, individually or in combination, have been and could be used to increase enzyme yields are listed below (215):

Synthesis of Enzyme

- o Optimize culture conditions: temperature, pH, carbon source, nitrogen source, aeration, agitation, induction of inducible enzymes, and maintenance of the secretory phase.

- o Genetic approaches (such as recombinant DNA) to remove restrictive control mechanisms.

- o Applied genetics approaches such as mutation and selection, rDNA, gene amplification, and gene fusion and use of cells with specialized cytomembrane structures.

Secretion of Enzyme

- o Addition of chemicals to make the cell wall/membrane complex more permeable ("leaky").

Rate of Reaction

- o Optimize substrate concentrations, pH levels, temperature, ionic strength.

- o Obviate end product inhibition of enzyme.

- o Immobilization of enzymes/cells to extend use (re-use) of biocatalyst, and facilitate removal of products.

Use of enzymes in organic chemical synthesis offers some important advantages over traditional methods of chemical synthesis. For example, enzymes are catalytic, highly reaction specific (even stereospecific), produce exceptionally pure products, and allow synthesis to be carried out under milder reaction conditions (216, 217). Enzymes also possess some qualities that have limited their industrial usefulness and reduced their economic potential. For example, enyzmes operate effectively only in a relatively narrow range of reaction conditions (aqueous solutions, moderate temperatures, pH sensitivity; 216). Also to use expensive enzymes once in reaction mixtures such as batch fermentation vessels has additional industrial economic disincentives:

- o the initial cost of the enzyme;
- o the need to remove the enzyme from the product;
- o extended reaction times to reduce enzyme costs; and
- o the need for large reaction vessels with concommitant high capital costs (218).

Thus, due to the incongruity between the relatively limited conditions associated with chemical reaction processes and the "delicate" nature of biocatalysts, enzymes had only limited applicability as catalysts in industrial processes until the early 1970s. Because of newer approaches which make enzymes insoluble while retaining their catalytic activity (analogous to in vivo association of enzymes with cell membranes, mitochondria and other cell organelles), industrial use of enzymes via immobilized biocatalytic (enzymes and microbial) systems has begun to expand (132, 219-221). Such enzymes are adsorbed, copolymerized, covalently-bonded, or otherwise attached to an inert water-insoluble material. This mechanism of attachment permits them to be separated physically from both substrate and reaction product, and also allows them to be recycled or reused easily. Immobilized enzymes have significantly enhanced processing and manufacture (for example, cheddar cheese) because they can be integrated into a continuous process and can be recovered for recycling (222).

In addition to use of immobilized biocatalysts to improve process economics and throughput, development of mathematical models to predict biosystem process behavior and to describe quantitative relationships between process variables will be essential to the success of optimization strategies for biotechnological

processes that are eventually used. As one starting point in developing process models, thermodynamic information is required to predict equilibrium (yield) and the concentration of enzyme-catalyzed reaction products; the heat absorbed or released from the reaction; and the conditions, such as temperature, fluid forces, pH, and substrate concentration, necessary to maximize a chemical process (for example, the enzyme-catalyzed hydrolysis of cellulose and hemicellulose to lower molecular weight products which can be converted by fermentation to commercially useful chemicals). A detailed knowledge of the thermodynamics and thermochemistry of enzyme-catalyzed reactions can be used to optimize biochemical processes and to minimize the side reactions which give unwanted by-products.

In order to develop predictive models of biochemical processes and to select the appropriate reaction pathways, equilibrium data are necessary. Data banks of evaluated thermodynamic data will be necessary to predict the equilibrium, yields, and heat liberated/absorbed in enzyme-catalyzed industrial reactions. These include heats of formation, entropies, and heat capacities of organic molecules. After the selection of the appropriate reaction, the rate of the reaction will depend on enzyme concentration, temperature, pH, metal ion concentration, etc. Therefore, chemical kinetic data will also be necessary for choosing the proper industrial process conditions.

Currently, at the laboratory scale, corn residues (corn waste) have been converted to both ethanol and acetic acid in significant yields by Clostridium thermocellum (an anaerobic bacterium). An understanding of the thermodynamics and energetics of the reactions under various conditions will be essential for the implementation of such a process on an industrial scale.

.1 Immobilized Enzymes. The basic enzyme immobilization methods are adsorption, cross-linking, entrapment, microencapsulation and covalent attachment (69, 217). The operational advantages of immobilized enzymes are:

- o reuseability and increased operational stability of enzymes;
- o possibility of both batch and continuous process operation modes;
- o rapid termination of reactions;
- o greater variety of engineering designs for continuous processes;

- o reduced cost of operation; and
- o potential for efficiency gains in multiple enzymatic reactions (218-220, 223).

Table 5.10 illustrates chemical and physical methods employed for the immobilization of enzymes.

Table 5.10

Methods for Immobilization of Enzymes

Chemical methods--(Covalent bond formation-dependent)

Attachment of enzyme to water-insoluble, functionalized polymer

Incorporation of enzyme into growing polymer chain

Intermolecular crosslinking of enzyme with a multifunctional, low molecular weight reagent

Physical methods--(Noncovalent bond formation-dependent)

Adsorption of enzyme onto water-insoluble matrix

Entrapment of enzyme within water-insoluble matrix

Entrapment of enzyme within permanent or nonpermanent semipermeable microcapsules

Containment of enzyme within special semipermeable membrane devices

Source: Reference 219.

Table 5.11 reviews the advantages and disadvantages of some immobilization methods, as well as prospects for their industrial utility.

There are some disadvantages to enzyme immobilization. A major one is that some enzymes are partially inactivated by immobilization. The key to the science (or art) of enzyme immobilization is to balance the loss of enzymatic activity that occurs upon association with support materials with the long-term

Table 5.11

Advantages and Disadvantages of
Some Methods for Immobilizing Enzymes

Method	Advantages	Disadvantages	Commercial Use
Enclosure in semipermeable membrane sac	High retention of initial activity.	No long term stabilizations of activity. Clean stream needed.	Uncertain
Entrapment in polymeric material, e.g., cellulose acetate	Easily used, long life (with suitably long lived enzyme).	Some enzyme inactivation on immobilization.	Yes. Glucose, isomerase, etc.
Adsorption to charged supports	Easily done. High enzyme activities immobilized. Often pH optimum change achieved.	Hydraulic properties of supports less than ideal. Impermanent enzyme-support link.	Yes. Glucose isomerase, penicillin acylase
Absorption into porous supports	Easily done. High activities achieved. Good hydraulic properties.	Diffusion of substrate/ product to/from enzyme difficult. Enzyme can "leak". Supports costly.	Uncertain
Absorption or adsorption plus cross-linking	Good retention of activity in use. Sometimes greater temperature stability.	Initial activities lowered. Most supports costly, need re-use.	Yes. Glucose isomerase, glucoamylase
Covalent attachment to activated support material	Good retention of activity in use. Good hydraulic properties. Sometimes greater temperature stability.	Possible leakage of activating chemical.	Uncertain

Source: Reference 218.

retention (half-life) of activity (218). For economically feasible industrial processes, extended enzymatic activity half-life is more valuable than high initial enzymatic activity. To improve enzymatic half-life activity and the diffusion of substrate into the enzyme (active site)--polymer support system, additional research is needed. Some examples of promising leads includes the use of magnetic fields to influence the orientation of polymers (224), the application of ultrasound for accelerating enzymatic activity (225), and the regeneration of the catalytic activity of thermoinactivated enzymes (226).

.2 Industrial Applications of Immobilized Enzymes. To date there are many more applications of immobilized enzymes in analytical fields than in large scale industrial processes (227-230). The largest in tonnage and most widely known current commercial application of immobilized enzymes is for the partial isomerization of glucose to fructose in the production of high fructose corn syrup (HFCS; 231). The major application of this technology in the pharmaceutical industry is the conversion (via fermentation with the enzyme penicillin acylase) of benzyl penicillin to 6-aminopenicillanic acid for the preparation of semi-synthetic penicillin (220). Table 5.12 summarizes industrial process and pilot plant developments in the application of immobilized enzymes.

Although a variety of approaches can be used to acquire and install an immobilized enzyme system in a commercial operation, the approach most commonly used is to obtain the complete catalytic system from an external supplier, such as Corning Glass Works, Monsanto, American Cyanamid, R.J. Reynolds, or Union Carbide (232). Experience has demonstrated that a close working relationship between system supplier and buyer is essential for the tailoring of the system to meet the user's specific needs. This experience may also allow biocatalytic system suppliers to forward integrate and to become producers of commercial products.

From a commercial immobilized enzyme system user viewpoint, objective means are essential to compare the industrial performance of different catalyst systems or similar catalytic systems from different sources is essential. Means for objective evaluation is often unavailable and the data that are available are difficult to compare. Thus, comparative evaluation of competing systems is

Table 5.12

Application of Immobilized Enzymes in Large-Scale Industrial Processes (I) and in Pilot Plants (P)

	Process*									
Company	a	b	c	d	e	f	g	h	i	j
Anheuser-Busch	-	-	-	-	-	I	-	-	-	-
Clinton Corn (USA)	-	-	-	-	-	I	-	-	-	-
Car-Mi (USA)	-	-	-	-	-	I	-	-	-	-
Corning (USA)	-	-	-	P	-	I	I	P	-	P
U.S. Army Natick Center (USA)	-	-	-	-	-	-	-	-	P	-
Denki Kagaku (Japan)	-	-	-	-	-	P	-	-	-	-
Diamond Shamrock (USA)	-	-	-	-	-	-	P	-	-	-
Gist Brocades (Holland)	-	-	-	-	-	I	P	P	-	P
Gulf Oil (USA)	-	-	-	-	-	-	-	-	P	-
Hameen Peruna (Finland)	-	-	-	-	-	I	-	-	-	-
ICI (England, USA)	-	-	-	-	-	I	-	-	-	-
Lehigh University (USA)	-	-	-	-	-	-	P	-	-	-
Novo (Denmark)	-	-	-	-	-	I	-	-	-	-
Sanmatsu (Japan)	-	-	-	-	-	I	-	-	-	-
Snam-Progetti (DeBi; Italy)	-	P	I	P	I	P	I	P	P	-
Tanabe Seiyaku (Japan)	I	I	I	I	-	-	-	-	-	-
Vallo (Finland)	-	-	-	-	-	-	P	-	-	-
CPC (USA)	-	-	-	-	-	I	-	-	-	-
Amstar (USA)	-	-	-	-	-	I	-	-	-	-
Suddeutsch Zucker (WG)	-	-	-	-	-	I	-	-	-	-

*(a) synthesis of L-aspartic acid with the use of immobilized microbial cells containing aspartase

(b) synthesis of L-malic acid with the use of immobilized microbial cells containing fumarase

(c) production of 6-aminopenicillanic acid with the use of immobilized penicillin acylase

(d) resolution of racemic mixtures of amino acids (produced by chemical non-enzymatic method) with the use of immobilized aminoacylase

(e) production of D-amino acid derivatives with the use of immobilized hydantoinase

(f) production of glucose-fructose syrups with the use of immobilized glucose isomerase

(g) use of immoblized lactase for production of nonlactose milk from milk sugars, and for production of glucose/galactose syrups from whey

(h) production of glucose from partial hydrolysates of starch with the use of immobilized glucoamylase

(i) production of glucose and/or ethanol from cellulose with the use of immobilized cellulase

(j) production of glucose-fructose syrups from sucrose with the use of immobilized invertase

Source: References 183, 216.

extremely difficult (231). Fundamental information on and standardized methods to evaluate biochemical, mechanical, hydraulic, etc. characteristics of processing parameters is essential. This would allow the development of performance data on a more unified basis. It would also be possible to establish kinetic requirements for multiple key process parameters for the efficient engineering design of commerical reaction systems. Examples of data needed include enzyme activity reaction half-life, catalytic packing density, external and internal mass transport efficiencies, reactor contact efficiency, and residence time distribution (Table 5.13; 231). Because of proprietary positions, however, enzyme producers and enzyme system suppliers may be reluctant to advocate standards in this area.

.3 Immobilized Cells. Serious work on immobilized cell research began in the early to mid-1970s only after the impetus given by advances in immobilized enzyme technology. Most of the research on immobilized cells (microbal or plant) has described techniques for entrapping cells in gels, although other procedures such as covalent attachment, adsorption, and cross-linking leading to insoluble aggregates have been used (131, 233). Some examples of cell immobilization procedures are given in Table 5.14.

Table 5.13

Parameters Important in Designing Biocatalyst System

I. Biochemical Characteristics

1. Activity
2. Operational Stability (half-life) and Activity Decay Profile
3. Productivity in Usage Life Time
4. Optimal Substrate Concentration
5. Effect of Oligosaccharides Concentration
6. Effect of Dissolved Oxygen
7. Minimum and Maximum Residence Times
8. By-Product Formation
9. pH and Temperature Sensitivity
10. Storage Stability
11. Protein-Enzyme Elution
12. Microbial Growth
13. Reactor Effluent Quality (Composition, Color, Odor, Protein Content, pH, etc.)

II. Mechanical Characteristics

1. Particle Size, Shape, and Size Distribution
2. Density (Dry Bulk Density and Wet Density)
3. Swelling Behavior
4. Compressibility
5. Cohesion
6. Particle Attrition

III. Hydraulic Characteristics

1. Pressure Drop
2. Mode of Flow (Upflow versus Downflow)
3. Bed Compaction
4. Axial Dispersion and Channeling
5. Residence Time Distribution
6. Stratification
7. Length-to-Diameter Ratio
8. Minimum Velocity for Onset of Fluidization

Source: Reference 231.

