14 PLANNING REPORT

NBS and Industrial Biotechnology:

Instrumentation and Associated Measurement Needs

Thomas C. O'Brien, Ph.D.

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Executive Summary

There is considerable scientific and commercial enthusiasm over the diversity of products that could be produced using the "new" biotechnologies. Markets most likely to be impacted by research and developments in biotechnology are pharmaceuticals; diagnostics, food additives, specialty chemicals, mineral recovery, and waste processing. Less attention has been given to: developments in biotechnology instrumentation and industrial instrumentation needs, opportunities for new applications of existing instrumentation in biotechnology, and applications of new instruments for bioprocess intensification and for bioprocess efficiency improvement.

This report describes some initial steps NBS could take to identify: industrial biotechnology's instrument needs and associated measurement-related problems, areas where there will likely be an industry underinvestment in resolving these needs/problems, and the appropriate research and service activities NBS could undertake in order to be responsive to some of this industry's infrastructure technology needs. The report accomplishes this by: (a) examining biotechnology instrumentation trends; (b) identifying specific instrumentation measurement-related R&D barriers and opportunities; and (c) providing examples of NBS scientific capabilities related to industrial biotechnology instrument needs and instrument development directions.

Report Conclusions

- Biotechnology instrumentation is a highly competitive and rapidly evolving market with several existing manufacturers and with many potential new market entrants. Although the overall instrument market is large, market projections for individual categories of instruments are relatively small. Major instrument market growth is projected for molecular and elemental analytical instruments, centrifuges, microscopes and balances.

- Regarding biotechnology market projections, some consistency is needed in defining biotechnology in order that more accurate data can be collected and systems for the collection of relevant data can be established. Consistency and comparable data are important because they are the basis for market projections and evaluations of technology and competitiveness impacts.

- Monitoring and control technologies, and separation technologies are two technology areas that will affect biotechnology instrumentation developments, and will make important contributions to bioprocess efficiency improvements and ultimately to the economic success of biotechnology outputs. Examples of process monitoring and control technologies emphasized in this report are biosensing electrodes and luminescence assay systems. Two examples of separation technologies highlighted in this report are flow cytometry and continuous flow electrophoresis. Discussed in this report are specific measurement-related problems associated with each of these technology areas, and the possible use of these technologies in an industrial setting. NBS scientific and service capabilities that could possibly address some of the measurement related problems identified have been outlined in this report.

- As a result of rapid developments in the biotechnology instrumentation industry and the many companies involved, the industry as a whole has not yet articulated what it perceives as the major measurement-related problems facing it in the commercialization and industrial use of the instruments it has or will produce. Thus, although NBS has some specific capabilities that could address some of the industry's measurement-related problems, it is difficult to identify specific research and service actions the Bureau could pursue at this time. However, this report suggests that the Bureau can take several steps now that will begin to identify the key measurement-related problems in biotechnology instrumentation requiring a future NBS response.

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1. REPORT OBJECTIVE

This is the fourth report* in a series of National Bureau of Standards (NBS) Planning Office reports on potential NBS contributions to biotechnology.

The most recent of these reports took a broad view of technology developments and trends in industrial biotechnology and identified several possible areas for NBS research and service activities in this field related to its mission (1). This report is not intended to be as comprehensive as the previous report. However, this report does focus on a subject area (biotechnology instrumentation trends and needs) not addressed in the previous report. Furthermore, this report provides examples (process monitoring and control technologies and separation technologies) of technologies that could have significant influences on biotechnology instrumentation developments, and identifies some possible measurement-related opportunities for NBS action that relate to industrial biotechnological process optimization. Specifically, this report:

- o lists instrumentation and equipment associated with biotechnology R&D;
- o provides market projections through 1990 on biotechnology instrumentation and equipment;
- o identifies certain instrumentation technological trends that may have significant impacts on industrial biotechnology process optimization needs: and
- o identifies biotechnological instrumentation measurement needs and possible NBS roles.

As with previous Planning Office Reports, this report will not define an operational plan or identify resource requirements. However, this report will serve as a guide for NBS scientist as they evaluate existing Bureau programs and develop new initiatives. In addition, this report will be useful to NBS managers as they identify research and service priorities, evaluate competence development projects, and plan for the allocation of available 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.

O'Brien, T. C., Planning Report 12: NBS and Industrial Biotechnology: Technical Developments and Future Measurement Needs. National Bureau of Standards, Department of Commerce, July 1982.

2. INTRODUCTION

.1 NBS and Biotechnology

The primary mission of the National Bureau of Standards is to provide a systematic basis for uniform and accurate measurements throughout the United States by means of precision measurement, evaluated data, measurement methods, and related services. In addition, NBS provides technical assistance to Federal agencies for solving high priority national problems that are measurement- and standards-intensive.

Within this context, NBS has been involved in a planning process related to biotechnology since 1980. The purposes of this process have been to: (a) identify barriers or problems in biotechnology related to commercial production of products; (b) project long-range biotechnology industry infrastructure technology needs; (c) identify NBS capabilities related to those needs consistent with the Bureau's mission; (d) anticipate biotechnology industry needs for NBS services in the future; and (e) establish a policy framework for future Bureau actions.

In order to be responsive in a timely manner to industrial biotechnology measurement related needs, the Bureau has developed a policy framework for Bureau research and service activities related to industrial biotechnology. It has begun to develop an internal knowledge base in this field through: (a) direct Bureau support of competence development initiatives in such areas as enzyme catalysis, two-dimensional electrophoresis, and condensed phase chemistry; and (b) interactions of Bureau technical staff with appropriate private and public sector organizations. These competence areas are exploratory efforts that will assess the prospects for longer-term Bureau involvement in such areas as:

- o understanding structure-function relationships in enzyme catalysis, emphasizing kinetic and thermochemical approaches primarily and theoretical (quantum and statistical mechanisms) calculations secondarily;

- o characterizing (in real-time) biological surface interactions and biotransformations of substrates at an elemental level;

- o improving the characterization of technologically important proteins:

- o assuring greater uniformity of data collected in technologically and industrially important biological reactions;

- o developing and standardizing analytical reagents that use bioactive molecules; and

- o providing key thermodynamic and other data that will be needed in the design design and optimization of important bioprocesses.

.2 Structure of the Report

The remaining sections of this report provide an overview of biotechnology R&D instrumentation and some examples of measurement related problems associated with certain instruments that may relate to Bureau research and service activities.

Section 3 collects and analyzes some projections on instrumentation requirements for biotechnology R&D, and discusses some inadequacies in the accuracy of and methods for data acquisition and analysis for industrial biotechnology.

Section 4 provides specific examples of instrumentation needs and associated technical developments that will have important implications for optimization of industrial biotechnology processes, e.g., process monitoring and control and separation technologies. Measurement related problems and possible NBS roles are also discussed.

Section 5 summarizes some comments by Bureau laboratory scientists on the application of NBS research and service capabilities to address some of the needs and opportunities illustrated in Section 4.

Section 6 discusses what steps NBS is taking or could take to strengthen its base capabilities in order to respond to industrial biotechnology needs within the context of the Bureau's mission.

3. BIOTECHNOLOGY INSTRUMENTATION MARKET PROJECTIONS

.1 Market Estimates for Biotechnology Products

Biotechnology is not a commercial phenomenon born in the late 1970s. As stated in a recent Scientific American article, . . .

> (Biotechnology) . . ."is not just a new field of entrepreneurial activity; it is a well-established factor in the world economy, responsible for a current annual production valued at . . . billions of dollars in the U. S. alone. Moreover, it is the outgrowth of a pervasive human activity with a rich history that goes back thousands of years . . ." (2).

This statement refers primarily to two industrial biotechnology application categories (craft applications and application of classical microbial genetic techniques), and not necessarily to the "new" biotechnology that includes or will include commercial applications resulting from the use of genetic manipulation techniques (for example, recombinant DNA and protoplast fusion), of large-scale animal and plant cell cultures, and of novel separation technologies. Microbial mineral leaching provides one example of the current economic importance of craft applications of biotechnology. In 1981, 25 percent of the copper produced in the United States was by microbial leaching (3). In reference to the application of classical microbial genetics to industrial processes in the 1960s and 1970s, there were two primary impacts. First, classical microbial genetic techniques allowed for significant bioprocess efficiency gains. This was particularly evident in the production of antibiotics. Second, these techniques increased the economic attractiveness of large-scale fermentation processes for the production of a wide range of products (for example, amino acids). Table 1 provides 1981 estimates for the global sales of fermentation products. These estimates indicate that the United States represents about 40 percent of all sales of fermentation products, and that secondary metabolites (for example, antibiotics and vitamins) represent about 50 percent of current global sales.

