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WORKSHOP ON BIOTECHNOLOGY IN AGRICULTURE:

Summary Report,

Jakarta, Indonesia
March 13–14, 1986

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PREFACE

In 1985, the National Research Council of Indonesia (DRN) invited the Board on Science and Technology for International Development (BOSTID) to join it in sponsoring a workshop on the application of biotechnology to agricultural development. This workshop was held in Jakarta, Indonesia, March 13-14, 1986. The primary objectives of the workshop were to exchange information and experience on applying biotechnology to agriculture, to discuss how the present state of and future prospects for biotechnology could lead to increased agricultural production in Indonesia, and to set priorities for the application of biotechnology to agriculture in four areas: embryo transfer and animal production, plant cell and tissue culture, biological nitrogen fixation, and bioconversion of agricultural by-products.

Agricultural development has high priority in Indonesia. It is key to fulfilling the basic needs of its people and providing prosperity for the country. Such development faces great challenges, however, since the demand for food--in relation to both quantity and quality--continues to rise. The tremendous amount of existing agricultural by-products, now underutilized, could be used to produce food, feed, and other industrial products.

Particular attention was given in the workshop to the industrial potential of products that may arise from the application of biotechnology as well as how to increase agricultural output. Attention was focused not only on near-term prospects, but also on the longer term which will be affected by the use of biotechnological techniques.

These activities were one activity in a larger program of cooperation between BOSTID and the Indonesian government. Begun in 1968, this program has featured a series of workshops on food policy, industrial and technological research, natural resources, rural productivity, manpower planning, and marine algae biotechnology. BOSTID's participation has been supported in the context of a science and technology loan from the U.S. Agency for International Development (USAID) to the government of Indonesia. The current two-year program with BOSTID calls for a number of activities (panel discussions, workshops, follow-up activities, or small advisory groups) to be organized each year.

ORGANIZATION OF THE WORKSHOP

This workshop was organized by the National Committee for Biotechnology of the Office of the Minister of State for Research and Technology under the sponsorship of the Indonesian National Research Council.

Prior to the workshop, a one-day visit was made to the Marihat area to meet with officials of the oil palm estate, research center, and palm oil factory located there. The next two days were spent in the Bogor area visiting officials of Bogor Agricultural University, staff of the Bogor Research Institute for Estate Crops, several dairy farms, as well as Tapos, the personal farm of President Suharto. All of these site visits were arranged by the staff of the Biotechnology Organizing Committee.

Dr. A. M. Satari, on behalf of the steering committee, convened the workshop in plenary session on the morning of March 13 (see Appendix A). Mr. Richard A. Cobb, chief of the Office of Agriculture and Rural Development of the USAID Mission in Indonesia, commented on the timeliness of these discussions given that biotechnology will likely become the foundation of a new revolution in agricultural research and development through the remainder of the century (see Appendix B).

Dr. Doddy A. Tisna Amidjaja, vice-chairman of the DRN, officially opened the workshop on behalf of Dr. B. J. Habibie, minister of state for research and technology and chairman of the DRN. He then described the long relationship between Indonesia and the U.S. National Research Council. He indicated that Indonesia's future development will depend heavily on its ability to use science and technology effectively, especially in the utilization of its endowment of natural as well as human resources. He expressed his hope that recommendations from this workshop would focus on program priorities, research and development needs, and manpower development (see Appendix C).

Papers prepared for the workshop compose Part I of this report. During the workshop, participants broke into four working groups which addressed the four specific areas of concern: embryo transfer and animal production, plant cell and tissue culture, plant nitrogen fixation, and bioconversion of agricultural by-products.

On March 14, the conclusions and recommendations of the four working groups were presented by the chairperson of each group. Dr. Charles C. Muscoplat, chairman of the U.S. National Research Council (NRC) panel, spoke on behalf of his U.S. colleagues about the various steps needed for Indonesia to further develop its capability in biotechnology. His comments are included in Part III of this report. The workshop agenda and a list of the participants are included as Appendixes D and E, respectively.