Table 5.14

Cell Immobilization Procedures

Covalent binding
- Hydroxyalkyl methacrylate (glutaraldehyde)
- Carboxymethylcellulose (carbodi-imide)

Entrapment
- Polyacrylamide
- Alginate
- Cellulose-triacetate
- Agar
- Carrageenan
- Chitosan
- Collagen (gelatin)
- Polystyrene
- Urethane
- Nylon (micro-encapsulation)

Adsorption
- Anion-exchange resin
- Dowex 1
- DEAE-cellulose
- Cross-linked pectate
- Metal oxide
- Bioadsorption: concanavalin A
- Ion-exchange cellulose
- Polyvinylchloride and porous bricks

Cross-linking
- Glutaraldehyde
- Albumin and glutaraldehyde
- Gelatin and glutaraldehyde

Source: Reference 233.

For further developments in this field, it will be necessary to understand the nature of the cells (viable-resting or growing, non-viable, permeabilized) once they are associated with a matrix (220, 233, 234). Although relatively simple reaction systems are employed currently for both immobilized enzyme and cell systems, immobilized cell systems (prokaryotic and eukaryotic) offer greater potential for developing reusable, multi-enzyme, complex reaction systems (153, 233-235). Thus, immobilized cells, exemplified by their use in water purification and pollution control systems, may have a broader range of industrial applications than immobilized enzymes.

Generally, most of the methods of immobilization employed for enzymes can also be used for cells; however, much greater care must be taken to prevent

inactivation of desired cellular activities (218). Also many of the same process design considerations noted for immobilized enzymes in Table 5.13 are also applicable to immobilized cell systems.

Table 5.15 highlights some advantages and disadvantages of methods for immobilizing whole cells, as well as the potential of the methods for using multi-enzyme systems. The best known immobilized cell process is that of the Tanabe Seiyaku Company. In this process, E. coli cells having a high aspartase activity were trapped in polyacrylamide. They were subsequently treated to disrupt the normal cell permeability barrier and, as a result, they stereo-selectively converted ammonium fumarate to L-aspartic acid (132).

Some specific advantages of immobilized cells over immobilized enzymes for continuous biocatalytic activity in industrial processes include (132):

- o high yields of biocatalytic activity are achieved on immobilization;
- o enzyme extraction and purification processes and costs are eliminated;
- o immobilized cells have higher operational stability;
- o actual cost of the "enzyme" is reduced;
- o intracellular enzymatic machinery of immobilized cells generally remains intact;
- o immobilized cells allow use of intracellular and generally unstable enzymes; and
- o immobilized cells appear well suited to multi-step biocatalytic conversions and coenzyme regeneration.

Immobilized cell systems have some disadvantages such as leakage of catalyst and support system, production of secondary products, potentially high capital costs, and fewer industrial successes. However, once particular

Table 5.15

Methods for Immobilizing Whole Cells

Method	Advantages	Disadvantages	Use with multi-enzyme systems
Enclosure in semipermeable membrane	Gentle	Fouling of membranes. No stabilization of activity.	Uncertain
Entrapment in polymeric material:			
1-Cellulose acetate	Easily used, long life.	Enzyme inactivation by organic solvent	Uncertain
2-Acrylamide	Inexpensive monomers, easily done.	Some enzyme activation by monomers, free radicals	Yes, after cell regrowth
3-Alginate	Very gentle, easily done. Good stabilization of cell activity.	Calcium alginate dissolved by chelating agents	Yes
4-Carrageenan	Gentle	Some heat shock to cells	Yes
5-Collagen	Inexpensive, easily done.	Cross-linking required	Yes
Adsorption to charged supports	Easily done. Some stabilization of cell activity.	Impermanent cell-support link-cell leakage	Yes
Absorption into porous supports	Easily done	Cells can leak easily	Yes
Covalent attachment to activated support material	Good retention of activity in use. Good hydraulic properties. Sometimes greater temperature stability. Permanent linkage.	Possible inactivation of enzymes	Uncertain
Cross-linkage of cells with or without support	Inexpensive. May be controlled to inactivate unwanted enzymes.	Reactivation by cell regrowth unlikely	No

Source: References 218, 233.

immobilized cell systems are evaluated regarding their potential for industrial use, it will be possible to lower capital operating costs of industrial biochemical processes by reducing the size of the reaction and/or fermenting vessels and by increasing the throughput of the substrate stream. The realization of the potential benefits of this technological "tool" will depend on identifying the appropriate role for it in an industrial process (for example, in the conversion of cellulosic material to commodity organic chemicals, in a specific biotransformation to produce a higher value added product, or in the replacement of a unit process step in a complex chemical synthesis).

.4 Research Opportunities. The following lists some areas of research opportunity for both immobilized enzymes and cells that could increase the potential industrial usefulness of this technology:

Immobilized Enzymes--

- o production of membrane associated forms of water soluble, labile, enzymes;.
- o stabilizing larger molecular weight enzymes by modification of the surface amino acids, by cross-bridging of the active site with neighboring molecules, by combined thermochemical and photochemical linkages, by designing semi-synthetic enzymes, etc.;
- o immobilization and/or microencapsulation of expensive and labile coenzymes for recycling or continuous use;
- o understanding physicochemical reactions for stabilizing enzymes to permit higher product yields;
- o co-immobilizing series of enzymes for sequential biochemical reactions;
- o developing kinetic models for individual reaction rates to achieve steady state in a complex reaction system;

- o reversing catalytic loss (enzyme rejuvination); and

- o immobilizing enzymes that will operate effectively in complex and/or "dirty" streams.

Immobilized Cells--

- o understanding how dissolved oxygen levels affect immobilized cells and their nutrient use;

- o operation of immobilized whole and permeabilized cell systems under harsh industrial conditions;

- o operation of immobilized cell or cell systems in substrate streams (slurries), and under solid state fermentation reaction conditions;

- o genetic manipulation for amplifying cellular synthetic pathways useful in specific industrial conversions at the expense of other "wasteful" pathways and enzymatic reactions; and

- o control of decay of biocatalytic activity.

In addition, modification and/or tailoring of polymers as biocatalyst support devices for--(a) improved process throughput and substrate loading, (b) enhanced biocatalyst orientation, half-life reactivity and stability, and (c) better coupling with product separation processes--could facilitate industrial development of more "solid phase" biotechnological approaches to industrial synthesis of organic chemicals (236).

5. Immobilized Cell Technology and Fermentation. Research in immobilized cell technology is beginning to affect bioprocess used in the fermentation of sugars to ethanol as a means to increase cell density, to reduce the volume of fluid used in traditional fermentation vessels, and to facilitate development of continuous processes.

Entrapment of cells in relatively non-toxic polymers such as polyacrylamide, collagen, agar, alginate, carrogeenan, polystyrene, cellulose acetate, etc. has been the method most widely used for the immobilization of cells. Calcium alginate and carrageenan have been used successfully to immobilize yeast cells for the production of ethanol. Listed in Table 5.16 are examples of some of the research activity and developments in this area.

Table 5.16

Research Activities Related to Immobilized Cells

Immobilization Polymer	Organism	Sugar Substrate (g/L)	% of Feed Sugar Use	Max. Ethanol Productivity (g ethanol/L/H)[a]	Reference
Ca-alginate	S. cerevisiae	glucose (100)	80	4.3	131
Ca-alginate	S. cerevisiae	cane molasses (175)	83	21.3	198
Ca-alginate	Z. mobilis	glucose	97	71	200
Ca-alginate	S. cerevisiae	glucose (30)	94	21	199
Carrageenan	S. cerevisiae	glucose (200)	100	40	237

[a]Reference 200.

Members of the Japanese Research Association for Petroleum Alternatives Development (Ajinimoto, JGC, Kansae Paint, Marnzen Oil, Sanraku-Ocean) have reported recently the completion of a pilot plant for the rapid, continuous conversion of biomass to ethanol via yeast cells immobilized on radiation cured polymers (238).

In addition to these studies, a variety of other approaches for stabilizing whole cells in polymer matrices are being explored regarding their utility for larger scale bio-reactor systems. Table 5.17 gives a sampling of experimental efforts in progress.

Table 5.17
Experimental Efforts to Stabilize Whole Cells in Polymer Matrices or on Surfaces

Immobilization System	Organism	Substrate	Reference
Ca-alginate + cross linking for stability (a. -polyethleneimine and glutaraldehyde; b. -carbodiimide and N-hydroxysuccinimide; c. -periodate and polyethyleneimine)	S. cerevisiae	--	239
Ca alginate	P. tannophilus	xylose	208
Ca alginate	K. fragilis	whey	240
Crude egg white + glutaraldehyde	C. acidophilia	--	241
Polyurethane foam and gel	--	--	242
Chitosan	E. coli	L-serine	243
Induced bacterial filamentous growth	E. coli S. typhimurium Bacillus species	--	244
Ion-exchange resins	S. cerevisiae	glucose	201
Titanium activated microporous inorganic supports	S. cerevisiae	sucrose	245
Fixed film reactor with treated (gelatin, glutaraldehyde) Rashig rings	S. cerevisiae	glucose	113

Continued advances in cell immobilization technology coupled to innovations in biotechnological processes will improve the economics for future use of such bio-systems in the industrial production of organic chemicals.

.b Separation and Purification Technologies. As fermentation is central to biotechnological processes, the ability to separate specific components from highly complex fermenter slurries is essential to the success of industrial fermentation processes.

The expanded potential for use of continuous fermentation and cell recycling processes in biotechnology offers cost reductions in comparison with batch fermentation, because the equipment may be smaller and more energy efficient for an equivalent throughput and fewer production delays are encountered. The realization of this potential, however, will require innovations in separation, extraction and purification technologies, since:

- o process technologies developed will depend upon the physical and chemical properties of the fermentation broths; and

- o these properties are difficult to measure in terms of quantifying the mechanisms of the process (246).

In terms of process economics, the cost of recovery procedures for high value added organic chemicals products developed through biotechnologies will not be as critical as those procedures developed for recovery of commodity organic chemical products from fermentation processes. If bioprocess substrates can be economically or politically as desirable as petroleum or coal and if microbial reactions are as economically and technologically attractive as chemical substrate conversions for producing commodity organic chemicals, new separation technologies using methods based upon low energy and high throughput per unit of capital cost are required (246).

The subsequent paragraphs in this section will highlight some important separation and purification technologies, some developments in the field, and the potential applicability of these technologies to industrial biotechnological processes for the production of organic chemicals.

.1 Membrane Technology. Membrane techniques or combinations of membrane techniques may have application in continuous alcohol removal and fermentation cell recycling. Membranes that are relatively permeable to solvents and impermeable to most other solutes present in the fermentation broth may allow enrichment of alcohol concentration to levels sufficient for recovery from biological systems with relatively low alcohol tolerance (162).

Technical interest in processes of mass transfer through membranes has increased substantially in the last decade. Table 5.18 lists technically important membrane separation processes and relevant properties, and, of these processes, ultrafiltration, hyperfiltration, dialysis and electrodialysis are presently of the most technical significance (247).

It is not possible here to discuss the advantages and disadvantages as well as applicability of each technology listed in Table 5.18 in this section of the report. Rather, this section of the report will illustrate the potential applicability of some of these technologies, such as ultrafiltration, to separation and purification processes in industrial biotechnological processes.

Ultrafiltration has important industrial applicability in the purification and concentration of biological molecules due to its ability to minimize denaturation and decomposition losses of labile, high value added products. A review by Michaels summarizes recent developments and limitations in the use of ultrafiltration (248).

Additional research and development in several areas of ultrafiltration technology will be essential in order to understand biological, chemical, physical, and hydraulic design parameters and before considering the broad application of this technology to large scale biotechnological and fermentation processes. Some general areas for technology improvement include:

- o modification of membranes to reduce or eliminate fouling;
- o improved fermentation broth conditioning techniques;
- o adaption for high volume, aqueous stream concentration and purification;
- o modification of the polymer chemical characteristics;
- o improved selectivity of membranes for separations;

Table 5.18

Technically Important Separation Processes

Process	Principle of Separation	Membrane Type	Driving Force
Ultrafiltration	screening effect	asymmetric porous membranes	hydrostatic pressure, 0.5 to 10 bar
Hyperfiltration	diffusion and solubility	asymmetric solubility membranes	hydrostatic pressure, 50 to 100 bar
Dialysis	diffusion	symmetrical porous membranes	concentration gradient
Electrodialysis	electrical charges of ions	ion exchange membranes	electrical potential difference
Piezodialysis	electrical charges of ions	mosaic ion exchange membranes	hydrostatic pressure gradient
Gas separation	diffusion and solubility	homogeneous or porous symmetrical membranes	hydrostatic pressure or concentration gradient
Liquid membrane permeation	diffusion and reaction reaction	mostly unsupported liquid membranes (oil or water type), multiple emulsions	concentration gradient, pumping effect in co- and counter-transport

Source: Reference 247.

- o deactivation of certain contaminating enzymes; and

- o removal and/or concentration of particulate solids such as cells/cell debris from fermentation broths (246, 248, 249).

The potential for hollow fiber ultrafiltration membranes in larger scale industrial processes is illustrated by the following two examples. First, Monsanto has developed a proprietary line of prism hollow fiber separators. These separators, currently being used to remove useful hydrogen from certain wastes, may have potential in separating and purifying fermentation products (250). Second, RPI has also developed a hollow fiber ultrafiltration process. It is currently being considered for use in the trapping of viruses and plasmids from waste control or separation processes, but may also be applicable to large scale fermentation process product separation (251). Possible barriers to the successful implementation on a large scale of either of these technological approaches are membrane fouling and fragility.

In addition to hollow fiber ultrafiltration, tangential flow filtration could potentially improve continuous biomass-conversion-to-ethanol processes by removing toxic materials, growth inhibitory substances, spent cells, as well as desirable reaction products while recycling viable cells to the fermentation vessel (252).