TABLE 1. SALES IN FERMENTATION PRODUCT MARKET - 1981*
($ Millions)

PRODUCTS	SALES UNITED STATES	SALES FREE WORLD
Primary Metabolites		
Alcohols (ethanol)	75	200
Organic Acids	400	870
Amino Acids	65	660
Nucleotides and Nucleosides	--	75
SUBTOTAL	$540	$1,805
Secondary Metabolites		
Vitamins	110	290
Antibiotics	1,433	2,990
SUBTOTAL	$1,543	$3,280
Polymers		
DNA and RNA Research Products	2	2
Proteins	248	600
Emulsifiers and Gums	75	175
Pesticides	15	30
SUBTOTAL	$340	$807
Living Cells		
Yeast	250	700
Starter Cultures	7	18
Soil Innoculants	10	15
Other Innoculants	8	10
SUBTOTAL	$275	$743
GRAND TOTAL	$2,698	$6,635

*S. King, Remora Associates, Palo Alto, CA (December, 1982).

It is expected that the "new" biotechnologies will continue to spur the demand for existing products (for example, amino acids and antibiotics) by improving upon industrial bioprocess efficiency gains made to date, and will open up new markets by increasing the number and diversity of products available. However, it is difficult to obtain accurate information on the future market value of biotechnology products. Table 2 provides some global estimates of the market impacts of this technology. The information in this table illustrates that: (a) this technology already contributes significantly to our economy; (b) this technology has the potential to make even greater and more cross-industry-sector contributions to our economy in the future; and (c) current estimates on the future economic impacts of this technology vary widely.

.2 Biotechnology Research Instrumentation

Table 3 illustrates the major capital equipment needed to conduct various types of biotechnology R&D (rDNA, hybridoma/monoclonal antibody, cell culture, fermentation, and core). This table does not reflect some of the specific equipment requirements for the industrial scale-up of systems developed in R&D laboratories. Much of the instrumentation and equipment listed in this table is relatively conventional. Even for some of the more sophisticated instruments listed (e.g., electrophoresis, chromatography, spectrophotometers, etc.), techniques for use are fairly well established. However, it can be expected that instrument suppliers will add some hardware and software ("bells and whistles") to meet some of the specific analytical requirements of biotechnology (4).

Exceptions to the above are recent instrumentation developments with oligonucleotide and polypeptide synthesizers (5, 6). These instruments, still not without performance problems, will have significant impacts on solid phase chemical instrumentation and on the timing of research outputs and technical developments in the field. It can be expected that there will be further developments and incremental improvements in the performance of these instruments. Ultimately, these and similar instruments will provide molecular biology's response to industrial automated flexible manufacturing processes.

TABLE 2. BIOTECHNOLOGY SALES (GLOBAL) PROJECTIONS

SECTOR	1979-1980 (EST.)[a]	PROJECTIONS FOR 1995-2000 (BILLIONS $) Genex[b]	T.A. Sheets[c]	IRD[d]	Chicago Pol. Res.[e]	Predicasts[f]
HEALTH CARE	4.0	2.4*	9.1	--	--	--
AGRICULTURE	2.0	4.0	8.6	--	50.00	103.3
CHEMICALS	1.8	25.0	10.6	--	--	--
FOOD PROCESSING	1.7	4.4	12.7	--	--	--
ENERGY/MINERALS	1.2	4.2	16.4	155.0	--	--

*Estimate based on partial listing of health care products.

References:

[a]Scientific American, September 1981; Impacts of Applied Genetics, OTA, April 1981.

[b]Chemical Weekly, April 27, 1982, p. 122.

[c]Eur. Chem. News, March 15, 1982, p. 17.

[d]Genetic Technology News, September 1981, p. 7.

[e]Genetic Technology News, July 1981, p. 6.

[f]Inside R&D, November 3, 1982, p. 2.

TABLE 3

MAJOR CAPITAL EQUIPMENT REQUIREMENTS FOR BIOTECHNOLOGY LABORATORIES*

rDNA

- o Laminar flow biohoods (total exhaust)
- o Large capacity refrigerators
- o Walk-in cold room (0°)
- o Freezers (-20°C)
- o Centrifuges:
  - preparative/collection
  - high speed
  - ultracentrifuge
- o pH meter
- o Balance
- o Scintillation counter
- o Gamma counter
- o Gel electrophoresis
- o Gel scanner
- o HPLC
- o Amino acid analyzer
- o Peptide/protein sequencer
- o Oligonucleotide synthesizer
- o Spectrophotometers (UV/VIS, IR)
- o UV monitor recorder
- o Computer/software
- o Microscopes (inverted, fluorescence)
- o Lyophilizer
- o Thin-layer chromatography
- o Water bath (temperature controlled)
- o Shake equipment (tier)
- o Vacuum pump (large)
- o Fraction collector
- o Hoods: radioisotope, fume
- o Ultrafiltration apparatus
- o CO_2 incubators

HYBRIDOMA/MONOCLONAL ANTIBODY

- o Laminar flow biohoods (total exhaust)
- o Microscopes (standard, inverted)
- o Centrifuge (preparative/collection)
- o Ultracentrifuge
- o Incubators: 37°C (walk-in)
- o CO_2 incubator
- o Balance
- o pH meter
- o Liquid N_2 Storage (-270°C)
- o Freezers: -70°C
  -20°C
- o Fraction collector/columns
- o Spinner flask + stirrer
- o Lyophilizer
- o Titrator
- o Containment room
- o Scintillation counter
- o Cell sorter (flowcytometer)
- o Refrigerator (large capacity)
- o Water baths (temperature controlled)

CELL CULTURE

- o Laminar flow biohoods (total exhaust)
- o Microscopes (dissecting, inverted, compound binocular)
- o Centrifuges (preparative/collection)
- o Incubators: 37°C (walk-in)
- o Roller apparatus (continuous)
- o Column chromatography (separate proteins from media)
- o Refrigerator (large capacity)
- o Freezers: -70°C
  -20°C (walk-in)
- o Vacuum pump (large)
- o Lyophilizer
- o pH meter
- o Balance
- o Liquid N_2 Storage (-270°C)
- o Process driers
- o Fermenter (10-20 L; 50 L)
- o Water baths (temperature controlled)
- o Filters (water filtration, cell separation)

*Containment room, laboratory bench space, cabinet and animal facility, reagent and consumable laboratory supply, and office requirements not included.

TABLE 3 (continued)

MAJOR CAPITAL EQUIPMENT REQUIREMENTS FOR BIOTECHNOLOGY LABORATORIES*

FERMENTATION

- o Fermenters (14 L)
- o pH meter
- o Balance
- o Peristolic pumps
- o Recorder
- o Control/monitoring/sensing equipments (pH, CO_2, redox dissolved O_2, temperature, etc. probes)
- o Mass spectrometer
- o Shaking equipment/incubator (controlled temperature)
- o Computer/software
- o Drying oven
- o Flow meter

CORE FACILITY

- o Autoclave and steam generators
- o Drying oven
- o Glassware washer
- o Ice machine (flake)
- o Water supply (5 gal/hr; purified; non-pyrogenic)

Sources: Genetic Engineering News, July/August, 1982.
Flow Laboratories Inc.
Mid-Atlantic Laboratory Equipment Company
Beckman Instruments, Inc.
Scherago Associates
Genex Corporation
Scientific Apparatus Manufacturers Association

*Containment room, laboratory bench space, cabinet and animal facility, reagent and consumable laboratory supply, and office requirements not included.

Table 4 provides a partial listing of some major suppliers of biotechnology instruments. As indicated from listings of scientific instrumentation and equipment suppliers, there are literally hundreds of current and potential suppliers to the biotechnology instrumentation and equipment market (7, 8).

With the considerable emphasis now being placed on biotechnology with the development and marketing of low volume, high value added products, equipment and instrument buyers are concerned with equipment quality and support services primarily, and, to a lesser extent, on equipment price. At this stage in the development of the industry, there is considerable interaction between suppliers, particularly suppliers of more sophisticated instrumentation, and potential users in order for: (a) both parties to maintain an awareness of technical developments, (b) users to articulate their specific instrument requirements, and (c) suppliers to better tailor their response to the specific needs of a sophisticated user community. These interactions and tailoring of instrumentation and equipment to meet industrial needs will be critical to surmounting the numerous problems anticipated in the design, scale-up, control, and optimization of industrial biotechnological processes (9-17)*. In this regard, two areas where significant technical advances in instrumentation will have far-reaching implications for the economic viability of industrial bioprocesses are in sensing/detection systems and in separation technologies. These areas will be discussed in a subsequent section of this report.

.3 Biotechnology Instrumentation Market Estimates

Table 5 presents several projections on future global markets for biotechnology instrumentation. As noted earlier, considerable competition can be expected among equipment manufacturers for these future markets because of the large number of potential equipment suppliers. Although the projected instrument market is relatively large, the market projections for specific categories of instruments are relatively small, particularly when compared with the overall biotechnology market projections in Table 2. From Table 5, separation and analytical instrumentation and equipment appear to be the major markets with significant growth potential. These instruments are measurement sensitive and may require measurement related services such as

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*Cooney, C. L., Science 219:728-733, 1983.