Ultrafiltration processes could also be coupled to immobilized biocatalyst (enzyme/cell) system reactors for the continuous: (a) flow through of substrate into the catalyst, (b) conversion of substrate to product, and (c) diffusion and convection of the reaction products out of the device (162, 248, 253, 254).

Advances in our understanding and application of applied genetic techniques to the induction of filamentous growth of industrially important microorganisms such as E. coli and Bacillus species could facilitate confinement (or immobilization) of microbial cells on membranes and which then could improve flow rates in continuous processes (244).

Liquid membrane (LM) technology may also have a potential range of applications to large scale biotechnological processes. LM technology involves those processes in which there is a simultaneous extraction/re-extraction process involving a selective separating phase or membrane. LMs are made by an emulsion of two immiscible phases and then dispersing the emulsion in a third phase, the continuous phase (255). This technology offers a new means for encapsulating materials and separating mixtures through a permeable membrane, although no full scale commercial plants using LM technology are currently on-line (247, 255).

LM technology may be particularly applicable to processes involving large volumes of fluids, for example, fermentation processes and liquid-liquid reaction systems (256). The principles of encapsulation of enzymes have been investigated and the results show the potential applicability of enzymes and this technology to organic chemical synthesis (255, 257). In addition, research on the conversion of cellulosic materials to ethanol indicates that LM technology has considerable potential: (a) for continuous extractive fermentation of ethanol; (b) to recover and reuse enzymes, coenzymes and microorganisms; (c) to increase the operational stability of biocatalysts; and (d) to reduce product inhibition, reaction volumes and operational costs (188, 194).

Two additional examples illustrate the range of possible applications of membrane technology to biotechnological processes for the production of organic chemicals. First, General Electric and the University of Pennsylvania are currently working to improve process ultrafiltration technology by developing a membrane reactor that will separate the reactant from an aqueous solution until the reaction takes place and to couple the reaction process to product purification (258). The membrane reaction is a sandwich of porous polymer membranes with a membrane on one side of the sandwich containing immobilized liquid organic solvent selective for the reactant and the other membrane has the catalyst. Thus the product stream is separated from the feed stream. Second, Engenics is considering use of bundles of capillary membranes on which microorganisms can be grown in high densities (258). Some product purification would be achieved by selective passage of products through the capillary membranes and thus separate the products on the basis of molecular weight or of hydrophobic or hydrophilic nature.

.2 Chromatography. Materials harvested from fermentation processes are usually aqueous. Traditionally, the first separation stage involves centrifugation. The second step involves ultrafiltration or microfiltration. This is followed by a purification step that hopefully will give a high yield in a short time at minimal cost.

Large scale liquid chromatography (LC) may offer possibilities for the isolation and purification of high value added proteins, for example, interferon and pheromone bioinsecticides from highly aqueous fermentation broths. Although large scale applications (e.g., >1000 liters) of LC have met with only limited successes in the past, Waters Associates believes that their approach using prepackaged cartridges of polyethylene or Teflon, where the pressure is applied radially to the cartridge, will eliminate spaces between particles and between the bed and the wall (259). This would eliminate a major problem of uniform packing. In related technical developments, Oak Ridge National Laboratory researchers are developing a pressurized continuous LC device that uses a slowly rotating bed or sorbent material, fixed multiple feed points, and fixed withdrawal locations (259).

A major industrial concern remaining with these and other new large scale LC techniques that are being developed is how well these techniques will operate over extended time periods and under industrial conditions.

In addition to these LC techniques, high performance liquid chromatography (HPLC) either individually or coupled with affinity chromatography could evolve into an industrially important technique for the rapid separation and purification of enzymes, peptides and other important biological molecules (260).

Another chromatographic technique that may have a broad range of industrial applications in the separation and purification of organic chemicals and biologically important macromolecules from crude plant/animal tissue extracts is countercurrent chromatography (261). This technique has already been applied on an experimental basis to the separation of glucose/fructose mixtures (262).

.3 Supercritical Gas Extractions. There is considerable research activity underway to examine the potential of dense gases at or near their critical points as an alternative to liquid solvents in the extraction of organic

compounds. Supercritical gas extraction depends upon unusual properties of gases in the "critical region" where no phase boundries between gas and liquid states are displayed. When increased pressure is applied to certain gases such as CO_2, usually an inefficient solvent, the extractive potential of CO_2 increases as well as its density. With higher density and temperature, the solubility of organic compounds in CO_2 increases. Above 31°C only a supercritical phase exists where liquid CO_2 becomes a highly selective solvent, for example, in the extraction: of caffeine from tea leaves and green coffee; of aromatic oils from crushed roasted coffee; of pyrethrins (esters) from gound pyrethrum flowers; of nicotine from raw tobacco; of tetrahydroxycannibinol from marijuana; of oils from sunflower seeds, soybeans, rapeseeds, and peanuts; of resins from air dried hops; and of cocoa butter from cocoa mass (263-266).

With advances in biotechnology, the potential exists for producing industrially useful organic chemicals from biomass via aqueous fermentation processes. In these processes, the selective extractive properties of super critical CO_2 extraction may be particularly useful in the separation and purification of such organic chemicals as:

- o low-to-medium weight oxygenated organic compounds, such as esters, ketones, alcohols, ethers and aldehydes, and

- o low molecular weight non-polar organic compounds, such as alkanes, alkenes, terpenes, as well as certain polar compounds, such as acetic acid (264).

In addition, this technology may also be applied to lignocellulose extraction (267).

Major barriers that will be encountered in the commercial application of this technology include: (a) a fundamental understanding of the thermodynamics of the process, (b) the mechanics of rate processes involved, and (c) the design of commercial scale operations (266).*

*NMR measurements at high pressures could aid our understanding of process dynamics and intermolecular interactions in supercritical gases (Science 216: 1179-1184, 1982).

.4 Monoclonal Antibodies. Monoclonal antibodies have obvious commercial potential in clinical laboratory diagnostic testing and in health care. In addition, monoclonal antibodies have potential both as probes of enzyme structure and function and as means for the rapid isolation and purification of enzymes or proteins from crude extracts, such as those encountered in dilute fermentation broths (268, 269).

In related developments in the commercial production of monoclonal antibodies, Damon Biotech used a microencapsulation technique that had previously been used in controlled drug delivery systems (liposomes; 270, 271). The Damon Biotech method developed is an in vitro model for producing pure monoclonal antibodies by encapsulating specific hybridoma cells in porous carbohydrate capsules and exposing the capsules to growth medium that allows the hybridomas to produce monoclonal antibodies within the capsules. At the end of the growth period, the nutrient medium is washed from the capsules, the microcapsules are opened with heparin, and the monoclonal antibodies are separated from the hybridoma cells by centrifugation (272, 273). The monoclonal antibody producing hybridoma cells are then recovered, encapsulated, and reused.

This encapsulation technique may also be applicable to large scale fermentation and biocatalytic systems, analogous to proposals for trapping enzymes in micelles (257), or the immobilization of microbial cells in liquid membranes (Micrococcus denitrificans used in the reduction of nitrate to nitrite; 274). Development in encapsulation technology could also potentially be coupled with sedimentation field flow fractionation for the separation of bio-macromolecules (275).

.5 Biological Separation/Genetic Engineering. An approach that potentially could be used in specific circumstances in fermentation systems to separate the desired product from the reaction system would be to genetically engineer the compartmentalization of the desired product within the producing organism. In industrial scale-up developments in the production of human insulin proteins, the genetically engineered E. coli strain employed morphologically demonstrated the accumulation of insulin proteins intracellularly within inclusion bodies (276). In a related development, ICI researchers

identified a microorganism, Alcaligenes eutrophus, that can concentrate the aliphatic polyester polyhydroxybutyrate (PHB)* in intracellular granules (277). In certain specific cases, these inclusion bodies or granules could be used as means to facilitate the isolation and purification of the desired product from other intracellular components.

Alternatively, rDNA techniques can be used to insert DNA information signalling sequences into a microorganism to trigger the extracellular secretion of a desired product (278). This approach not only would facilitate subsequent product separation and purification, but also would allow such microbial cells to be immobilized and used in continuous processes.**

.6 Dry Separations. Although more closely associated with efforts to reduce particulate pollution from industrial sources and to remove water impurities, "dry" separation techniques, such as electrostatic, electromagnetic, and screening, may have some specialized applicability in industrial biotechnological processes used for producing organic chemicals. These separation methods differentiate between particles on the basis of such properties as size, shape, electrical characteristics, magnetic characteristics, and density (279, 280). Possible applications of "dry" separation techniques include the following:

- o recovery or removal of trace minerals;
- o mechanical separation of biomass fractions based on size differences (if problems of moisture can be overcome);
- o dialectrophoretic separation of specific cell populations (281); and

*Possible example of future "non-traditional" commodity chemical (biopolymer/plastic) with additional small volume, high value added applications resulting from PHB's piezoelectric and biodegradable properties (European Chem. News, May 3, 1982, p. 15).

**Another approach would be to manipulate genetically the production of certain intracellular metabolites in microorganisms having no cell walls. Intracellular metabolites would be released from the microorganism when cells were placed in a hypotonic solution (Biotechnology Letters 4: 217-222, 1982).

Regarding this latter point, a more fundamental understanding of microbial magnetotaxis and of the synthesis of magnetosomes by certain microbial species may prove useful in considering future separation technology alternatives (286). In addition, application of magnetic polymers as supports for immobilized enzymes or of magnetic affinity chromatography techniques may also be useful for the rapid isolation of proteins and enzymes from batch and fluidized bed and/or continuous reaction vessels (287-289). Separations of cells from reaction vessels could also be accomplished by means of antibodies or lectins associated with magnetic microspheres that would bind specifically to cell surface receptors (290, 291).

An alternative to both electrophoresis and chromatography in separation and purification techniques that also may be applicable to biotechnological processes is electromolecular propulsion (EMP) or the chemoelectronic mobilization of chemical species in low conductivity fields (292, 293). Although currently applied only to lipoprotein analysis and to purification of gold, silver and platinum ores, this technique could induce the movement of non-polar molecules, such as aromatic hydrocarbons, in a separation process.

The preceeding discussion illustrates only some of many opportunities for the potential application of a broad range of innovative separation and purification technologies to large scale industrial biotechnological processes. For this potential to be realized, however, considerable fundamental research is needed on the integration of these technologies with industrial process design parameters and variables.

.c Process Monitoring and Control

As industry expands its use of biotechnological and fermentation processes for improving product yields and system productivity, greater demands will be made on process control and monitoring functions and on the automated, real-time

and continuous assessment of process parameters. Information generated from the development of and/or improvements in more sophisticated computerized process control and monitoring systems could serve as the basis for development of predictive models of biotechnological processes and of process parameters. Areas for improvement include:

- o technical developments in, evaluation and performance comparison of, and standards for control and monitoring instrumentation and techniques;

- o fundamental understanding of the applicability of on-line sensing, measurement and analytic techniques; and

- o availabilty of critically evaluated data on key process parameters.

Information and developments in these areas will assist in establishing reproducible and consistent manufacturing conditions, in quantitatively improving product quality, in reducing human errors and raw material waste, in increasing process accuracy and energy efficiency, and in increasing process productivity.

Although there have been advances in automation of off-line measurement techniques, these techniques are relatively slow in comparison to on-line methods that involve measurement directly on or within a biologic system (294). However, many on-line sensing and measurement systems provide only an indirect measure of the process parameter under consideration. Table 5.19 illustrates some examples of measurement approaches currently being used to monitor biotechnological processes. Since major objectives of capital intensive industrial biotechnological and fermentation processes are system productivity and product yield, a barrier to controlling such process for improving process efficiencies is in the continuous quantitative assessment of substate and product concentrations. However, sensing systems for direct and continuous measurement of substrate concentration, for example, biomass, and resulting process conversion products and product concentration are generally lacking for large scale industrial biotechnological processes (295).

TABLE 5.19

Approaches to Measurement (and Control) of Biotechnological Processes

Process Parameters	Measurement Approaches
Temperature	o cooling requirements o flow calorimetry o dynamic calorimetry
pH	o glass-reference electrode o base addition
CO_2	o off-gas analysis--infrared absorbance o dynamic assessment: tubing method, mass spectrometer (and gas chromatography)
O_2 concentration and transfer	o off-gas analysis (gas flow rate, inlet O_2 concentration, outlet concentration) o dynamic assessment (air flow temporarily stopped and change in dissolved O_2 measured over time) o mass spectrometer
Cell mass	o oxygen uptake rate o cummulative CO_2 production o total culture fluorescence (NAD+ - NADH) o electrode system o total amount of ATP
Cell growth	o flow viscometer o power requriements of impeller system o scattered light o substrate consumption o production of CO_2 o rate of base addition (load cells) o quantitation of O_2 demand
Cell metabolism	o respiratory quotient (RQ) o electrical conductance
Nutrient uptake	o enzyme probe o dialysis membrane probe
Ionic strength	o electrical conductance
Cell morphology - population differentiation	o cytofluorometry

Source: Reference 294.

The following discussion provides some examples of technological developments that may improve quantitively the continuous and more direct on-line measurement of process parameters.

Advances in immobilized enzyme technology have aided considerably in the design and industrial applicability of enzyme electrodes as biosensors. The advances have also indicated the potential for use of multi-enzyme systems to expand the analytical capabilities of enzyme electrodes (283, 296). Such enzyme electrodes would allow the rapid, specific and sensitive analysis of various components in complex mixtures. In addition to enzymes, immobilized (or permeabilized) prokaryotic or eukaryotic cells in electrodes may extend analytic capabilites beyond sensing biochemical substances, and, via cell regeneration, may increase the operational life of biosensors (297). Immobilized whole cell membrane reactor sensing systems could also be employed to measure substrate diffusivities within biosystems (298).

Both immobilized cell and enzyme electrodes in association with a thermistor could improve industrial microcalorimetric measurement capabilities (299). Developments in flow microcalorimetric techniques may also potentially expand industrial process control capabilities by providing: (a) predictive methods for screening polymers, (b) measurements of interactions of biological molecules, (c) information on surface properties in purification of proteins, and (d) data on the activity of immobilized biocatalytic systems (300).

NMR techniques could be applied to the monitoring and measurement of specific nutrients or other components in complex fermentation broths (301, 302). Advances in semipermeable membrane technology may offer sensing alternatives for the on-line measurement of fermentation product concentration (303). In addition to these more macro-sensing technical possibilites, developments in X-ray laser and microdensitometry and microfluorimetry technologies could potentially provide information on biochemical activity and other functional parameters on individual cells in complex biotechnological processes (304, 305).

These examples represent just a sampling of the many avenues being investigated to improve the monitoring and control of industrial bio-processes. As industry begins to scale-up bio-processes over the next several years, its demands for better measurement techniques will serve as a stimulus for technological innovation and developments in this area.

d. Genetic Engineering

In addition to traditional industrial microbiological tools such as mutation and selection for improving product yields from bioprocesses, recent research and developments using new applied genetics-genetic engineering tools, such as recombinant DNA (rDNA), protoplast fusion, and gene amplification via plasmids and bacteriophages, have expanded industrial options for fermentation and other biotechnological processes.

Preceeding sections of this report have illustrated several examples of genetic engineering and rDNA research focused on product yield improvements in the conversion of cellulosic materials to ethanol. Some of these molecular manipulations attempted to increase microbial ethanol tolerance, to improve the thermostability of fermenting mircroorganisms (metabolic pathways-enzyme systems), to transfer genes for multi-step enzyme systems for more direct conversions, etc.

It is unlikely in the near term that NBS research activities will include genetic engineering of microorganisms. However, it is important to realize in planning Bureau research activities that genetic engineering will be an important industrial tool. In addition, genetic engineering of industrial microorganisms will have important influences on industrial scale-up considerations and on the design of industrial biotechnological processes. Some examples of these influences include (51):

- o strain stability;
- o type of fermenter-fermentation process used;

- o nonsubstrate nutrient requirements and costs;
- o separation technologies complementary to microbial properties;
- o operating conditions such as cell immobilization and cell recycle; and
- o efficiency of substrate utilization.

Thus, the future success of biotechnology related research and service activities at NBS will require the cooperation and intergration of many scientific disciplines. Further, a basic working knowledge at the Bureau of molecular genetics and of genetic engineering techniques will be essential in the long-term for understanding and for being responsive to industry needs in biotechnology within the framework of the Bureau's mission.

6. DISCUSSIONS WITH INDUSTRY AND TECHNICAL GROUPS

.1 Purpose of Discussions and Listing of Meetings

An important element in the process of anticipating future industry needs for NBS services in biotechnology and of guiding NBS competence building efforts in this area is establishing a dialogue with industry and with individuals knowledgeable in the biotechnologies. Such dialogue provides an opportunity to obtain information on barriers or problems that industry has or will have in developing products via biotechnological processes, and on how these problems may relate to traditional Bureau infrastructure services.

Based on the Bureau's projections regarding the importance of biotechnology in the future production of organic chemicals and of biocatalysts in the economics of biotechnological processes, the initial Bureau focus for discussions with industry was the organic chemical industry. In addition to discussions with individuals at firms in the chemical industry, discussions were also held with individuals from technical associations, academia, other industries using or developing biotechnological processes, and a high-technology investment firm. Table 6.1 provides a listing of the individuals with whom meetings were held as well as their affiliations.*

.2 Summary of Industry Views on Needs in Biotechnology

The following discussion provides a summary of comments by individuals with whom NBS staff met from January through April 1982. Summary comments are grouped under two major headings, Standards and Measurement Needs and Fundamental Science and Information Needs. These summary comments reflect external preceptions of future industry needs in biotechnology and of the relationship of these needs to possible roles for NBS in this area. Sections 8

*NBS participants varied from meeting to meeting. With the exception of the meeting with CPC International, the author of this report participated in all meetings. Other NBS participants included Drs. Peter Heydemann, William Kirchhoff, Merrill Hessel, Stanley Abramowitz, Elio Passaglia, and Robert Goldberg.

Table 6.1
NBS-Industry and Technical Group Meetings on the Role of NBS in Biotechnology

1982 Meeting Date	Organization Representative(s)	Address	Telephone	Field(s) of Interest
Jan. 7	Bernard Wolnak	Bernard Wolnak & Associates Suite 706 360 North Michigan Avenue Chicago, IL 60601	312/782-4926	enzymology; industrial biotechnological processes
Jan. 18	Zsolt Harsanyi	E. F. Hutton 1 Battery Place (12th floor) New York, NY 10004	212/742-7470	biotechnology; high technology investments
Jan. 20	Harvey Price	Industrial Biotechnology Assoc. 2115 E. Jefferson St., Suite 504 Rockville, Maryland 20852	301/984-9599	association management; biotechnology
	Asger Langlykke	Genex 6110 Executive Blvd. Rockville, MD 20852	301/770-0650	biotechnology - fermentation process technology
Jan. 26	Charles Cooney Arnold Demain Anthony Sinskey Daniel Wang James Weaver	Massachusetts Institute of Technology Cambridge, MA 02139	617/253-3108	industrial microbiology; biotechnology process engineering; biologic measurement technology
Feb. 18	Riley Housewright	American Society for Microbiology 1913 I Street, N.W. Washington, DC 20006	202/833-9680	association management; microbiology
	Robert Stevenson	American Type Culture Collection 12301 Parklawn Drive Rockville, MD 20852	301/881-2600	culture collection director; microbiology; hybridoma technology
	Juan Menjivar	Bethesda Research Laboratories 8717 Grovemont Circle P.O. Box 6009 Gaithersburg, MD 20877	301/840-8000	biotechnology process engineering

Table 6.1 (continued)

1982 Meeting Date	Organization Representative(s)	Address	Telephone	Field(s) of Interest
Feb. 18	Morris Danzig Raffaele Bernetti Jonathan Mielenz Fred Armbruster	CPC International Moffett Technical Center P.O. Box 345 Summit-Argo, IL 60501	312/458-2000	biotechnology; biomass fermentation; enzymology; food processing
Mar. 11	William Riley William Dowd Robert Hefner	Dow Chemical U.S.A. Bio-Products 1701 Building Midland, MI 48640	517/636-5243	organic chemicals; biotechnology; process engineering
Mar. 15	Oskar Zaborsky	National Science Foundation 1800 G Street, N.W. Washington, D.C. 20550	202/357-9606	immobilized enzymes; biomass conversion
Apr. 2	Clifford Hesseltine Robert Detroy Morey Slodki W. L. Wang Robert Silman	Northern Regional Research Center Fermentation Laboratory U.S. Department of Agriculture Peoria, IL 61601	309/685-4011	microbiology; fermetation technology; biomass conversion; biotechnology process engineering
Apr. 8	Charles C. McDonald Robert M. Busche	Central Research & Development Experimental Station E.I. DuPont DeNemours & Company Wilmington, DE 19898	302/772-3933	organic chemicals; biotechnology; biosciences research planning
Apr. 19	William T.H. Chang Howard H. Weetall Wayne Pitcher	Corning Glass Works Sullivan Science Park Corning, NY 14830	607/974-4393	biotechnology; immobilized enzymes; hybridoma and cell culture technology; biosystem marketing
Apr. 26	Zachary S. Wochok L. Edward Klein F. B. Zienty George Glover Ned Seigle	Corporate Research & Development Monsanto Company 800 North Lindbergh Blvd. St. Louis, MO 63167	314/694-8643	biotechnology; immobilized enzymes; agriculture and plant cell research; health care research

and 9 of this report will describe possible NBS responses to industry needs should the Bureau decide to develop certain capabilities in this area in addition to its on-going competency development efforts.

.a Standards and Measurement Needs

.1 Raw Material Substrates/Feedstocks. Lignocellulosic materials and hydrolyzed starch are the two biomass feedstocks that are being seriously considered as substrates in industrial processes for the production of commodity organic chemicals. Reference standards for these and other substrates are inadequate. Usually, only a functional definition of a substrate is available. For both of the above substrates, but especially for lignocellulose materials, basic information for the development of standards is limited. To develop SRMs for lignocellulose and major chemical components of lignocellulose (cellulose, hemicellulose and lignin), these feedstocks need to be characterized on a molecular level and appropriate assay systems need to be developed. In addition, questions of lignocellulose source heterogeneity, of source lot variability, of feedstock storage, and of pretreatment and isolation methods need to be addressed in developing SRMs. Municipal and bio-wastes will probably not serve as feedstocks for industrial production of organic chemicals via fermentation. However, according to some industry spokepersons, NBS experience in developing standards in this area could assist in developing standards for lignocellulosic materials or other substrates used as feedstocks for biotechnological processes. On the other hand, some industry spokespersons indicated that because of the unique, specific, and usually proprietary substrate/feedstock requirements for an industrial process, feedstock standard reference materials may not be required.

.2 Biocatalysts/Enzymes. Most of the enzymes used in commercial processes are obtained from microbial sources, are extracellular, and are impure. Thus, one of the major problems in the commercial use of enzymes in biotechnological processes is that one must deal with product (enzyme) functionality rather than with product composition and homogeneity. This problem will become even more acute as enzyme or biocatalytic systems assume greater industrial process and commercial significance in the future.

In general, buyers of commercial enzymes, with the possible exception of those in the clinical chemistry diagnostics area, are not sophisticated regarding the possible benefits of standard methods for measuring enzyme activity. Although standardization of enzyme activity appears reasonable in academic laboratories, this capability and the extensive supporting literature has not yet been transferred effectively to the commercial sector. A possible exception may be the producers of "enzyme tools" (restriction endonucleases, ligases, etc.) for rDNA research.

Currently, there is considerable variation between commercial enzyme producers not only in the methods of enzyme production (for example, different substrates and microbial strains) to produce the same enzyme but also in the analytic approaches to measuring enzyme activity. For the buyer of "bulk enzymes," this makes difficult the accurate comparison of the relative cost of enzymes of specific activities from different sources. According to some industry spokespersons, examples of enzymes where standards are needed include carbohydrases, proteases, lipases, and pectinases.

Standards of enzyme activity would be helpful to buyers by establishing the equivalency of products used in commerce. However, it is uncertain whether the respective proprietary positions of enzyme producers, mostly foreign owned companies, would limit the market impact of NBS development of standard assay procedures and SRMs in this area.

.3 Microorganisms and Cell Cultures. Currently, the American Type Culture Collection (ATCC) and USDA's Northern Regional Research Laboratory are major organizations responsible for serving as repositories of microbial cultures and for maintaining culture collections. Additional manpower and resources are required to maintain and to expand these culture collections. This will become even more critical in the future as patented rDNA microorganisms are deposited in these culture collections and as microbial cultures, both wild type and genetically modified, become more important as articles of commerce.

According to industry spokespersons, the standardization and characterization of microbial strains in culture collections is vital to industry plans for expansion into and investment in biotechnology. Some industry spokespersons

suggested that the responsibility for support of this "national resource" should reside in the Department of Commerce, since this activity would provide generic, infrastructure support to the new biotechnology industry.

Also, as microorganisms become more important as articles of commerce, buyers will need some assurance that they are obtaining not only the microbial strain they are seeking, but also the appropriate passage of the microorganism that will express certain well documented morphologic and biochemical characteristics, and, possibly with rDNA organisms, verifiable genetic sequences. According to industry spokesperson, well characterized and standardized measurement and analytic methods for testing and certifying the similarities and differences between microorganisms at the molecular level will become more important in the future. For example, methods will be needed for evaluating the degree of homology between gene sequences of patented rDNA procaryotic or eucaryotic cells involved in patent disputes.

In addition to microorganisms, advances in cell culture technology (animal and plant) will provide additional industrial alternatives for the production of organic chemicals. The establishment of cell culture repositories (cell banks), the characterization of cell cultures in cell banks, and the on-line availability of cell culture data will assume increasing importance in industry's plans for developments in biotechnology. Investment of resources and development of standards and of evaluated data sources in this area will also be required.

.4 Proteins and Nucleotides. As the biotechnology industry grows, the commercial demand profile for specific enzymes (for example, endonucleases), for peptides (for example, as synthetic nutrients), and for oligonucleotides (for example, as gene sequence linkers and probes) will shift from academia to industry. Currently, a number of manufacturers produce these products, and little has been done in the way of developing measurement methods or standards for assuring the buyer of product purity, equivalency, etc. In addition, analytic techniques and measurement methods for assessing these newer articles of commerce are varied. Existing techniques for comparing products from different firms are either complex or lacking.

.5 Biopolymers/Polysaccharides. With increased interest in applications of biotechnology, microbial biopolymers/polysaccharides will have increased importance as articles of commerce as lubricants, food additives, surface sealants, controlled substance release vehicles, surfactants in tertiary oil recovery, etc. However, progress has been slow in developing measurement methods, standards, and evaluated data sources for polysaccharides including standarized nomenclature and analytic and measurement techniques for batch comparisons. To overcome these barriers limiting the expanded role of polysaccharides as articles of commerce, efforts in the following areas are needed: (a) critically evaluated information base on biopolymer/polysaccharide characteristics including thermodynamic properties, (b) standardized measurement techniques; (c) tests for product purity and for certification of product equivalency; and (d) SRMs.