TABLE 4. SELECTED MAJOR SUPPLIERS OF BIOTECHNOLOGY EQUIPMENT

Equipment Type	No. of Companies	Examples of Some Major Suppliers*
Chromatography	64	Pharmacia, Waters, Bio-Rad
Ultracentrifuges	10	Spinco (Beckman), Sorvall (DuPont)
Electrophoresis	27	LKB, Hoeffer
pH Meters	60	Orion
Fermenters	10	New Brunswick, Biolafitte
Membranes & Filters	36	Millipore, Nalge, Pall
Cryogenic & Low Temperature Freezers	49	Revco, Forma
Lyophilizers	16	Virtis
Autoclaves	20	American Sterilizer
Incubators	40	New Brunswick, Napco

Sources: Inside R&D, October 13, 1982.
Analytical Chemistry, August 1982.
AAAS 1982-1983 Guide to Scientific Instruments.

*These companies are mentioned for illustrative purposes, and their listing does not represent an endorsement of their products by NBS.

TABLE 5. BIOTECHNOLOGY EQUIPMENT SALES PROJECTIONS*

CATEGORY	Near Term Estimates (millions)2 1980[a]	1980[b]	1981[e]	1982[d]	PROJECTIONS (1980 constant dollars--millions) 1985[b]	1985[c]	1985[e]	1987[d]	1990[b]	1992[d]
Analytic Instruments										
o Molecular analysis										
- Separative (incl. chromotography)	262.6	362	513	11.7	738	20	1064	18.1**	1345	23.8
- Spectrometric	274.5	225	--	--	361	--	--	--	539	--
o Elemental analysis										
- Spectroscopic	192.7	178	--	--	298	--	--	--	480	--
- Surface analysis	26.9	67	--	--	113	--	--	--	169	--
- Other	382.5[f]	55	--	--	121	--	--	--	259	--
Laboratory Equipment										
o Microscopes	63.3	137	--	--	167	--	--	--	193	--
o Fermenters	--	--	--	4.9	--	37	--	15.0	--	21.0
o Centrifuges	118.5	69	--	67.6	88	20	--	78.7	107	89.5
o Electrophoresis	42.9	--	--	18.4	--	--	--	28.0	--	27.8
o Balances	39.5	94	--	--	158	--	--	--	233	--
o Lyophilizers	--	--	--	--	--	7	--	--	--	--
o Fluid handling	--	39	--	--	60	--	--	--	84	--
o Automated Sequence/Synthesis	--	--	--	6.8	--	--	--	12.2	--	19.2
o Titrators	--	25	--	--	37	--	--	--	52	--
o pH meters	36.3	--	--	--	--	--	--	--	--	--
o Other (incl. tissue culture)	--	18	--	7.7	25	15	--	11.2	34	12.5
Laboratory Appliances										
o Refrigerators/freezers	9.4	46	--	34.5	62	5	--	51.9	79	58.1
o Ovens/furnaces	50.4	22	--	--	27	--	--	--	33	--
o Sterilizers/autoclaves	23	8	--	--	9	5	--	--	10	
o Incubators	36.5	23	--	32.9	32	--	--	45.2	44	51.7
o Baths/Circulators	--	19	--	--	24	--	--	--	30	--
o Hot Plates/Mantles	3.5	19	--	--	23	--	--	--	27	--
o Membranes and Filters[g]	--	--	--	3.5	--	15	--	7.3	--	14.2
o Water Purification	31.5	26	--	--	40	4.5	--	--	59	--
o Other	--	73	--	--	98	--	--	--	125	--
Other Test/Measurement Equipment	--	18	--	--	25	--	--	--	34	--

[a]Selected Instruments and Related Products, Bureau of Census, U.S. Dept. of Commerce, January 1982. No specific designation as to biology or other uses.
[b]Scientific Apparatus Manufacturers Association, 1982.
[c]Chemical and Engineering News, September 6, 1982, p. 22.
[d]International Resource Development, Inc., 1982.
[e]Anal. Chem. 54: 323R, 1982.
[f]Includes some surface analysis instruments.
[g]Annual market size is currently estimated at $550 million (Chemical and Engineering News, November 8, 1982, pp. 7-12).
*Sales--global; public and private sectors.
**Chromatography only.

calibration services and standard reference methods, data and materials. One source estimates that the United States market for biotechnology instrumentation will be one and one-half times greater than the rest of the world (18).

An underlying assumption in these projections is that there will be continued Federal support for university, Federal laboratory, and small business innovation research in the basic sciences at, for example, HHS, NSF, DOE, and, increasingly, USDA, DOD, and NASA in high priority research areas such as molecular biology, biochemistry, genetics and immunology (19-21). Currently, approximately 40-45 percent of R&D expenditures by the leading university research performers are in the life sciences (22, 23). In addition, institutional efforts to upgrade research facilities in these fields will help to sustain current enthusiasm for industry-university links in biotechnology. However, it can be expected that the magnitude and the frequency of these links will moderate in time as industry expands its internal knowledge base and R&D capabilities in biotechnology, and as economic and other forces influence the growth of industrial biotechnology. Thus, it can be expected that the not-for-profit segment of the biotechnology instrumentation market, currently approximated at 55-60 percent, will fall to about 45-50 percent in the longer term (18). Further, the equipment market is rather decentralized presently with the considerable number of university and Government laboratories, and the over 200 new and established firms involved in research in these areas. However, this market may become more centralized and easier to target by suppliers as major recipients of R&D funds expand their research capabilities and subsequently their ability to attract research resources, and as the industry begins to shake-out over the next 3-4 years.

.4 Definition of Biotechnology and Information Needs for Future Market Projections

As illustrated by the tables on market projections for biotechnology, it will be essential for industry, Government, and academia to develop a common working definition of biotechnology. Such a definition will be required in order to establish the framework for:

- better quantifying economic projections:

- developing systems for collecting accurate industrial data and making statistical comparisons on biotechnology product and economic impacts;

- improving the focus of government science and technology, regulatory, etc. policy responses to this technology;

- developing voluntary standards;

- facilitating international agreements; and

- improving domestic and international trade and other buyer-seller relations.

The following are examples of candidate definitions of biotechnology:

- "Integrated use of biochemistry, microbiology, and engineering sciences in order to achieve the technological application of the capacities of microorganisms, cultured tissue, cells, and parts thereof." (24)

- "The collection of industrial processes that involve the use of biological systems. For some of these industries, these processes involve the use of genetically engineered microorganisms." (25)

- "The application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services." (26)

As illustrated from these definitions, biotechnology is a general term and is applied broadly. Among other things, it refers to:

- industrial use (products, processes, and services);

- integration of biology with several scientific and engineering disciplines;

- application of biological systems (microorganisms, plant and animal cells, biocatalysts) to accomplish a variety of functions; and

- use of such tools as genetic engineering (mutation, recombinant DNA, somatic hybridization, etc.) to accomplish industrial objectives.

To complicate our ability to make projections on/or to quantify the impacts of biotechnology, these definitions also imply that there will be cross industry sector applications of this technology. However, this phase in the development of this new industry is an opportune time for Government to work with industry and technical and trade associations to identify the information that should be collected and the analyses that would be important to enhance this industry's market potential, forecasting capabilities, and ultimately its international competitiveness.*

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*Some initial activities to collect more accurate information are being undertaken by an Office of Science & Technology Policy (OSTP) Interagency Working Group on Biotechnology.

4. BIOTECHNOLOGY INSTRUMENTATION DEVELOPMENTS AND ASSOCIATED MEASUREMENT NEEDS

.1 Overview

One important aspect of the vitality of R&D is the practical importance of its applications. Biotechnology is no exception. Laboratory and pilot plant instrumentation developments are essential to maintaining the flow of research finding's through commercial operations to the market place. NBS fundamental research in measurement technology has formed the basis upon which instrumentation has been developed (for example, instrumentation for SO_2 detection)*. NBS, however, is not involved in the development of commercial instrumentation.

The Bureau views the relationship between its traditional measurement related activities and the measurement needs associated with biotechnology instrumentation from several perspectives: First, the Bureau has existing analytical capabilities and active research programs in such areas as bioanalysis, electrochemistry, separation techniques, organic analysis, metal speciation and optics. Second, it is anticipated that there will be an underinvestment by the private sector on those activities and services generic to improved methods of measurement**. Third, NBS believes that the conduct of these activities and services will enhance industry's capabilities to optimize processes and to compete more effectively in international markets. Separation technologies and process monitoring and control technologies are two technological areas that involve instrumentation, are perceived as generic to industrial biotechnological process optimization and to the commercial success of industrial bioprocesses, and are prime areas of opportunity for instrument and equipment development.

Developments in separation technologies will improve opportunities for bioprocess intensification and for isolation and purification of lower volume, higher value added products (for example, protein products from recombinant DNA systems, and glycoproteins, steroids, natural pesticides, artifical sweetners, essential oils, etc. from cell culture systems). Separation technologies will also contribute to maintaining sterility in continuously operating bioprocesses,

*Schwarz, F.P., Kabe, H.O., and Whittaker, J.K., Anal. Chem. 46:1024, 1974.
**Tassey, G., Tech. Forecasting and Soc. Change 21:163-180, 1982.
Tassey, G., Research Policy 11:311-320, 1982.
Nelson, R.R., and Langlois, R.N., Science 219:314-818, 1983.

particularly those involving flow systems and plant, animal and hybridoma cells where maintaining cell viability and vitality and insuring limited variance in cell yields are critical. An example of an approach to maintain long-term system sterility is the use of continuous spin filters to selectively and gently retain larger mass densities such as plant and animal cells while removing contaminating bacteria and mycoplasmas from the system (27).