.6 Public-Private Sector Discussion of and Consensus on Standards. In addition to establishing closer working relations with industry scientists and with technical associations such as the Industrial Biotechnology Association (IBA) and the American Society for Microbiology (ASM), it was suggested that some other actions could be taken by the Bureau to facilitate the public-private sector consensus process necessary for the development of effective measurement methods and standards in biotechnology. For example, NBS and NSF could collaborate in a workshop or a workshop series on the necessity and advisability of developing standards for biomass or other biotechnology raw materials. (The Bureau has already offered technical support on standards in this area with the ASTM. In addition, the Bureau could offer technical support to such groups as the Enzyme Ad Hoc Committee or other voluntary standards organizations. Bureau scientists, in cooperation with international technical organizations, have begun discussions on approaches to developing international standards in this area, for example, biothermodynamics--Inter Union Commission on Biothermodynamics of the IUPAC-IUB-IUPAB.)

.b Fundamental Science and Information Needs

.1 Enzymes. .a Enzyme Characterization. Industry spokespersons believed that the Bureau's base of expertise in physical and analytic chemistry,

microcalorimetry, materials science, instrumentation and catalysis would be helpful in providing a fundamental science base for better understanding of the parameters of enzymatic reactions and of biocatalyst immobilization in industrial biotechnological processes. The extent to which enzymes or biocatalysts are used in biotechnological processes will depend on the purity of the enzyme and on the development of appropriate measurement methods. Examples of types of information that would be useful to industry in characterizing enzymes under different conditions include activation energies, molecular conformation of the active site, molecular and thermodynamic aspects of binding to and cleaving of substrate, kinetics, efficiency of enzyme systems, molecular attachment to and catalytic properties in association with polymers (immobilized systems), and evaluations of isolation and measurement methods.

In addition to benefiting existing bioprocesses, the development of basic information on enzymatic processes, on enzyme active sites and conformational relationships, and on models of enzyme action will be of importance to industry's development of modified or synthetic enzymes tailored for specific and possibly novel industrial bioprocesses in the future. Fundamental information on enzyme structure-function relationships will assume even greater importance to industry in the longer-term future, since rDNA technique will be able to modify specific enzyme molecules and, thereby, change enzyme function.

.b Enzyme Catalogue. In addition to Methods in Enzymology, an information source or catalogue of known enzymes that would centralize critically evaluated data on catalytic reactions; thermodynamic and kinetic reactions; enzyme properties, activities, and characteristics; standardized assays and measurement methods; international units for the conversion of specific substrates; methodology to determine purity; properties of isoenzymes; enzymes requiring cofactors, etc. would be useful in the long-term for the application of enzyme technology to industrial biotechnological processes. Such an on-line information system would be particularly helpful in developing predictive models and in evaluating chemical and biological process alternatives for product synthesis. For immobilized biocatalysts, some industry spokespersons indicated that handbook information on diffusibility and orientation, on rates of reaction, on biocatalyst half-life and support systems, and on approaches for catalyst rejuvenation would also be important for optimizing industrial processes.

.2 Fermentation. A major industrial consideration in fermentation processes is the amount of water in the reaction vessel. Economically feasible industrial bioprocesses for the production of organic chemicals will require fermentation process improvements so that reactions can take place in reduced water environments. In addition to the general lack of critically evaluated information on reduced water and/or "solid substrate" fermentation processes, there is also, according to industry, a general lack of information on properties of "slurry" streams of biomass substrate on fermentation process parameters (flow characteristics, power for agitation, shear forces, mass transport of gases, thermodynamics and product heats of formation, cell density, foaming), as well as on subsequent separation of the product from the reaction vessel, on cell recycle, and on biocatalyst recovery.

.3 Separation and Extraction. Bioprocesses usually involve producing desired end-products in lower concentrations than those obtained via other process routes. In scaling-up, substantial difficulties will be encountered in separating desired products from the fermentation milieu. The traditional distillation approach is quite energy intensive, and, therefore, expensive. Thus, newer separation technologies that are amenable to the scale-up and other requirements of industrial processes will have to be developed. According to industry spokespersons, considerable input from materials science research will be required for developing improved and durable commercial separation processes, as well as processes that will allow fermentation process steps to be coupled to and be compatible with chemical synthetic steps. Evaluation of newer separation and extraction procedures will be essential prior to industry investment in capital intensive biotechnological processes. There will be a need for accurate data on thermophysical properties of alternative separation approaches, on phase diagrams of constituents of mixtures, on properties of various solvents, and on predictive models of properties of separation approaches and of possible range of applications. Membrane, liquid chromatography, super critical CO_2 were some of the separation technologies mentioned by industry spokespersons that may be applicable to large-scale industrial bio-processes.

.4 Process Monitoring and Control. Development of real-time, continuous process monitoring and control instrumentation and probes will be important for the expansion of industry use of biotechnological processes (for example, continuous fermentation and cell culture). Although there are a number of indirect approaches to measuring such process parameters as O_2, CO_2, pH, viscosity, diffusivity, etc., little has been developed for the continuous measurement of biological products and secondary metabolites in complex fermentation broths, for monitoring microbial cells, and for monitoring plant and animal cell cultures. Examples of instrumentation development needs mentioned by industry spokespersons include: (a) non-specific biomass sensors, (b) substrate sensors with high sensitivity and rapid response times, (c) and sensors that can measure production of a specific chemical in a complex reaction environment.

.5 Chemical Engineering. In addition to the specific chemical engineering examples cited above, general experience in chemical engineering will be essential to the successful scale-up of industrial biotechnolgoical processes. According to industry spokespersons technological improvements in biotechnological process design and control will be important to the economic feasibility of bioprocess approaches for the production of organic chemicals. A fundamental understanding of critical scale-up parameters, of the kinetics of production, and of fermentation processes will be required.

7. NBS RESEARCH CAPABILITIES RELATED TO BIOTECHNOLOGY

With the possible exception of activities in (1) clinical chemistry, (2) analytical measurements, and (3)dental biomaterials development and evaluation, NBS is not involved in areas specifically related to biotechnology. However, the Bureau does have fundamental expertise and capabilities in the non-biological sciences that may relate to long-range industry infrastructure support needs in biotechnology.

To determine what this expertise is and where these capabilities reside in the Bureau, an assessment of current NBS research activities was undertaken. The objective was to identify areas of fundamental research strength and traditional NBS missions and roles that could possibly relate to industry needs in biotechnology. Table 7.1 summarizes the results of this assessment. Biotechnology-related research capabilities are dispersed throughout the technical centers of the Bureau. Organizationally, the National Measurement Laboratory (NML) contains most of the Bureau's fundamental research capabilities in the non-biological sciences that potentially relate to: (a) future industry needs in biotechnology, and (b) the development of a core of biotechnological expertise. Specifically, the Centers for Chemical Physics, for Analytical Chemistry, and for Materials Science provide a base of scientific, measurement and standards expertise in physical chemistry, organic chemistry, analytical chemistry, polymer sciences and related fundamental disciples, as well as instrumentation technology that appears, based upon discussions with industry (Section 6), to be particularly important in industrial biotechnology process development.

Potential also exists within the National Engineering Laboratory (NEL), specifically the Center for Chemical Engineering (CCE), to transfer to industrial biotechnology problems some of the expertise they have and are acquiring in flow dynamics, modeling of unit operations, slurry and continuous process technology, process control sensing systems, and separation technology. Examples of industrial problems relevant to CCE expertise include: (a) characterization of and process engineering design requirements for slurries of biomass substrate, and (b) measurement and evaluation of alternative extraction

Table 7.1

Identification of NBS Generic Research Capabilities Potentially Related to Industry Biotechnology Needs

NBS-wide Research Activity Areas[a]	NBS GENERIC RESEARCH CAPABILITIES Complementary to Core Biotechnology Competency Initiative	Performer(s)	NATIONAL MEASUREMENT LABORATORY (500)	Abs. Phy. Quant. (520)	Rad. Res. (530)	Chem. Phys. (540)	Anal. Chem. (550)	Matl. Sci. (560)	NATIONAL ENGINEERING LABORATORY Chemical Engineering (770)
Chemical Analysis (17)	o Quantitative molecular analysis of complex mixtures by laser molecular spectroscopy;	DeVoe, Haurilla**					X		
	o Instrumentation and analytical methodology (electrochemistry, neutron activation analysis, spectroscopy) and fundamental studies of high level sensitivity/ acccuracy multielement analysis;	Garner					X		
	o Measurement of organic constituents in environmental matrices, for example, biowaste;	Garner					X		
	o Ion, laser, electron probe microanalysis	Newbury, Etz					X		
	Enzyme/protein chemistry; bioanalytical characterization; stabilization studies;	Reeder					X		
	o High resolution spectrometry/chromatographic techniques for identifying and quantifying organic compounds in complex mixtures;	Hertz, Chesler, Margolis, May					X X		
	o NMR spectroscopy of carbohydrates, steroids and other small biological molecules;	Coxon, Davidson**					X		
	o Aerosol and particulate characterization and micro analysis (X-ray fluorescence, non-linear optical spectroscopy, etc.) for improved control of industrial processes;	Pella, McKenzie, Rook, Chabay					X		
	o Development of advanced organic electrochemical methodology for basic studies of reaction kinetics, mechanisms of complex reactions, and interfacial phenomena--for example, catalysis; provide thermodynamic and kinetic data base;	Durst, Bunding**					X		
	o Chemical and physical microprobe analysis and characterization, including organic tomography, of particles;	Newbury, Downing**					X		

Table 7.1 (continued)

NBS-wide Research Activity Areas[a]	NBS GENERIC RESEARCH CAPABILITIES Complementary to Core Biotechnology Competency Initiative	Performer(s)	NATIONAL MEASUREMENT LABORATORY (500)	Abs. Phy. Quant. (520)	Rad. Res. (530)	Chem. Phys. (540)	Anal. Chem. (550)	Matl. Sci. (560)	NATIONAL ENGINEERING LABORATORY Chemical Engineering (770)
Chemical Kinetics (18) and Thermochemistry/ Thermophysics (99)	o Chemical kinetic information at molecular level and thermodynamics research of, for example, reactions involving aromatic, heterocyclic cellulosic compounds, and aqueous solutions;	Hule, Herron, Scheer, Martinez, Abramowitz				X			
	o Phase behavior of complex mixtures in industrial processes;	Morrison							X
	o Development of methods for understanding mechanisms of hydrocarbon transformation and of data base of key processes	Tsang				X			
	o Ultrasonic techniques for NDE	Linzer						X	
	o Thermodynamics and spectroscopic studies of small molecules; reaction calorimetry, thermodynamics of biochemical processes	Abramowitz, Goldberg				X			
	o SRM's for clinical chemistry/diagnostics	Rasberry	X						
	o Temperature sensing techniques	Mangum		X					
	o Laser chemistry and study of ion-molecule reactions	Leone		X					
	o Physico-chemical properties of glass; immobilization of enzymes on glass surfaces for catalysis, micro-analytic sensors	Haller						X	
	o Adsorption, desorption, and catalytic processes on surfaces	Madey, Egelhoff				X			
	o Laser techniques to study chemical reactions on pico-second time scale	Stephenson, King, Shapiro				X			
	o Chemical thermodynamic properties of organic compounds	Domalski				X			
	o Thermometry in resource recovery	Reilly		X					
	o Continuous industrial processes; new/efficient separation methods, for example, membrane processes	Hord							X
	o Thin film sensor (temperature, humidity) technology	Dils							X
	o Solution thermodynamics and structure	Abramowitz				X			
	o Aqueous solution thermodynamics	Wasik				X			
	o Solution kinetics	Hule				X			
Atomic/ Molecular Spectroscopy (05)	o Quantum mechanics of complex organics	Krauss				X			

Table 7.1 (continued)

NBS-wide Research Activity Areas[a]	NBS GENERIC RESEARCH CAPABILITIES Complementary to Core Biotechnology Competency Initiative	Performer(s)	NATIONAL MEASUREMENT LABORATORY (500)	Abs. Phy. Quant. (520)	Rad. Res. (530)	Chem. Phys. (540)	Anal. Chem. (550)	Matl. Sci. (560)	NATIONAL ENGINEERING LABORATORY Chemical Engineering (770)
Clinical Chemistry (57)	o Development of enzymatic assay procedures and of more accurate clinical chemistry analytical methodology	Hertz					X		
Corrosion (28)	o Quantitative measurement for molecular characterization of chemical basis for species specific biodegradation/biotransformation	Brinckman, Iverson						X	
Ionizing Radiation (65)	o Radiation and free radical and kinetic chemistry; model systems in biological electron transport	Simic			X				
Neutron Scattering/ Analysis (73)	o Measurement (light scattering detector for gel permeation chromatography, neutron scattering) of diffusion in, degradation of, and interface properties of polymers.	Smith, McCrackin						X	
Polymer Characterization (81)	o Polymer SRM's; specification and quality control; thermodynamics of polymers	Sanchez, Amis**, Lodge**						X	
	o Phase transitions in polymer physics and self-assemble processes (e.g., membranes) in biology	Dimarzio	X						
Biomaterials (58)	o Performance of polymer materials and modeling; substitute identification; conformational properties (Raman scattering)	Fanconi						X	
	o Microstructural analysis and NDE&T of biological materials (neutron diffraction, radiographic methods)	Rush, Wlodawer						X	
Reference Data (85)	o Data prediction methods	Garvin				X			
	o Basic data center								
	-thermodynamics	Garvin, White				X			
	-electrolytes	Staples, White				X			
	-kinetics	Harron, Gevantman				X			

Table 7.1 (continued)

NBS-wide Research Activity Areas[a]	NBS GENERIC RESEARCH CAPABILITIES Complementary to Core Biotechnology Competency Initiative	Performer(s)	(500)	NATIONAL MEASUREMENT LABORATORY Abs. Phy. Quant. (520)	Rad. Res. (530)	Chem. Phys. (540)	Anal. Chem. (550)	Matl. Sci. (560)	NATIONAL ENGINEERING LABORATORY Chemical Engineering (770)
Health/ Medical (56)	o Polymer durability, characterization of polymer material porosity; adsorption of polymer on surfaces	Cassel, Dehl						X	
Recycling/ Materials Substitution (84)	o Organic analysis of recycled materials	Hertz					X		
Surface Science (97)	o Surface characterization (spectroscopy, sputtering, etc.); measurement of surface composition to study mechanisms in surface reactions, for example, catalysis	Fine				X			
Fluid Mechanics (46)	o Fluid dynamic modeling; flow/fluid mechanical measurements; mixing in reacting systems; shear flows	Whetstone, Klebanoff, McMichael							X
Instru-mentation (64)	o Process measurement and control; sensing systems	Whetstone							X

**Post-doctoral

[a]Based upon task information system keywords.
No tasks categorized as 07 (biotechnology).

techniques for the separation of specific biological molecules from relatively aqueous reaction mixtures. In addition to the CCE, the Center for Electronics and Electrical Engineering has expertise that could contribute to bioprocess sensing systems and to the on-line electronic control of industrial biotechnological processes.

Although the preceeding discussion illustrates that the Bureau has several bases of technical strength that could relate to industry needs in biotechnology, it is also apparent that there are a number of gaps in the Bureau's fundamental capabilities related to biotechnology.

Should the Bureau decide to invest its resources to provide infrastructure support to industrial applications of biotechnology, in addition to on-going competency development efforts, NBS needs to acquire or develop scientific capabilities in the following areas: enzymology, molecular biology/biophysics, microbial/cell biochemistry, and bioprocess engineering. In order to effectively link existing in-house Bureau scientific expertise and capabilities with future biotechnology needs of industry related to the Bureau's mission, the Bureau will need to begin developing, over the next few years, core expertise in fundamental biosciences. This will allow the Bureau to: (a) sustain the dialogue that has already begun with industry in biotechnology, (b) accurately and effectively tailor Bureau responses to industry needs, and (c) develop the core science base for collaboration with and, to some degree, aggregating existing Bureau research capabilities related to biotechnology. This competency development process will also provide the in-house technical credibility necessary for a sustained Bureau presence in this area.

8. DEVELOPMENT OF NBS KNOWLEDGE BASE AND CAPABILITIES RELATED TO LONG-TERM INDUSTRY NEEDS IN BIOTECHNOLOGY

In this section of the report, NBS scientists and technical staff* identify nine possible NBS research initiatives and services that might be undertaken to provide infrastructure support for long-term industry needs in biotechnology. In general, these technical descriptions by NBS staff focus on two areas: (a) fundamental science (knowledge development) opportunities and possible roles for NBS; and (b) possible areas where NBS knowledge base and services could be applied to long-term industry needs in biotechnology as these needs relate to the Bureau's mission.

The identification of these technical areas was influenced by industry's perceived needs in biotechnology in relation to the mission of the Bureau (Section 6), and by existing Bureau scientific and service capabilities (Section 7). Bureau scientific staff in providing input to this section of the report were influenced not only by industry needs and by the scientific opportunities in areas related to industry needs, but also by Bureau resource limitations and competing demands on existing resources. The summation of these influences is reflected in a cautiously optimistic assessment by Bureau scientific and technical staff.

It is emphasized that the contributions which follow are only examples of a range of possible technical areas where NBS scientific and technical staff might contribute to meet long-term industry infrastructure support needs in biotechnology. Further, these technical descriptions by Bureau staff represent a cross-sectional view of Bureau responses to industry needs for applying the outputs of a dynamic technology. Only through continued interactions between Bureau scientists and industry researchers will industry be better able to articulate its measurement infrastructure support requirements in biotechnology, and NBS be better able to define its role and tailor its response in this area.

* Stanley Abramowitz, Robert Alverez, Sherman Fivozinsky, Ruth Haines, John Herron, Morris Krauss, David Lide, Cedric Powell, Stanley Rasberry, William Reed, Dennis Reeder, Leslie Smith, and George Uriano.

Decisions to allocate Bureau resources to any of the following technical areas must, of course, be made through the Bureau's competitive program and budget process where comparison with other priority areas can occur.

.1 Predictive Models

The structure of bio-organic molecules, such as the active site of enzymes, might be analyzed and even predicted by various molecular quantum mechanics models (306). Ab initio quantum mechanical methods are still not adequate for such large systems (>100 atoms), but the ab initio methods are used to determine the interaction energy parameters and to investigate the reaction mechanism by studying model systems (307). A vast amount of calculating has been done with emphasis on hydrogen-bonding interactions. There is still a need for an accurate yet inexpensive method for calculating the van der Waals interactions. The inclusion of pseudopotentials permits the inclusion of heavy atoms which play an important role at the active site of many enzymes. Accurate pseudopotentials that also include relativistic efforts were recently developed and their use will certainly become more widespread.

Using the hydrolysis of polysaccharides by lysozyme or the hydration of CO_2 by carbonic anhydrase as guiding reactions, quantum mechanical methods could be developed at NBS for applications to the following problems:

- Determination of the energy surface describing the transition complex for reaction at the active site of an enzyme by parametrizing the ab initio energy surface for models of the active site and incorporating parametric representation into classical methods of calculating the conformations of ground state enzymes in order to extend methods to the reaction complex.

- Determination of the effect of solvation on changing the character of a gas phase reaction by using the multi-configuration self-consistent-field method including analytic evaluation of gradients with particular attention given to (a) the "catalytic" effect of metal ions, and (b) systematic calculation of reaction surfaces for classical organic reactions to provide background for catalytic calculations.

- o Critical analysis and evaluation of present methods of calculating conformations, energies, and spectra of intermediates important in those enzymatic reactions that possibly will be of industrial process significance.

- o Development of new methods for calculating the van der Waals interaction between molecules.

Development of these fundamental research capabilities and data resource within the Bureau will allow, in the long-term, for the exploitation of predictive models in evaluating on a molecular level biocatalytic reactions that might be useful in industrial biotechnological processes.

.2 Microanalysis of Complex Mixtures in Bioprocess

Use of highly sensitive analytical techniques utilizing enzymes as reagents has been a slowly developing field until the past four years when the techniques of immobilization of enzymes began to be used extensively (230, 308, 309). Enzymes that were normally free or water-soluble were immobilized or made insoluble by either entrapment in a matrix or by covalent bonding of the enzyme to a solid phase. Enzymes thus bound showed retention of activity for long periods of time. For example, a single sample of immobilized glucose oxidase has been used for several thousand determinations of glucose in samples.

Widespread use of immobilized enzymes and a proliferation of automated instruments that utilize this technology are expected to be of particular importance in the field of clinical chemistry in the next five to ten years (310, 311). Limitations and technological barriers are mainly related to the understanding of the mechanisms of optimal binding of the enzyme to solid phase supports and to practical ways of further protecting the activity of the bound enzyme.

The role of NBS relative to this technology could be several fold. First, from an analytical standpoint, gaining familiarity with immobilized enzymes as analytical reagents is an imperative. One cannot understand the problems of the

technology until an intimate working knowledge of the strengths and pitfalls of the use of these reagents is gained. Second, improvements in reagent sensitivity and selectivity can be made if fundamental knowledge is gained on the mechanism of enzyme-substrate interactions, the conformation of the enzyme active site, and the proper amino acid residues that may be used in covalent binding for optimal preservation of activity.

Of the current applications of immobilized enzymes, one of the most interesting is in the use of enzyme electrodes. The coating of electrodes with an organic polymer containing immobilized enzymes yields an electrochemical sensor that may be useful in applications where sample preparation has been a major problem. By eliminating extraction and clean-up steps, better analyses will be feasible.

Development of more reliable sensors, understanding conditions for immobilizing enzymes, and providing materials definition for reagents required in the binding steps are all applications that will be needed by industry in a variety of biotechnological applications.

.3 Thermodynamics of Bioprocesses

One of the fundamental questions faced in all chemical processing is that of knowing the energetics of the chemical reactions involved. Traditional work in thermodynamics has focused on hydrocarbons, inorganic materials and aqueous solutions of simple electrolytes. Bioprocesses on the other hand involve complex molecule and electrolyte interactions. Today there are several calorimetric, theoretical, and separation techniques available which can be successfully modified and used to obtain the thermodynamic properties important in bioprocesses. Four of these are summarized here.

Biological calorimetry is presently mostly involved with scanning calorimetric studies of reactions. These measurements are made on complex reacting systems and do not in general yield precise and accurate heats of formation ($\Delta_f H$) of biological substances (312). Precise and accurate $\Delta_f H$ of

biological materials are needed. In particular measurements of heats of combustion ($\Delta_c H$) and heats of solution ($\Delta_s H$) are needed so that heats of formation are available for the aqueous phase. Key measurements of purine and pyrimidine bases and nucleosides could be made at NBS so that correlation methods for estimating these parameters could be developed. Similar correlations could be made for heat capacities (Cp) and entropy (S) so that equilibria could be predicted for various temperatures. This activity once developed could be coordinated with a data evaluation effort to extend the 1971 NBS compilation of thermodynamic properties of compounds containing carbon, hydrogen, nitrogen, oxygen, phosphorus and sulfur.

During the last decade NBS developed state-of-the-art apparatus for microcalorimetry. These techniques were applied to problems as diverse as heats of cell division, heat output due to the internal resistance of batteries, and enzyme catalyzed reactions of serum. Today microcalorimetry is being used for some biothermodynamic studies. Equilibria of enzyme catalyzed reactions could be studied at NBS by using the methods of microcalorimetry. This technique could also be extended to the measurement of rates of enzyme catalyzed reactions.

With the advent of gas chromatography (GC) and high pressure liquid chromatography (HPLC) the solubility and partition coefficients of trace organics in the aqueous and condensed phases can be measured. In other programs at NBS these techniques have been developed for environmental concerns. These techniques could now be extended by NBS to biological systems. Measurements made at other than room temperature will allow the determination of heats of solution by this method, an important quantity for industrial biotechnological process design. Comparison with established (time consuming) calorimetric studies could be made in order to develop rapid techniques for measuring heats of solution.

Gibbs Energy maps are of importance in determining product yields for biochemical reactions. These maps are contour diagrams which show the composition of complex mixtures as a function of pH and metal ion concentration. To date all these maps have been produced assuming ideal solutions (313, 314).

Using expertise already established at the Bureau in the aqueous thermodynamics of electrolytes and coupling this expertise with recent developments at NBS on an equilibrium approach to the computation of activity coefficients, thermodynamic properties and compositions of complex mixtures could be constructed using these maps to more accurately reflect the non-ideal solutions found in biochemical systems. Specifically, the maps for the several energy transfer reactions of importance to biochemical systems are among the ones which could be constructed.

This information base would serve as a valuable resource to industry applications in biotechnology by allowing development of predictive models of alternative biotechnological approaches and by improving industry predictive capabilities on process economics.

.4 Chemical Kinetics of Enzyme Reactions

The chemical kinetics of enzyme reactions is a well developed discipline in terms of measuring overall enzyme rate constants (or enzyme activity) and determining the effect of pH and temperature on reaction processes.

Studies of the detail of the processes, for example the nature of the transient intermediates or specific configuration transformations at the enzyme catalytic sites, are made extremely difficult by the requirement that we monitor what is happening in some small isolated part of an extremely large molecule possessing thousands of individual bonds. There is thus a requirement for site-specificity and transient analysis capability.

At the forefront of the methods being applied to this problem is resonance Raman spectroscopy which has been successfully used to detect enzyme-substrate transient species, and which has great promise for carrying out time resolved studies on these transient intermediates (315, 316). Another method which has potential is the method of extended X-ray emission fine structure spectroscopy (EXEFS). Extended X-ray absorption fine structure (EXAFS) spectroscopy has already been applied to biochemical systems (317). It provides information on the structure of the enzyme system. EXEFS provides more direct information on

the environment of the valence electrons and thus potentially provides direct chemical information concerning the active site.

A third method potentially applicable to enzyme systems is the laser induced lanthanide ion luminescence probe technique (318). This approach is applicable to systems containing metal ions and involves substitutions by the lanthanide ion and its laser induces luminescence. The luminescence spectra provide information on the nature of metal-coordinated water molecules, characterization of bonding sites, and other data on enzyme-substrate interations.

Strengthening existing NBS competences to apply and to extend these techniques and to provide time-resolved site-specific chemical information on enzyme-substrate interactions will be a valuable resource for industry to draw on in optimizing biotechnological process. As an initial step in enhancing NBS capabilities in this area, laser enhanced Raman spectroscopy and the EXEFS methods could be used to study systems ranging from simple organic compounds to enzyme catalyzed reactions.

Along with the development of experimental strategies, the Bureau could begin an overall evaluation of the status of chemical kinetic data compilation needs in this area.

.5 Surface and Interface Characterization

The characterization methods of surface science are being widely utilized for a large variety of problems of both scientific and technical importance (319). Surface analysis, in particular, is now an integral part of many technologies and industries (e.g., catalysis, coatings, corrosion, semiconductor devices, computer, automobile, communications) for many different applications (e.g., failure analysis, quality control, process and device development). Surface properties and processes are similarly important in many areas of public concern (e.g., defense, energy, health, environment).