In addition to separation technologies, research and development advances and trends in process monitoring and control technology will provide the opportunity for the automation of industrial bioprocess via real-time and continuous assessment of key processes (for example, system integrity and sterility, influence of environment on cells, substrate and product concentrations, oxygen transfer, heat production and transfer, CO_2 requirements for cell metabolism, pH effects on cell growth and system contamination, system viscosity)*.

These two as well as other technological areas considered essential to the success of industrial biotechnological processes were discussed in depth in a previous Bureau report (1). Subsequent sections of this report will, therefore, highlight certain subject areas, for example:

- o advances in separation technologies such as flow cytometry and continuous flow electrophoresis that may have industrial process optimization applications; and

- o technological developments in bio-electrochemistry and luminescence chemistry and the possible impacts of these developments on improved bioprocess monitoring and control.

.2 Biotechnology Process Separations

Separation technologies will be an important component in the optimization and economics of biotechnological processes. A previous Bureau report has addressed in detail: (a) the importance of this technology to industry, (b) recent developments in separation technologies, and (c) technological barriers that must be addressed for successful industrial bioprocess use of

*Hochhauser, S. J., High Technology, February 1983, pp. 55-60.

these technologies (1). Separation technologies discussed in the report included: membranes, chromatography, supercritical CO_2, monoclonal antibodies, biological separations, and "dry" separations.

This report will highlight two separation technologies, continuous flow electrophoresis and flow cytometry, that may have significant impacts on biotechnology instrumentation and on the optimization and economies for certain industrial biotechnology processes.

a. Flow Cytometry

High speed flow cytometry systems provide an opportunity to analyze and/or to separate large numbers of individual cells based upon their optical properties. In principle, 10^4 to 10^5 cells in aqueous suspensions can be measured per minute. A key to the discrimination between individual cells is the light scattering of stained or unstained cells in a narrow laminar flow stream exposed to a focused beam of exciting light (laser). The resulting fluorescent light pulses are viewed by a photomultiplier tube. The amplitude of the electrical pulses generated by the fluorescent light pulse is proportional to the fluorescence intensity. There are several commercial instruments available for single cell analysis and/or cell sorting. Measurement of light scatter, fluorescence, and light absorption is usually described in relative terms and not quantitative measures. This has resulted in the need to establish some biological (for example, leukocytes) and non-biological (for example, plastic beads) particles for staining and instrument standards (28).

Since some earlier work on the development and initial applications of this technique (29, 30), there have been several reports on the use of this research tool to distinguish between cell populations, particularly in clinical, cell biology and immunological research areas (31-33). Specific examples of the use of flow cytometry include: cancer cell diagnosis and classification (34-38), fluorescent antibody (monoclonal) studies of lymphocyte subpopulations and of cancer cells (39-42), assessment of the characteristics of normal cell populations (43-45), cell analysis and concentration in transfusions and transplantations (46, 47), evaluation of cell parameter changes resulting from parasite

invasion of blood cells (48, 49), and study of cell activation phenomena (50). In addition to clinical applications, there is some potential for applying flow cytometry to continuous industrial bioprocesses for measurement of process variables such as cell size (as an indicator for adjusting nutrient flows), cell viability, and internal components and composition of cells (51), and for using fluorescent metal dyes in conjunction with flow cytometry for calibrating bioprocess streams (52).

There have been recent attempts to correlate light scattering at multiple wavelengths, using appropriate optical filters, to further enhance the specificity of this technique: (a) in distinguishing between T and B lymphocyte cell populations, and (b) in providing an approach for rapidly screening and subsequently characterizing hybridoma cells with specific monoclonal antibody production capability (53).

Regarding this latter point, the commercial impacts of monoclonal antibodies (hybridoma technology) in diagnostics, therapeutics, imaging, and separations have been discussed (54-58). In order to effectively sort large populations of hybridoma cells for those few hybrid-myelonoma clones producing the desired monospecific antibody, individuals are taking advantage of the availability of flow cytometry instrumentation with cell analysis and sorting capabilities. In addition, the increasing body of information on cell size and surface characteristics and on the quantitative expression of certain molecules on the cell surface is aiding this effort. This has facilitated to some extent the search for desired hybridoma clones by dividing large cell populations into subpopulations on the basis of size and cell surface characteristics.

One of the limitations with this approach is that, with existing cell sorting instrumentation, considerable emphasis is placed on cell sorting by cell function rather than by the physical parameters of cells. When one is searching for one in 10^3 to 10^4 hybridoma clones, greater instrument precision is needed, than is currently available with flow cytometry instrumentation schemes, to ensure that all the desired hybridoma cells are sorted into one specific compartment to the exclusion of all other cells (27)*.

*Laser-induced fluorescent analysis coupled with flow cytometry may provide opportunities to detect attogram quantities of liquid samples, specific cell surface binding sites, and single molecules in a probed volume (Science 219:845-847, 1983).

.b Continuous Flow Electrophoresis

Electrophoretic methods are used for many biochemical separations of basic and acidic molecules, for example, proteins, peptides and nucleic acids. The resolution of the separation will depend on the type of electrophoretic technique used and the nature of the supporting matrix. Several matrices have been used to facilitate electrophoretic separations. These include matrices such as agar, starch and polyacrylamide gels, cellulose and paper.

A more recent application of electrophoretic techniques having possible biotechnology industrial process implications involves continuous flow electrophoresis. In this technique, a free flowing buffer curtain or carrier ampholyte is used in a separation chamber. A constant voltage electrical field is applied to this carrier ampholyte to form a pH gradient perpendicular to the continuous flow of the ampholyte stream. When a sample consisting of a mixture of proteins, cells or cell components is placed in this environment via a separation chamber inlet port, the individual proteins and subpopulations of cells in this sample will migrate to a specific isoelectric point based upon the net charge of the protein and the pH gradient in the separation chamber. Some attractive features of this electrophoretic technique over other electrophoretic and separation techniques is the short residence time of the sample in the separation chamber, the potential capability of the system to effectively separate biologically and commercially important molecules in large sample volumes, the avoidance of non-specific interactions with solid surfaces and the continuous operation. In addition, the surface charge density of the molecules can be altered and the separation capabilities of the sample improved by reaction with antibody (59).

As a result of apparatus developments in the early 1970s (60), clinically related applications of continuous flow electrophoresis, particularly in the areas of cell and cell component separations and isoelectric focusing of proteins, have predominated (61-65). Examples of research and experimental uses of this technique include: separation of T and B lymphocyte cell populations

(59); isolation of normal cells or parasites at different stages in development (66-68); separation of cell membrane fractions (63, 69-71); analysis of the surface properties of cancer cells (72); purification of human lysosomes (73); separation and purification of histocompatibility antigens (74); early detection of remission in cancer patients (75) and enhanced cell separations using immunomicrospheres (76, 77). With this technique, flow rates of 10^7 to 10^8 cells per minute have been achieved.

With the emergence of rDNA as a powerful biotechnology industrial tool involved in the production of low volume high value added products such as proteins and peptides, there is considerable research and industrial interest in developing high throughput and relatively low cost schemes for the isolation and purification of commercially useful products developed through genetic engineering. NASA and industry are currently cooperating in the evaluation of the commercial utility of a vertical continuous flow electrophoresis system in separating biological materials and in obtaining commercial quantities of high purity pharmaceutical products (78, 79). If this separation technology is to be other than a space-based process, several technological barriers need to be addressed. These barriers include, for example: sample overconcentration at the inlet port; particle stream convection; bandspread of adjacent particle streams; interaction of carrier ampholytes with metal ions; pH gradient stability; complex formations with polyanions or other molecules; cell and cell component aggregation, instability and interaction with the carrier ampholyte; particle sedimentation and carrier ampholyte densities; and the balancing of throughput and separation capabilities with voltage increases.

.c NBS Role

Flow Cytometry. Examples of some areas where NBS could contribute to improving the precision and selectivity of flow cytometry instrumentation and, ultimately, the process by which critical hybridoma cells are identified include:

- development of standard artifical cells, for example, synthetic or immunomicrospheres, that could mimic the size, density and other important characteristics of critical hybridoma cell populations;

- development of individual cell identification and separation schemes that involve closer correlations between cell functions and physical parameters;

- evaluation of the optical imaging and detection systems to increase the sensitivity and resolution of the system optics, to improve the differential capabilities of multiple detection schemes, and to optimize optical filter collection of emitted fluorescence and rejection of laser light;

- exploration of non-standard fluorochromes (for example, red or near-infrared excited dyes) and energy transfer from one dye to another;

- evaluation of alternatives for improving laminar flow system stability for more precise regulation of pressure/flow rate, and for automating and miniturizing sample handling devices;

- development of standardized and well-calibrated flow cytometers for Government agency use; and

- development of alternatives for improved data handling and storage (for example, optical disk storage of collected data, display of multidimensional data, compression of gathered multiparameter data, kinetic analysis).

Continuous Flow Electrophoresis. Within the Bureau's Center for Analytical Chemistry, there is considerable ongoing research activity that may relate to some of the technological barriers associated with continuous flow electrophoresis (80). Examples of areas where NBS could contribute to reducing the technological barriers to broader industrial applications of this separation technique include:

- evaluation and development of carrier ampholytes with a view also to ampholyte cost, possible recycling and standardization of reagents;

- o development of sensing/detector systems, for example, the use of lasers, to continuously monitor the adequacy of the separation process; and

- o provision of evaluated physico-chemical data on proteins, and protein-protein and protein-material interactions.

.3 Biotechnology Process Monitoring and Control

On-line and continuous monitoring and control of commercial biotechnological process parameters will be essential for process optimization and for commercial and economic success of the products derived from such processes. Rapid developments in immobilized biocatalysis* and in membrane technologies offer attractive alternatives to existing analytical capabilities for the rapid, specific, and sensitive analysis of valuable components and of system component inhibitors, often in trace amounts within complex aqueous mixtures (1). Two categories of biosensing systems that merit further discussion because of the potential they may offer for optimizing bioprocesses are biosensing electrodes and luminescence analytical systems.

.a Biosensing Electrodes

In addition to many existing opportunities for biocatalysts to improve industrial biotechnological reactor process throughout (1), increasing physico-chemical knowledge on (a) the binding of biocatalysts to solid surfaces or macromolecules, (b) the immobilization of biocatalysts within polymer matrices, and (c) the retaining of biocatalysts within selective membranes is providing an increasing number of novel solutions to industrial biochemical analytical problems (81-89). A specific example of biocatalysts being used as analytical tools is the coupling of their substrate-selective reaction capabilities with electrochemical sensors. In such systems, the reactant being sensed can be related to initial substrate concentration.

Biosensing systems coupled to electrodes can be classified into two main types of reaction systems, i.e., potentiometric (measure change in concentration of an analyte produced by a biocatalytic reaction, for example, ion-selective

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*Klibanov, A. M., Science 219:722-727, 1983.

and gas sensing electrodes) and amperometric (measure oxidation or reduction as a linear function of the concentration of an analyte in a biocatalytic reaction, for example, an oxygen electrode; 90, 91).

Immobilized biocatalytic electrode sensor/detector systems offer several advantages as analytical probes:

(a) ease in introduction of the biocatalyst into and separation from a reaction mixture;
(b) reuse and applicability to continuous measurement systems;
(c) selectivity of biocatalytic processes;
(d) increased availability of a wide variety of biocatalysts;
(e) enhanced electrode ability to discriminate against interfering ions in complex biological fluids;
(f) enhanced biocatalyst pH and temperature stability;
(g) extended biocatalyst half-life;
(h) extended range of species that can be measured;
(i) rapid sample analysis;
(j) reduced sample preparation;
(k) economic use of reagents;
(l) in situ and continuous monitoring of bioprocesses; and
(m) probably most significant in comparison with other analytical techniques, direct conversion of chemical information to an electrical signal.

Specific goals in the use of these bioanalytical electrode probes include: sensitivity, high selectivity, good stability, rapid response, and electrode-biocatalyst system component compatibility (87, 92).

Initial applications of biosensing electrodes have been in clinical medicine. Single enzyme-electrode (ion- selective electrodes; ISE) have been used to measure the concentration of certain inorganic ions (potassium, calcium, sodium, magnesium) and organic molecules (glucose, galactose, sucrose, maltose, lactose) in physiological fluids (91, 93, 94).

Single enzymes, however, represent only one class of biological materials that can be used as biocatalysts in electrode biosensing systems. The use of multiple enzymes and cofactors, microorganisms, immuno-reactive materials, organelles, tissue slices, and liposomes in solution or as immobilized biocatalysts in conjunction with bioselective electrodes has extended significantly the measurement potential of biosensor electrodes (81, 87, 88). Table 6 illustrates the considerable research activity in this area, and provides some examples of a range of applications for such analytical systems, for example, clinical medicine, industrial fermentations, food processing, environmental monitoring, and pharmaceutical analysis.

Biosensing electrodes have potentially a broad range of biotechnology industry process applications and may offer attractive alternatives to existing, more indirect approaches for the continuous and, possibly, in situ (for example, metal processing) measurement of process parameters (1). Some current R&D trends, illustrated in Table 6, suggest several areas for industrial bioanalytical application, for example:

- o allow direct measurement of relative amounts of stereoisomeric (d, l) forms of compounds (important in analysis of pharmaceuticals such as ephedrine, chemical and food process components such as amino acids, and other optically active compounds; 91);

- o provide stable, self-contained, highly selective molecular probes for specific analyses of a wide range of organic compounds from amino acids to large organic molecules with pharmacologic activity (106, 133);

- o monitor enzymes and enyzme catalyzed reactions in situ (92, 134);

- o provide sensitive temperature measurement probes using thermophilic bacteria or their enzymes (81);

- o determine nutrients (nutrient uptake), products, and whole cells in fermentation broths for in situ process control and optimization (116);

TABLE 6. SELECTED APPLICATIONS OF BIOSENSING ELECTRODES

SUBSTANCE MEASURED	SPECIES SENSED	BIOCATALYST(S)	SYSTEM COMMENTS	CURRENT/POTENTIAL APPLICATIONS	REFERENCE
• ENZYME					
ethanol	O_2	alcohol oxidase	immobilization on silanized glass beads; flow cell apparatus for automation	clinical chemistry	95
methanol (primary alcohols)	O_2	methanol dehydrogenase (phenazine ethosulfate)	enzyme solution in anode compartment	industrial fermentations	96
L-lysine	CO_2 gas	L-lysine decarboxylase	immobilization in protein gel	food processing	82
glucose	H_2O_2	glucose oxidase	enzyme grafted to glassy carbon electrodes	chemical analysis	97
1, 2 dihydroxy benzene	p-benzo-quinone	mushroom tyrosinase	rotating disc electrode	enzyme assays, clinical chemistry	98
galactose; galactose and glucose	H_2O_2	galactose oxidase	enzyme sandwiched between cellulose acetate membranes	chemical analysis	99, 100
alpha-benzoyl-arginine amide	NH_3 gas	trypsin	--	clinical chemistry	101
L-amino acids	H_2O_2	L-amino acid oxidase	covalent bonding of enzyme to graphite (carbon) surface-electrode	detectors/sensors coupled to analytic systems	90, 102
xanthine	ferricyanide, uric acid	xanthine oxidase	covalent bonding of enzyme to graphite-electrode; dual (ampero-metric and potentiometric) electrode	food processing, chemical analysis	103
wastewater (chlorinated phenolic compounds)	anions	neutral phosphatase enzymes (*Pseudomonas* sp.)	immobilization in polyacrylamide	environment monitoring	104
glucose	H_2O_2	glucose oxidase	covalent bonding of enzyme to derivatized membrane	industrial fermentations	105

TABLE 6. SELECTED APPLICATIONS OF BIOSENSING ELECTRODES (continued)

SUBSTANCE MEASURED	SPECIES SENSED	BIOCATALYST(S)	SYSTEM COMMENTS	CURRENT/POTENTIAL APPLICATIONS	REFERENCE
ENZYME (continued)					
creatinine	NH_3 gas	creatininase	immobilized enzyme	clinical chemistry	106
uric acid	CO_2 gas	uricase	immobilized enzyme in flow system	clinical chemistry	106
penicillin	pH	penicillinase	--	industrial fermentations	106, 107
urea	ammonium-NH_4 (nonactin-PVC)	urease	enzyme sandwiched between dialysis membranes	clinical chemistry	106
lactate lactate dehydrogenase	O_2 H_2O_2	lactate oxidase	enzyme sandwiched between filter and dialysis membranes; pyruvate and NADH added to sample solution	clinical chemistry	108
• MICROBIAL CELLS					
nitrate, wastewater	NH_3 gas	Azotobacter vinelandii	bacterial cell layer between dialysis and gas permeable membrane (nitrate reductase, nitrite reductase)	potable water analysis, chemical analysis, cofactor (NAD^+) regeneration	109
BOD (wastewater)	O_2	Hansenula anomala	immobilized yeast cells	industrial environmental monitoring	110
mutagens	O_2	Bacillus subtilis (Rec-) or Salmonella typhimurium (Rec-)	bacterial cell suspension between membrane filter and teflon membrane	mutagen, antibiotic, respiration inhibitor, bactericide screening	111, 112
3 keto-4-eno-steroids	reduced 2, 6-dihydrophenol-inophenol	Nocardia opaca	cells immobilized in 15 % polyacrylamide between two glass plates (carbon electrode)	clinical chemistry	113
BOD (wastewater)	hydrogen, formate	Clostridium butyricum	immobilized cells (anaerobe)	industrial environmental monitoring	114, 115
Vitamin B_1	--	Lactobacillus fermenti	cell suspension	food processing, industrial fermentations	116

TABLE 6. SELECTED APPLICATIONS OF BIOSENSING ELECTRODES (continued)