Surface analysis has been used in recent years to characterize chemical treatments of teeth and wool fibers; similarly, surface analysis has been utilized for assessments of implant materials (e.g., the presence of toxic metals on the surfaces of plastics) and for determining corrosion mechanisms and the durability of implanted prostheses. It is believed that these types of applications will grown and be extended to a wider range of materials (e.g., composites, rubbers, adhesives, plastics), properties (microbial corrosion, stress corrosion, biocompatibility, toxicity, adhesion, wear, immunological response), and processes (compatability, controlled porosity, artificial organs).

There is a growing interest and demand for improved methods of interface characterization (320). For many scientific and technical applications including those listed above, it is desirable to measure the composition and other properties of solid-solid, solid-liquid, and solid-gas interfaces with the sensitivity and specificity now available for surface characteriztion (i.e., usually a solid-vacuum interface). Ideally, interface characterizations should be obtained in situ or non-destructively as artifacts can frequently occur when one phase is removed to expose the interface region.

Bureau expertise in surface and interface characterization techniques could be applied to several problems in industrial applications of biotechnology. One area where surface and interface effects are critical is bioattachment. A variety of physical, chemical, and biological methods are being applied to study the attachment of bacteria to solid surfaces, molecular film adsorption on solids from a bacterial culture, adhesion of cells to collagen and plastics, and the adhesion of proteins to polymers. Development of new and evaluation of existing methods for their industrial process applicability appears essential.

Another area of high potential impact is the characterization of lipid membranes. Theory has been developed recently to describe the interaction of van der Waals molecules with dielectric films. From this it might be possible to determine how the transport of macromolecules through membranes could be controlled and/or facilitated. This could in turn lead to improvements in the separation of specific biological molecules from complex reaction mixtures.

.6 Separation and Analytic Techniques

The main method currently being used in biotechnology for separating and analyzing protein and recombinant DNA fractions is gel electrophoresis and associated techniques. Recent developments in this area have created a great deal of interest in establishing data banks based on the information that can be derived from these separations.

Examples of recent developments in this field include the reporting of new staining methods that have extended the sensitivity of the analytical gel techniques to detect proteins and peptides at sub-nanogram quantities, with detection limits nearing that of autoradiography. Better computer algorithms and advanced concepts of graphic display have helped to handle the large amount of data generated by gel electrophoresis, particularly two-dimensional methods.

Future developments in analytical gel technology may include the interfacing of high-performance liquid chromatography (HPLC) to analytical gel separation and detection. The newer HPLC gel-filtration columns have capabilities of performing initial separations of proteins. Further separation of components in HPLC peaks by the combination of isoelectric focusing and polyacrylamide gel electrophoresis in the presence of a detergent, should provide another order of separation, characterization and measurement of important biomolecules.

NBS has a possible role in the research and development of these techniques. Capabilities are being developed in order to establish protein marker compounds that will help standardize the two-dimensional electrophoresis technique. Use of HPLC in the materials definition phase should provide an additional dimension to the separations procedures.

Application of these results could provide a basis for standardization of results between laboratories involved in biotechnology-related research activities. Extension of the data to include sequencing of recombinant DNA would require minor reconfiguration of the electrophoretic equipment and development of staining methods to detect DNA/nucleotide patterns.

.7 Separation Technologies -- Membranes

Efficient separation techniques developed for industrial biotechnological processes will be critical to the economic success of these processes. Exisitng separation methods will have to be tailored to the separation requirements of large-scale industrial process (for example, the production of commodity organic chemicals via biotechnological processes, or the coupling of biological and chemical synthetic process steps). Membrane separation methods are attractive for industrial processes of this type, and advances in membrane technology will have a significant influence on the development and implementation of commercial processes.

NBS has considerable expertise and fundamental research capabilities in polymer science, microscopy, structure determinations in polymer systems, and synthetic polymers. A possible opportunity for the Bureau could be to use this base of expertise for developing a fundamental understanding of membrane measurement and characterization techniques in aqueous solutions in order to better understand the effect of processing variables on membrane structure and performance. Information generated in these areas would begin to provide a scientific basis to otherwise empirical approaches in membrane separation technology.

In addition to these approaches, NBS could develop fundamental research capabilities for studying: (a) the permeability of polymer films, (b) the molecular structure and morphology of membrane structures, (c) membrane selectivity and performance, (d) aging processes in membranes, and (e) the interrelationships between these research areas.

Development of information in this area would be of particular interest to industry in evaluating separation technology alternatives for and in the design of biotechnological processes.

.8 Standard Reference Materials (SRMs)

If past NBS experience with developments in other emerging technologies (for example, analytical chemistry) and industries of national importance (for example, health care-clinical laboratory testing) serves as a reliable guide, the need for Standard Reference Materials (SRMs) in the industrial applications of biotechnology is certain.

Because of the dynamic state of this technology and of the rapidity of technological advances in this area, it is not clear at this time what specific SRMs should be developed to meet the long-term needs of industry in its application of biotechnologies. To a considerable extent, Bureau development of SRMs in this area will depend upon development of Bureau scientific and technical capabilities and expertise in areas related to biotechnology, as well as upon outside scientific expertise to guide SRM program efforts and resource investment decisions.

Several initial steps, however, could be undertaken by the Bureau in order to identify and evaluate industry's specific needs for SRMs in biotechnology. These could include:

- o an education effort by NBS with prospective industry clients to explain the need for accurate and reliable measurements and SRMs as one vehicle for improving industry process operating efficiency and also for improving quality control over intermediate and/or final products;

- o encouragement by NBS for industry participation in setting SRM planning priorities in biotechnology; and

- o evaluation of the applicability/compatibility of existing (or currently planned) Bureau SRMs with measurement technology needs of industrial bioprocesses (for example, temperature; calorimetry; pH, basimetry, acidimetry; sugars; CO_2 and O_2 off-gas analysis; rheology; and light scattering).

In the longer-term, a compatibility must exist between the measurement technology and the SRMs that are developed in biotechnology. Since measurement technology specifically applicable to industrial process applications in biotechnology is either lacking or only in its formative stages, SRM development must follow development in measurement technology areas. Specific industrial biotechnology SRM needs will become more apparent when: (a) measurement systems needs to be "functional", (b) the optimization of process parameters becomes critical to efficiencies and yields, and (c) well-defined trade specifications are developed for end products as commodities or articles of commerce. However, some possible targets of opportunity for Bureau SRM development could include SRMs applicable to the following:

- measurement of the initial and half-life activity of enzymes and enzyme systems used in industrial biotechnological process;

- determining the "dimensional specificity" of industrial bioprocess filtration techniques using aerosal/particulate dimensional metrology;

- development of standard feedstocks/substrates or of organic constituents of substrates used in industrial bioprocesses;

- development of standard reaction pre-tests;

- determining the purity of products (for example, proteins, peptides, and polysaccharides) from biotechnological processes and quantifying impurities within products; and

- filling in the existing gaps for thermometry SRMs covering temperatures at and just above room temperature (for example, 3-5 new fixed point SRMs might be developed covering the range from 29.77°C, the existing gallium melting point SRM, up to about 85°C; two SRMs are currently under development that would provide fixed points in this range).

.9 Standard Reference Data (SRD)

As industrial biotechnological processes become more operational, the need for critically evaluated data will be essential in order to predict the effectiveness and the efficiency of the processes.

The Bureau's Standard Reference Data Program supports a number of major data base development activities which could match expanding industrial biotechnology concerns (321). Existing programs in chemical thermodynamics, chemical kinetics, transport properties, phase diagrams, as well as other areas, could be expanded to supply important input to the industrial use of bioprocesses.

The SRD program would significantly expand its substance coverage to enable response to information requirements on the complex chemicals and processes associated with projected industrial applications in biotechnology. SRD outputs could consist of compilations of selected properties of important pure substances, predictive methods for properties of mixtures and other hard-to-measure data, and computer-readable data bases for use in industrial bioprocess models.

In the long-term, as industrial bioprocesses are further developed and biological mechanisms become well characterized, the SRD Program might expand its scope to include evaluation and compilation of more purely biological data.

The temporal progression of an SRD activity directed at industrial biotechnology evaluated information needs might be viewed in three steps:

- Initially, selected compilations could provide evaluated data in the traditional areas of SRD expertise which overlap important biotechnology needs. Rate constants of enzymatically-controlled reactions and thermal and transport properties are examples.

- Predictive methods could be constructed to provide needed properties which have not been measured over the required range of process parameters; for

example, the thermodynamic properties of substrates for enzymatically-controlled reactions and properties of the mixtures found in process streams.

- Comprehensive computer-readable data bases could be made available for use in major industrial bioprocess predictive models.

9. NBS ROLE IN SUPPORT OF INDUSTRY NEEDS IN BIOTECHNOLOGY

This section of the report outlines a number of steps the Bureau could take to provide infrastructure support to industry needs in biotechnology. The selection of these steps for possible Bureau action takes into consideration biotechnology developments and trends (Section 5), industry perception of needs in relation to NBS's mission (Section 6), existing Bureau capabilities (Section 7), and Bureau scientific and technical staff ideas on research initiatives and services that could be undertaken or developed to provide infrastructure support for industry needs in biotechnology (Section 8).

Table 9.1 provides a framework for possible Bureau action and identifies a range of scientific, measurement and standards opportunities for NBS that are specifically related to long-term industry needs in biotechnology. To implement the action items identified in Table 9.1, considerable, but not exclusive, reliance would be placed on expanding and/or refocusing existing Bureau capabilities in physics, chemistry, materials/polymer science, chemical engineering, measurement techniques and instrumentation, standards, and evaluation and management of technical information. To complement existing Bureau capabilities in the short-term, staff expertise in enzymology would be required. In the intermediate to longer-term, expertise in molecular biology/biophysics and microbial/cell biochemistry and eventually in bioprocess engineering would be required.

To effectively address many of the problems related to industry needs in biotechnology, interdisciplinary scientific approaches would be necessary. Should the Bureau decide to implement part or all of the framework illustrated in Table 9.1, Bureau management would have to provide a climate that would encourage interdisciplinary cooperation and collaboration among Bureau scientists and technical staff.

Table 9.1

Areas of Scientific, Measurement, and Standards Opportunity
for NBS in Biotechnology

NBS ACTIVITIES	ACTIONS AND/OR OUTPUTS SHORT TERM (1-2 YEARS)	INTERMEDIATE TERM (2-5 YEARS)	LONG TERM (5-15 YEARS)
Standard Reference Material (SRM)	o Organize workshop(s) on characterization of and standardization requirements for bioprocess feedstocks/substrates o Interact with industry (domestic and foreign) on SRM needs for industrial bioprocess o Use currently available methodology and readily prepared SRMs (phosphorus and nitrogen compounds, certain organic chemicals, certain sugars, calorimetry standards)	o Develop and evaluate markers (pI, molecular weight) for separation methods (e.g., 2-dimensional electrophoresis) o Develop SRMs to meet short-term critical measurement problems (temperature, dimension specificity of filtration techniques, initial and half-life of enzymes) o Determine applicability of rDNA products to replace some existing SRMs	o Provide definitive standards which meet major requirements of the biotechnology industry (proteins, peptides, nucleic acids, enzymes, bioprocess feedstocks/substrates, product purity)
Evaluated Data; Standard Reference Data (SRD)	o Update selected current compilations of critically needed kinetic, thermochemical, and transport properties o Expand international cooperation in data compilation and evaluation in biotechnology o Interact with industry to assist in setting priorities	o Develop an integrated experimental, theoretical data evaluation program to deal with the interdisciplinary information needs associated with industrial applications of biotechnology o Develop predictive techniques for properties of mixtures o Refine theoretical approaches to begin predictive properties of biosystems (for example, Gibbs Energy Maps) o Establish kinetic data bank on fundamental biochemical reactions o Develop base of membrane selectivity and maximum flux data	o Develop information base on membrane performance characteristics in polymer/protein systems o Use prediction schemes to generate data of known accuracy for biotechnology needs o Provide computer-readable comprehensive, evaluated data bases for industrial bioprocess modeling

Table 9.1 (continued)

NBS ACTIVITIES	ACTIONS AND/OR OUTPUTS		
	SHORT TERM (1-2 YEARS)	INTERMEDIATE TERM (2-5 YEARS)	LONG TERM (5-15 YEARS)
Measurement/Analytic Techniques & Instrumentation	o Develop measurement techniques for study of membrane selectivity in aqueous solutions	o Standardize 2-dimensional electrophoresis reagents and materials	o Develop/evaluate methods for determining/mapping gene (DNA) structure and homology between gene (DNA) sequences
	o Understand and measure variables in determining membrane structure and processing characteristics	o Understand and measure influence of techniques (spacer-arm, ligand technology) for manipulation of enzymes on surfaces	o Provide rapid analysis techniques for proteins, nucleic acids and glycoproteins
	o Extend thermodynamic techniques (solution calorimety, bomb calorimetry, microcalorimetry) to biosystems	o Measure key bioprocess parameters via sensing and other techniques	o Develop techniques and model systems for studying the interactions between proteins and surfaces; immobilized biocatalytic systems
	o Develop optical methods and picosecond measurements for characterizing the activities and structural changes of biological molecules in solution	o Measure in aqueous systems the morphology of complex membrane structures and of composite membranes	o Characterize and develop techniques for characterization of surfaces of immobilized biocatalytic systems
	o Expand solution kinetic measurement capabilities	o Develop techniques for measurement of polymer absorption and conformation on low energy surfaces	o Develop solution kinetic technology for measuring rates of reaction
	o Determine applicability of storage technology to long-term cell culture maintenance	o Draw on gas-solid phase experience for development of protein-surface interface and membrane characterization techniques which operate in situ and non-destructively	o Understand non-aqueous technologies and their applicability to biotechnological processes

Table 9.1 (continued)