SUBSTANCE MEASURED	SPECIES SENSED	BIOCATALYST(S)	SYSTEM COMMENTS	CURRENT/POTENTIAL APPLICATIONS	REFERENCE
MICROBIAL CELLS (continued)					
cell population numbers	--	--	cell suspension (S. cerevisiae) in growth medium	industrial fermentations	116
nicotinic acid	pH	Lactobacillus arabinosis	immobilized cells	food processing, industrial fermentations	107, 116
BOD (wastewater)	O_2	microorganisms from activated sludge	cells immobilized in collagen membrane	industrial environmental monitoring	116
glucose, fructose and/or casein	Δt	Saccharomyces cerevisiae; Enterobacter cloacae, Proteus rettgeri, Klebsiella pneumonia	yeast cells immobilized in 15 % polyacrylamide	industrial fermentations, process control, analysis of single components or complex solutions, microbial identification via growth patterns, clinical chemistry	117, 118
L-aspartate	NH_3 gas	Bacterium cadaveris	cell regeneration at electrode surface	clinical chemistry, industrial fermentations	81
L-cysteine	H_2S gas	Proteus morganii	cell regeneration at electrode surface	clinical chemistry, industrial fermentations	81
serine	NH_3 gas	Clostridium acidiurici (anaerobe)	--	clinical chemistry	116
histidine	NH_3 gas	Pseudomonas sp.	--	clinical chemistry	116
glutamine	NH_3 gas	Sarcina flava	continuous flow system	clinical chemistry	106
cephalosporin	pH	Citrobacter freundii	--	industrial fermentations	116
nitrilotriacetate	NH_3gas	Pseudomonas sp.	cells between dialysis and gas permeable membranes	environment, agricultural, industrial	119

TABLE 6. SELECTED APPLICATIONS OF BIOSENSING ELECTRODES (continued)

SUBSTANCE MEASURED	SPECIES SENSED	BIOCATALYST(S)	SYSTEM COMMENTS	CURRENT/POTENTIAL APPLICATIONS	REFERENCE
• ENZYME SEQUENCE/COMPETITION ELECTRODES					
NAD^+	NH_3 gas	NAD^+ nucleosidase and *E. coli* (nicotinamide deaminase)	coimmobilization of enzyme and bacterial cells at electrode surface (hybrid electrode)	chemical analysis	81
bilirubin	H_2O_2	glucose oxidase, horseradish peroxidase	coimmobilization by entrapment in gelatin of enzymes between two dialysis membranes	clinical chemistry	120
aminopyrine	O_2	catalase, horseradish peroxidase	alternating polyethylene and dialysis membranes separated by enzyme entrapped in gelatin	clinical chemistry	120
glucose	H_2O_2	microsomal P-450 (rabbit liver), glucose oxidase	coimmobilization in gelatin and fixed to rotating disc electrode by dialysis membrane (organelle-enzyme hybrid electrode)	avoid NADPH cofactor replacement, determine several substrates in mixed redox	121, 122
glucose, maltose	H_2O_2	glucose oxidase, glucoamylase	immobilization of glucose oxidase in gelatin; glucoamylase immobilized by glutaraldehyde cross-linking	clinical chemistry, industrial fermentations	123
ATP substrates	H_2O_2	hexokinase, glucose oxidase	coimmobilization in gelatin	clinical chemistry, industrial fermentations	123
NAD^+ substrates	H_2O_2	glucose dehydrogenase, glucose oxidase	coimmobilization in gelatin	clinical chemistry	123

TABLE 6. SELECTED APPLICATIONS OF BIOSENSING ELECTRODES (continued)

SUBSTANCE MEASURED	SPECIES SENSED	BIOCATALYST(S)	SYSTEM COMMENTS	CURRENT/POTENTIAL APPLICATIONS	REFERENCE
ENZYME SEQUENCE/COMPETITION ELECTRODES (continued)					
glucose	O_2	glucose oxidase, glucose isomerase	immobilized in individual layers with solubilized collagen fibrils	clinical chemistry, industrial fermentations	124
tyrosine	CO_2 gas	L-tyrosine decarboxylase, porcine small intestine	hybrid immobilized system (enzyme and tissue)	clinical chemistry, food processing	125
• ORGANELLES					
NADPH	H_2O_2	microsomes (rat liver)	organelles entrapped in gelatin	chemical analysis	122
thyroxine	I^-	microsomes (rat liver)	--	--	116
glutamine	NH_3 gas	mitochondria (kidney)	--	clinical analysis	106
• TISSUE					
guanine	NH_3 gas	rabbit liver	tissue placed between two dialysis membranes	chemical analysis	126
glutamate	CO_2	yellow squash	--	extend range of sensing systems	81, 127
AMP	NH_3 gas	rabbit muscle	--	clinical chemistry	81
glutamine	NH_3 gas	porcine kidney cortex	continuous flow system	clinical chemistry	106

- o sense various process parameters on line before actual fermentations (135);

- o quantify low level presence of toxic materials using enzyme inhibition sensors, for example, immobilized acetylcholine esterase and pesticide detection (85);

- o measure and provide process data on thermal effects via flow calorimetry of immobilized enzyme deactiviation and regeneration as a consequence of use in industrial processes (136);

- o differentiate metabolic pathways in and improve selectivity of whole cell systems (81, 88);

- o screen mutagens with microbial cell-electrode systems (111, 112);

- o provide static, ion-selective surface measurement systems for clinical analysis (inorganic ions and trace metals) and for environment toxic/ hazardous material detection system, possibly including the use of monoclonal antibodies or hybridoma cells for worker safety monitoring or for detection of biological organisms in pollution monitoring (106, 137-140); and

- o couple biosensing electrode detection systems to continuous flow, flow injection, liquid chromatographic and other analytical instrumentation for on line, continuous measurement of substances (ions, gases, organic molecules, organometals) present in low concentration in flowing liquid system, for example, clinical, water, soil, food processing, fermentation process analysis (89, 91, 106, 134, 141-146).

In addition to these possible opportunities for commercial application of biosensing electrodes, there are several problem areas where additional R&D will help to enhance both the sophistication and commercial potential of these analytical tools. Examples of areas of research need include:

use of genetic manipulation for producing specific as well as novel biocatalysts in sufficient quantities for biosensing electrode systems;

- o improved stabilization of multi-biocatalyst components of sensing systems;
- o understand molecular aging and enzyme denaturation and regeneration phenomena;
- o improve system response times and detection levels;
- o reduce inputs of toxic materials to poison sensing system;
- o improve regeneration of cofactors, cell design and electrode performance;
- o understand system interfaces, e.g., energetics and structure influences on catalytic activity, enzyme behavior and electrochemical reactions; and
- o couple the specificity of monoclonal antibodies with the sensitivity of biocatalysts for improving the sensitivity of biosensing electrode probes for organic molecules (86, 133, 147-149).

In the intermediate-term, development of highly specific and sensitive miniturized and continuous multicomponent sensors for detection of chemical and conformational changes in complex environments could be achieved with the coupling of integrated circuit, membrane, immobilized biocatalysis, material/polymer, and monoclonal antibody technologies*.

In the long-term, our ability to understand structure-function relationships and to predict 3-dimensional structural conformations of biologically important molecules could provide the technical basis for designing molecular probes for highly precise and selective intracellular monitoring or sensing of important bioprocesses (150)**.

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*Meyerhoff, M.E., and Fraticelli, Y.M., Anal. Chem. 54:27R-44R, 1982.
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**Pabo, C., Nature 301:200, 1983.
Ulmer, K. M., Science 219:666-671, 1983.

.b Luminescence Assays

Luminescence systems are beginning to attract considerable attention as highly specific, sensitive, and rapid detection systems for organic compounds in aqueous environments, and as possible alternative detection schemes to fluorescence and spectrometric systems (151-155). There are several applications of luminescence assays including, for example, trace metal detection, monitoring biomass conversions, measurement of microbial contamination in waste water and food products, monitoring immunoassays, monitoring microbial populations in clinical and ecological analyses, measurement of substrates in redox and dehydrogenase reactions, and monitoring cell mediated immune responses (154, 155). Major initial impacts of luminescence detection systems will be in clinical analyses (151, 153, 155, 156).

Bioluminescence. The ability of organisms (bacteria, fish, firefly, etc.) to emit light in the presence of oxygen is known as a bioluminescence. The importance of the use of bioluminescence for analysis of biologically important molecules has been well documented (154, 155).

There are three bioluminescence systems that are important from an analytical perspective: (a) bacterial luciferase and oxidoreductase, (b) firefly luciferase, and (c) aequorin luciferase (151).

The reactions catalyzed by the bacterial luciferase/oxidoreductase bioluminescence system can be viewed as a branch of the electron transport pathway in which electrons from reduced substrates are shunted to oxygen at the

level of flavin (Figure 1; 157).