NBS ACTIVITIES	ACTIONS AND/OR OUTPUTS SHORT TERM (1-2 YEARS)	INTERMEDIATE TERM (2-5 YEARS)	LONG TERM (5-15 YEARS)
	o Review measurement technology in conjunction with existing standards in areas (temperature, rheology analytic techniques/standards) that could relate to industrial biotechnology needs	o Adapt solution kinetic techniques to biological systems o Determine applicability of slurry and continuous process technology to industrial biotechnological process o Organize workshop on standardization of 2-dimensional electrophoresis	o Determine applicability of microelectronic technology to sensing systems and to on-line bioprocess monitoring and control
Expanding knowledge Base; Non-proprietary, Generic Research	o Organize workshop series on: - biomolecules in analytic chemistry - separation and extraction technologies for industrial bioprocesses - analytic applications of membrane technology - on-line process monitoring and control of industrial biotechnological processes - protein-surface interfaces - flow dynamics in industrial bioprocesses o Study permeability of polymer films and relation to molecular structure o Determine applicability of gas phase expertise to study of hydrogen bonding in liquid phase (high resolution spectroscopy, raman spectroscopy) o Determine applicability of quantum mechanics methods to biological systems o Acquire enzymology expertise by direct hire	o Determine relationship of morphological structure to molecular structure and processing variables o Evaluate structure determination technique(s) interface with kinetics, thermochemistry and theory for detailed study of a key enzyme catalyzed reaction o Study weak interactions and their applicability to biological systems o Acquire molecular biology/biophysics and microbial/cell biochemistry expertise by direct hire o Encourage industry participation in NBS Research Associates Program	o Determine relationship between polymer adsorption and membrane performance o Study aging processes in membranes (fouling, pore structure collapse) o Study molecular relaxation mechanisms in polymers o Study and develop fundamental models of key systems which relate enzyme structure to function and properties o Develop predictive models of unit operations in industrial biotechnological process o Physically and chemically characterize prokaryotic and eukaryotic cells o Acquire bioprocess engineering expertise by direct hire

Finally, Table 9.1 reflects an implementation strategy based upon a current but dynamic technology. Therefore, it will be necessary for Bureau scientific, technical and management staff to evaluate this approach periodically versus industry infrastructure support needs over the life cycle of this technology. Further, the action items listed in Table 9.1 do not extend the Bureau's mission into the biological sciences but rather concentrate on NBS strengths in the chemical, physical and engineering sciences, and on traditional NBS lines of communication with the private sector.

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Glossary*

Aerobe. An organism that requires air or free oxygen to maintain life processes.

Anaerobe. An organism that does not require air or free oxygen to maintain its life processes.

Amino Acid. An organic compound that contains one or more basic amino groups ($-NH_2$) and one or more acidic carboxyl groups (-COOH). They may be polymerized to form peptides (short chains of amino acids) and more complex forms (proteins) that are joined together in a strictly ordered sequence which determines the primary (sequence), secondary (chain folding), and tertiary (3-dimensional) structure. There are 20 common amino acids.

Antibody. A protein that is a component of the immune system. It originates either normally or in response to a foreign substance or antigen, and is characterized by a specific reactivity with its complementary antigen.

Antigen. A molecule, usually a protein or polymeric carbohydrate, which when introduced in the body stimulates the production of an antibody that will react specifically with it.

Biomass. Includes many plant species, various animal wastes, and concentrated masses of unicellular organisms. It can serve as a feedstock for the production of organic chemicals.

Biosynthesis. The production of a chemical compound by a living organism by either synthesis or degradation.

Biotechnology. The collection of industrial processes and of technologies that involve the use of biological systems. Some of these processes may involve the use of genetically engineered microorganisms.

Carbohydrates. The family of organic molecules consisting of simple sugars such as glucose and sucrose, and sugar chains (polysaccharides) such as starch and cellulose.

Catalyst. A substance that alters the velocity of a chemical reaction and remains essentially unaltered in form and amount at the end of the reaction.

Cell culture. An in vitro method of propagating cells from animal, plant, and insect tissues; technique can be applied to the production of complex proteins, polysaccharides, and hormones.

*Sources. Impacts of Applied Genetics, Office of Technology Assessment, Washington, D.C., 1981; "Summary of Genetics Briefing," Congressional Clearinghouse on the Future, April 23, 1980; Dictionary of Scientific and Technical Terms, Second Edition, McGraw-Hill, 1978.

Cell fusion. The integration of the components of two or more cells to become a single cell; represents a pooling of genetic information that would not occur under natural circumstances.

Cellulase. A group of extracellular enzymes produced by microorganisms and some lower animals that hydrolyze cellulose to simpler sugars such as anhydro-glucose.

Cell membrane. A thin layer of protoplasm, consisting mainly of lipids and proteins, which is present on the surface of all cells (plasma membrane). This cell structure is involved in regulating the flow of substances into and out of cells.

Cell permeability. The permitting or activating of the passage of substances into and out of cells through the cell or plasma membrane.

Cellulose. A polysaccharide composed entirely of several glucose units linked end to end; it constitutes the major part of cell walls in plants.

Clone. An individual organism derived asexually from a single cell through mitosis. Phenotypic or genotypic homogeneity is usually but not always a characteristic of the resulting population.

Coenzyme. A nonprotein portion of an enzyme that can be associated at the active site of an enzyme. It is a prosthetic group which functions as an acceptor of electrons or functional groups (for example, nicotinamide adenine dinucleotide-NAD).

Cytoplasm. The protoplasm of a cell, external to the cell's nuclear membrane.

DNA (deoxyribonucleic acid). The genetic material found in all living organisms. DNA is composed of four deoxyribonucleotide building blocks (nitrogenous base + sugar + phosphate). The nitrogenous bases of DNA are paired: adenine (A) + thymine (T) and guanine (G) + cytosine (C). The base pairs are joined by hydrogen bonds. The four bases form the alphabet of the genetic code. The sequence of each base in a linear array along the sugar-phosphate backbone of DNA determines the information content of the gene. Every inherited characteristic has its original somewhere in the code of each individual's complement of DNA.

Enzyme. A functional protein (e.g., protein with a tertiary structure and active site) that catalyzes a specific chemical reaction without being affected by the reaction. Enzymes are the active agents in fermentation processes, and control the rates of reaction in the vast majority of chemical reactions in all living organisms.

Eukaryote. A higher, compartmentalized cell characterized by its extensive internal structure and the presence of a nucleus containing the DNA. All multicellular organisms are eukaryotic. The simpler cells, the prokaryotes, have much less compartmentalization and internal structure; bacteria and some algae are prokaryotes.

Feedstock. The raw material furnished to a particular process.

Fermentation. The process of transformation by enzymes or microorganisms of large molecular weight organic substrates (or feedstocks), especially carbohydrates, to lower molecular weight chemicals. It is used in various industrial processes for the manufacture of products such as alcohols, acids, and certain dairy products (such as cheese) by the action of yeasts, molds, and bacteria.

Gene. Basic hereditary unit; a sequence of DNA which codes for a specific protein.

Genetic code. The biochemical basis of heredity consisting of codons (nitrogenous base triplets along the DNA sequence, e.g., ATA, GAT, AGA, etc.) that determine the specific amino acid sequence transcribed and eventually translated into proteins. The genetic code is a universal code for all forms of life studied so far.

Genetic engineering. A technology used at the laboratory level to alter the hereditary apparatus of a living cell so that the cell can produce more or different chemicals, or perform completely new functions.

Gene mapping. Determining the relative locations of different genes on a given chromosome.

Genotype. The genetic constitution of an individual or group.

Hybridoma. The cell product of the fusion of two different types of cells which possesses new combinations of properties inherited from both distinct parent cells. For example, in the prepartion of monoclonal antibodies, one parent is a cell (lympocyte) which produces a specific antibody. The other parent is a tumor cell from a myeloma, a cell which can propagate indefinitely ("immortal") in cell culture. Once these two parent cells are combined/fused, a hybridoma (hybrid melanoma) is formed that after cloning and selection, can propagate indefinitely in cell culture and can product large amounts of monoclonal antibody. This antibody recognizes only one specific antigen.

Hydrocarbon. Compounds composed of carbon and hydrogen, and are commonly found in petroleum, coal and gas.

In vitro. Outside the living organism and in an artifical environment.

In vivo. Within the living organism.

Lignocellulose. A group of substances in woody plants consisting of cellulose, hemicellulose, and lignin.

Liposome. Synthetic microscopic phospnolipid vescicle or carrier that can encapsulate individual cells, enzymes, antibiotics, etc. and can serve as controlled substance release vehicles and as vehicles for transferring genetic material into plants.

Metabolism. The sum of the physical and chemical processes involved in the maintenance of life and by which energy is made available.

Mitochondria. Structures in the cytoplasm of higher cells that serve as the "powerhouse" for the cell, producing chemical energy.

Monoclonal antibodies. Antibodies derived from a single source or clone of cells (for example, a hybridoma) which recognize only one kind of antigen.

Mutants. Organisms whose visible properties with respect to some trait differ from the norm of the population due to mutations in its DNA.

Mutation. Any change that alters the sequence of bases along the DNA, changing the genetic material.

Nucleic acid. A polymer composed of DNA or RNA subunits.

Nucleotides. The fundamental units of nucleic acids. They consist of one of the four bases--adenine, guanine, cytosine, and thymine (uracil replaces thymine in the case of RNA)--and its attached sugar-phosphate group.

Organic compounds. Chemical compounds composed of carbon and hydrogen, and also may contain oxygen, nitrogen, and various other elements.

Phenotype. The sum total of the expression of morphological characteristics (and the manifestation of physiological, enzymatic, etc. processes) of an organism. Represents the output from the interaction of an organism's genotype with its environment.

Plasmid. Hereditary material that is not part of a chromosome. Plasmids are circular and self-replicating. Because they are generally small and relatively simple, they are used in recombinant DNA experiments as acceptors of foreign DNA, and as vectors for insertion of foreign DNA into organisms.

Polymer. A long-chain molecule formed from smaller repeating structural units.

Polysaccharide. A long-chain carbohydrate containing many molecules of simple sugars linked together; examples would include cellulose and starch.

Prokaryote. Cells of bacteria or blue-green algae which are characterized as being rather small, having a single chromosome that is not enclosed by a nuclear membrance, and lacking cytoplasmic organelles.

Protein. A linear polymer of amino acids; proteins are the products of gene expression and are the functional and structural components of cells.

Protoplast. Cell without a wall (for example, certain enzymes such as pectinases and cellulases can dissociate tobacco leaves into living but wall-less plant cells).

Protoplast fusion. A means of achieving genetic transformation by joining two protoplasts or joining a protoplast with any of the components of another cell.

Recombinant DNA. The hybrid DNA produced by joining pieces of DNA from different sources.

Restriction endonuclease. Enzyme isolated from a varity of bacteria recognizes and degrades DNA from foreign organisms, thereby preserving the genetic integrity of the bacterium. In recombinant DNA experiments, restriction endonuclease enzymes are used as tiny biological scissors to cut up foreign DNA before it is recombined with a vector. These enzymes have a unique specificity for one specific nucleotide sequence.

Saccharification. The conversion or enzymatic hydrolysis of more complex sugars (e.g., disaccharides-sucrose) to simpler sugars (e.g., monosaccharides-dextrose and levulose).

Vector. A transmission agent; a DNA vector is a self-replicating DNA molecule that transfers a piece of DNA from one host to another, for example, a plasmid.

NBS-114A (REV. 2-80)

U.S. DEPT. OF COMM. **BIBLIOGRAPHIC DATA SHEET** (See instructions)	1. PUBLICATION OR REPORT NO. NBSIR 82-2549	2. Performing Organ. Report No.	3. Publication Date July 1982

4. TITLE AND SUBTITLE

NBS AND INDUSTRIAL BIOTECHNOLOGY: TECHNICAL DEVELOPMENTS AND FUTURE MEASUREMENT NEEDS

5. AUTHOR(S)

Dr. Thomas C. O'Brien

6. PERFORMING ORGANIZATION (If joint or other than NBS, see instructions)

NATIONAL BUREAU OF STANDARDS
DEPARTMENT OF COMMERCE
WASHINGTON, D.C. 20234

7. Contract/Grant No.

8. Type of Report & Period Covered

9. SPONSORING ORGANIZATION NAME AND COMPLETE ADDRESS (Street, City, State, ZIP)

10. SUPPLEMENTARY NOTES

☐ Document describes a computer program; SF-185, FIPS Software Summary, is attached.

11. ABSTRACT (A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here) Biotechnology will be a significant industrial technology in the future. NBS's role with respect to this technology is examined; and infrastructure support requirements needed by future biotechnology based industries are identified in this report. This report also describes steps NBS could take to meet future industry infrastructure support needs in biotechnology by: examining commodity organic chemical industry trends; identifying R&D opportunities and barriers to commercialization of biotechnology products; and evaluating current NBS capabilities in relation to long-term industry needs.

Report conclusions indicate that: (a) the commodity organic chemical industry will undergo structural changes in the next two decades; (b) early applications of biotechnology will be in higher value added products; (c) "traditional" commodity chemicals synthesized from petroleum feedstocks will be difficult to displace via biotechnological process; (d) biotechnology offers significant opportunities for production of "non-traditional" commodity organic chemicals; (e) biomass may become an important feedstock for production of organic chemicals; and (f) existing NBS capabilities could meet many of industry long-term infrastructure support needs in biotechnology.

This report provides a framework for possible short- and long-term actions NBS could take to meet industry needs in biotechnology. Report includes an extensive bibliography.

12. KEY WORDS (Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)

Biomass conversion R&D; bioprocess engineering; biotechnology; chemical industry trends/strategies; commodity organic chemicals; measurement/evaluated data needs; NBS research capabilities.

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14. NO. OF PRINTED PAGES

180

15. Price

$16.50