(1) $NADH + FMN + H^+ \xrightarrow{\text{oxidoreductase}} NAD^+ + FMNH_2$

(2) $FMNH_2 + RCHO\ (\text{aldehyde}) + O_2 \xrightarrow{\text{luciferase}} FMN + RCOOH + H_2O + Hv\ (\text{light})$

Figure 1. Schematic Representation of Bacterial Bioluminescence System

Some of the substances that have been assayed by these bioluminescence systems include, for example, ethanol, glucose, ATP, CA^{++}, biotin, pyruvic acid, 3-hydroxybutyric acid, malic acid, myristic, oxaloacetic acid, hormones, antigens, and cell populations (151, 155). Additional examples of bioluminescence assay systems are provided in Table 7. Experimental efforts to immobilize luciferase with other appropriate system enzymes (co-immobilization) have not only enhanced the stability of these enzymes but also increased the efficiency of the reaction scheme (158).

Chemiluminescence. Chemiluminescence is the measurement of light produced by a chemical reaction. This analytical approach is particularly useful in the detection of hydrogen peroxide (H_2O_2). The most widely used chemiluminescent reagent is luminol (3-aminophthalhydrazide) which reacts with H_2O_2 in the presence of microperoxidase (metal catalyst) to produce light (Figure 2; 163).

$$\text{luminol} + H_2O_2 \xrightarrow{\text{metal catalyst}} \text{3-aminophthalic acid} + \text{light (hv)}$$

Figure 2. Chemiluminescence Reaction Scheme

Examples of substances measured by chemiluminescence assays are provided in Table 8.

TABLE 7. EXAMPLES OF BIOLUMINESCENCE ASSAY SYSTEMS

SUBSTANCE MEASURED	BIOCATALYST(S)	SYSTEM COMMENTS	CURRENT/POTENTIAL APPLICATIONS	REFERENCE
androsterone, glucose-6-phosphate, lactate, alcohol, or bile acid	bacterial luciferase/ oxidoreductase coimmobilized with dehydroxenase enzyme	coimmobilization on Sepharose particles	clinical chemistry industrial fermentations	151, 158
direct mutagens, DNA damaging agents, DNA synthesis inhibitors, or DNA intercalating agents	dark varient of *Photobacterium leiognathi*	10^5 cells + test chemical in scintillation vials	mutagen screening	159
methotrexate	firefly luciferase covalently linked to methotrexate (antigen)	derivitized enzyme bound to anti-methotrexate (antibody) on Sephadex particles; nonisotopic immunoassay; single and double antibody methods	clinical chemistry	160, 161
pheronomes	luciferases from *B. harveyi* and *P. phosphoreum*	qualitative differentiation between aldehyde pheronomes; measurement of airborne hormones	insect control agriculture	162

TABLE 8. EXAMPLES OF CHEMILUMINESCENCE ASSAY SYSTEMS

SUBSTANCE MEASURED	BIOCATALYST(S)	SYSTEM COMMENTS	CURRENT/POTENTIAL APPLICATIONS	REFERENCE
glucose	glucose oxidase (immobilized), luminol-ferricyanide	amenable to automation	clinical chemistry	168, 169
trihalomethanes	luminol-H_2O_2 (chlorine dioxide)	membrane flow cell system	water analysis	170
glucose	glucose oxidase, luminol - H_2O_2	enzymes in flow streams (non-immobilized); microporous membrane flow cells	clinical chemistry	163, 171
water (trace H_2O_2)	luminol - potassium hexacyanoferrate	portable system	water analysis	172
cortisol	steroid-luminol conjugates with horseradish peroxidase, luminol - H_2O_2	antibody solid-phase separation technique (competitive luminescent enzyme immunoassay, LEIA)	clinical chemistry	152, 161, 173
acetylcholine	acetylcholinesterase, choline oxidase, luminol - H_2O_2	multienzyme system	clinical chemistry	152
drugs	blood platelets - luminol + arachidonic acid	cell-drug inhibition assay system	drug screening (effectiveness/toxicity)	152
hepatitis B surface antigen (HBsAg)	HBsAg + anti-HBsAg, anti-HBsAg + isoluminol, microperoxidase + peroxide	luminescent immunoassay (LIA)	clinical chemistry	161

For luminescence assays (bioluminescence and chemiluminescence), commercially available photometric (photomultiplier tubes) instruments suitable for low-level and other light emissions are available (152, 164). Luminescence assays are:

- providing measurements of substances at picomole and sub-picomole levels (151, 158, 160).

- attracting considerable interest as a post-column photochemical reaction detection method for increasing the sensitivity and selection of detection for trace substances in complex mixtures in HPLC separations (152, 165, 166);

- enhancing the ability to follow the kinetics of sub-cellular molecular events continuously and non-destructively (159); and

- improving the sensitivity of luminescence reactions as molecular probes by their integration into immunoassays, (alternatives to use of radioisotopes and fluorescent molecules in such assays) and by use of monoclonal antibodies (151, 153, 167).

In addition to these advantages of luminescence assays, additional research in a number of areas may improve the sensitivity and specificity of these assay systems. Examples of areas of research opportunity that are measurement related include:

- developing and applying new photochemical reagents;

- coupling luminescence detectors to electrochemical detectors;

- improving photomultiplier sensitivity;

- improving the purity, standardization, and quantity of luminescence system reagents (for example, use of genetic manipulation techniques as a tool for production of luciferase);

- optimizing the flow rate to achieve maximum photochemical response;

- coupling luminescence reactions to flow injection and continuous flow for automated and continuous analysis;

- varying the ratios of enzymes in coupling mixtures to optimize performance;

- exploring the use of membrane versus solution and immobilized enzyme luminescence techniques; and

- standardizing reagents and producing reference materials (144, 145, 152, 158, 165, 171, 172, 174-178).

.c Other Developments in Process Monitoring and Control Instrumentation

In addition to the above biotechnology processes monitoring and control research and technological developments, some additional biotechnology instrumentation developments merit further evaluation because of their potential importance to the monitoring of continuous industrial bioprocess systems. These developments include, for example:

- the expanded use of nondestructive infrared (IR) spectroscopy for the on-line analysis and monitoring of low level concentrations of components of biological systems in aqueous, continuous flow processes, e.g., food processing, organic chemical fermentation process steps, and pharmaceutical processes, (179-181)*;

- the greater use of HPLC (measurement of proteins, peptides and amino acids) and microflow cells and metal dyes in conjunction with multiple detection systems in clinical and pharmaceutical areas of biotechnology (52, 89, 141, 182, 185); and

- the applications of laser chemistry for analyzing optical parameters of biological materials, e.g., the monitoring of microbial population changes in

*Infrared analysis may also be applicable for nondestructive assay of solid substrate fermentations (Biotechnol. Bioeng. 25:606-607, 1983).

biotechnological processes (186, 187), if reliable and less costly and complex instrumentation becomes available (188).

.d NBS Role

Within the Bureau's mission, there appear to be several steps the Bureau could take to provide infrastructure technology support to industry process monitoring and control needs in biotechnology. These steps could include:

- evaluating compilations of electrochemical and thermodynamic data, and the applicability of this information to predictions on bioelectrical analytical systems;

- providing a basis for standardizing reagents and other materials used in biosensing analytical systems for improved intra- and inter-laboratory comparison of findings;

- evaluating the compatibility of components in biosensing analytical systems;

- understanding stabilizing influences and thermodynamic aspects of system interfaces in multicomponent biosensing systems;

- providing the technical basis for development of standards (for example, sodium, potassium and other standards in ion-selective electrodes) for evaluating the operational efficiency and effectiveness of both flow liquid and static surface biosensing systems and for measuring/validating the real-time/ continuous performance of such systems;

- measuring profiles of species concentration in composite membranes for optimizing biosensing membrane systems;

- evaluating and/or developing photochemical methods for picomole and subpicomole measurements of substances in complex aqueous environments, and

- assessing alternatives for coupling luminescence assays with optical imaging systems for the real-time monitoring of functions of immobilized cells in fluidized beds.

5. NBS KNOWLEDGE BASE AND CAPABILITIES RELATED TO LONG-TERM INDUSTRY INSTRUMENTATION/MEASUREMENT-RELATED NEEDS IN BIOTECHNOLOGY.

In this section of the report, NBS scientists and technical staff* identify some examples of on-going Bureau research and measurement-related activities that could provide infrastructure support to industrial biotechnology instrumentation development needs. The technical descriptions of the examples selected by NBS staff focus on two areas: (a) knowledge development opportunities; and (b) areas where an existing Bureau knowledge base could be applied to industrial biotechnology instrumentation needs as these needs relate to the Bureau's mission.

The technical contributions which follow represent only a few examples from a range of possible research areas where NBS staff might contribute to meeting industry infrastructure technology needs. A previous NBS report identified several other areas where Bureau research initiatives and services could be undertaken to provide this industrial infrastructure support (1).

.1 Electrochemistry and Biosensing Systems

The use of potentiometric sensors (ion-selective electrodes) spans almost two decades. Research accomplishments in this area include the miniaturization of ion-selective electrodes for microanalysis, the application of these sensors to the analysis of environmental and biological samples, the development of continuous-flow measurement systems, and an automated discrete sample analyzer for sodium and potassium in whole blood. Studies have also been performed using both potentiometric and amperometric gas-sensing electrodes and several types of enzyme electrodes.

The Organic Electrochemistry Group within the Bureau's Center for Analytical Chemistry has developed a broad range of expertise applicable to the design, development, and application of electrochemical sensors for biological

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monitoring and analysis. A research program initiated several years ago has been actively concerned with the development of chemically modified electrodes and with the immobilization of enzymes and other biocatalysts onto the surfaces of appropriate electrode sensors. The primary emphasis of this work has been on the attachment of electrocatalytic sites to electrodes modified with polymer films. This investigative approach allows considerable flexibility in modifying surface characteristics, e.g., film thickness, permeability, and site density. In addition to these experimental studies, mathematical modeling of the charge transfer and mass transport processes occurring has been investigated. Information from these studies has provided new insights into the behavior of electroactive sites and mobile charge carriers in polymer films. Such models will be of considerable assistance in exploring the problems associated with optimizing the performance characteristics of polymer-modified electrochemical sensors--problems which previously had, for the most part, been approached empirically.

Considerable effort by this group has also resulted in the development and application of hydrodynamic electrochemical detectors for HPLC. The approaches taken for these low-volume detectors have included dual-electrode designs for increased sensitivity, differential-pulse excitation for increased selectivity, and optimization of the cell parameters for efficient operation. Coupling these detectors with biocatalytic sensors could provide a means for continuous-monitoring detectors in biomedical and industrial applications (for example, pharmaceutical manufacturing process).

.2 HPLC in Protein, Peptide and Amino Acid Analysis and in Separations

HPLC has been used in protein, peptide and amino acid analytical biotechnology for the past several years. The use, development and application of new methods in this area is still in an exponential phase of growth. New support materials are being rapidly developed for both reverse phase separations and ion exchange chromotography. The precise mechanism of many separations, however, is still poorly understood. The major methods of detection utilize either ultraviolet or fluorescent techniques. The ultraviolet techniques are less sensitive than the fluorescent techniques which frequently require sample

derivatization, but result in permanent destruction of the sample. There is a need to employ several methods of detection sequentially, utilizing different principles of analysis such as the binding of specific substances, and biochemical and electrochemical reactions. When these types of analytical procedures are combined they will lead to a greater degree of specificity and possibly enhance sensitivity. Within the limitations of these methods, proteins, peptides or amino acids can either function as the analyte or as a reagent for the detection of a specific analyte.

Within the Center for Analytical Chemistry, a research program on the analysis of peptides and amino acids was initiated five years ago. This program has focused on the separation of peptides differing in sequence bv only a single amino acid or in the isomeric configuration of a single amino acid. The techniques used in these studies, which led to the certification of an angiotensin I standard reference material (SRM), in conjunction with newer separation methods and multidimensional detection systems could be utilized to develop more efficient and more sensitive methods for the measurement of proteins and peptides of clinical and pharmaceutical significance.

Instrumentation such as the programmable excitation/emission wave length fluorometer, the diode array ultraviolet detector, the electrochemical detector, and the mass spectrometer could be valuable in developing more sensitive and more specific detection systems.

.3 Biotransformations

In contrast to the carefully controlled microbial fermentations used in the food processing, brewing, and pharmaceutical industries, the use of microorganisms for the leaching of metal ores and for the bioaccumulation of metals from process streams is in a primitive state. Nevertheless, microbial leaching currently accounts for 25 percent of U. S. copper production. and shows proven potential for recovery of strategic metals. Many organizations are actively investigating or funding research directed toward understanding and improving the processes in microbial leaching of metal ores (Biogen, Genex, Chevron, Advanced Mineral Technologies, Inc.), treatment and biorecovery of metals from waste effluents (ARCO, Eastman Kodak), and biodesulfurization of coal (DOE).

The goal of these bioprocesses will be cost effective field performance. Therefore, field sensing or monitoring measurements of aqueous effluents, of leaching liquors, and of chemical composition of participating micrcroorganisms will be needed to assess processing effectiveness and to detect toxic/ interfering element species that, at trace levels, may be rate-limiting. For example, sub-parts-per-million levels of mercury can totally inhibit microbial oxidation of ferrous iron (at g/L concentrations) to the ferric iron solutions that are effective copper ore solubilizers (189). Additionally, more complete knowledge of the range of specific elements taken up and transformed by microorganisms and of biocatalytic reactions on ore surfaces is needed. These needs require the development and application of instrumentation capable of speciating metals at trace concentrations in solution and on surfaces.

Over the past decade at the Bureau's Center for Materials Science, a research program in ultratrace metal speciation measurement has supported studies on uptake and biotransformations of metals that have made significant contributions to knowledge of microbe-metal interactions. For example, advances in speciation measurement techniques and monitoring instrumentation provided an early demonstrations of: biomethylation of tin (190), the occurrence and the enzymology for toxic metal resistance in ore leaching bacteria (189, 191), and the use of molecular speciation techniques in the study of the mechanism of microbial accumulation of an organometal (192).

Currently, the Bureau's Chemical and Biodegradation Processes Group is developing quantitative measurement methods to permit metal speciation on surfaces using metal fluorescent dyes and epifluorescence microspectro-photometry. These methods and associated monitoring instrumentation (for example, the coupling of a microbore HPLC effluent to a fluorescence detection system) will provide the capabilities for real-time observations on the localized uptake or biotransformation of specific metal species on natural surfaces (ores, immobilized cells), and carefully designed standardized substrates (for example, metal doped ceramics or polymers). This could in turn provide a means of assessing bioprocessing effectiveness in situ.

6. NBS ROLE IN SUPPORT OF INDUSTRY'S MEASUREMENT-RELATED NEEDS IN BIOTECHNOLOGY

A previous NBS report outlined several areas of scientific measurement and standards opportunity for NBS that were related to long-term industry needs in biotechnology (1)*. This section of this report takes into consideration only some of these areas, and, in addition, includes biotechnology instrumentation trends in order to outline possible steps NBS could take at present to respond to evolving industry needs for infrastructure support associated with biotechnology instrumentation.

It is clear from the previous sections of this report that NBS has specific capabilities that could address some of the measurement-related problems identified for the development of biotechnology instrumentation. Because biotechnology instrumentation companies have not yet articulated what specific measurement-related problems or needs have not been or will not be addressed adequately by the industry, it is difficult to identify now what specific research and service actions the Bureau should take in this area. However, in spite of the lack of a clear mandate for specific, long-range Bureau action and within the ever-present constraints associated with resource limitations and Bureau competing priorities, the following are examples of steps that could be taken now by NBS to futher clarify specific technical needs and to start the multi-year process to obtain results:

- o convene a series of NBS internal workshops to exchange ideas and information on in-house research developments and to adequately reflect the Bureau's capacity and ability to address key interdisciplinary measurement problems related to biotechnology instrumentation;

- o convene workshops at NBS, as part of the Bureau's Biotechnology Seminar Series, in cooperation with industry and Bureau Advisory Panels for assessing critical bioprocess parameters and variables and the instrumentation needs associated with their measurement;

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*Section 9, pp. 146-148.

- convene a series of meetings between NBS scientists and R&D scientists with biotechnology instrument manufacturers on generic measurement-related problems perceived as barriers to the development and use of improved instrumentation;

- encourage Bureau scientists to participate in and to present research findings at biotechnology-related scientific meetings;

- initiate discussions with other Federal agencies on where collaborative research efforts and the provision of other services related to biotechnology could be started (for example, Bureau capability to evaluate biological and non-biological mediation in measurement process);

- initiate a process to identify non-clinical standard reference material and data needs for industrial biotechnology instrumentation; and

- begin a research effort in bioprocess measurement technology with an emphasis on the characterization of monitoring/control and separation systems.

Implementation of these steps would rely primarily on existing Bureau capabilities. Many of the capabilities required for implementing these steps reside in NBS Centers for Analytical Chemistry, Chemical Physics, Materials Science, and Chemical Engineering. The success of Bureau activities in this area will depend on inter-center cooperation and interdisciplinary communication, and on cooperation and collaboration among Bureau scientists and technical staff and between NBS and industry researchers. A projected outcome of these initial activities will be better Bureau and industry perceptions of: (a) where industry will underinvest in the measurement-related problems identified in this report, (b) the generic nature of the measurement problems to be addressed, and (c) specific NBS program activities to address these problems within the context of the Bureau's mission.

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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)*

Instrumentation developments in biotechnology are essential to maintaining the flow of research findings through commercial operations to the marketplace. This report identifies several measurement-related problems associated with industrial biotechnology instrument needs, and discusses appropriate research and service activities NBS should undertake in order to be responsive to some of this industry's infrastructure technology needs.

The report highlights several areas: (a) the highly competitive and rapidly evolving biotechnology instrumentation market; (b) biotechnology instrumentation market projections; (c) instrumentation developments (for example, monitoring and control technologies and separation technologies) considered essential for bioprocess optimization; (d) specific NBS scientific capabilities in bioanalysis, electrochemistry, etc. related to instrumentation measurement-related needs; and (e) steps NBS could take to begin to address these needs.

An extensive bibliography is provided.

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

biotechnology; market projections; measurements; monitoring and control technologies; National Bureau of Standards; scientific instruments, separation technologies.

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