This workshop report was prepared by Rose Bannigan of the BOSTID staff using papers written by the Indonesian and NRC workshop participants. The papers have been edited to eliminate duplication, but they accurately reflect the discussions. The final draft was reviewed and approved by the members of the NRC panel and the Indonesian organizing committee. Sabra Bissette Ledent, BOSTID consultant, edited the report.

Participants would like to acknowledge the valuable contribution of the workshop's organizing committee to the final arrangements for the workshop as well as to the site visits to Marihat and Bogor. The organizing committee was chaired by Dr. Haryanto Dhanutirto. Special thanks is due to Ir. Oestara Wiradinata, director of Utama PT Perkebunan, and his colleagues for receiving the visitors to the oil palm estate and industry and for their generous hospitality. Appreciation is also extended to officials at Tapos for hosting the visit to their cattle breeding experiment station.

Finally, the participants would like to thank the members of the workshop secretariat for the excellent organization of the workshop. The secretariat was under the supervision of Drs. Jana Anggadiredja.

CONTENTS

PART I

Overview

NATIONAL PROGRAM FOR THE DEVELOPMENT OF BIOTECHNOLOGY

Didin S. Sastrapradja
Assistant II Minister for Research and Technology/
Chairman, National Committee on Biotechnology

Biotechnology, which integrates several sciences, plays a significant role in development worldwide. Research in this field has moved rapidly, and its application has produced new biotechnologies or bioindustries. Many technological problems remain, however, in applying biotechnology.

The government of Indonesia has determined that the development of biotechnology is a national priority. It will be utilized for supporting national development needs on an industrial scale in the areas of agriculture, health care, chemical manufacturing, food production, and environment.

APPLICATION OF BIOTECHNOLOGY IN INDONESIA

In Indonesia, biotechnology is part of the activities traditionally associated with the fermentation of solids or liquids to produce food and beverages. This traditional technology has several characteristics: small investment, labor intensive (using skilled and unskilled labor), small scale, low budget, and low value added. Currently, medium-scale industries using biotechnological techniques produce alcohol, beer, citric acid, monosodium glutamate, liquid sugar, and single-cell protein.

Biomedical products for humans have been produced for decades in Indonesia at Biofarma in Bandung, but the production process used presently needs improvement by introducing recently developed, sophisticated technologies. Biomedical products for animals have also been produced in Indonesia, with the use, however, of old technological processes.

BIOTECHNOLOGICAL RESEARCH IN INDONESIA

In general, research is dominated by efforts to develop materials standards for use in the traditional fermentation processes; to develop inoculum through isolation, identification, and screening of domestic microbes; and to learn more about the variables of the fermentation processes.

Recently, research has been expanded to cover the sectors of health care, agriculture, and industry. This research has centered around the search for a microbial strain that produces a secondary metabolite, bioengineering (fermentation conditions, reactor design, the post-fermentation process, the computerized fermentation process), tissue culture, biogas, and waste processing. In the future, this research will be directed toward large-scale applications.

Research units supporting biotechnology in Indonesia include:

- The Agency for the Assessment and Application of Technology
- Universities such as the University of Indonesia, Bogor Agricultural University, Bandung Institute of Technology, and Gadjah Mada University. These universities form the Inter-University Center of Biotechnology.
- Research institutes under the Indonesian Institute of Sciences, including the National Institute of Chemistry, National Institute of Biology, and Center for Research and Development in Biotechnology at Cibinong (planned)
- Research institutes under the Department of Agriculture, including the Sugar Research Institute in Pasuruan, Estate Crops Research Institute, and Food Research Institute.

Organizations involved in the production of biotechnologies include the Biofarma in Bandung (state owned), Veterenary Farma in Surabaya (state owned), various food and pharmaceutical industries (privately owned), and the research institute in the Department of Health.

NATIONAL PROGRAM ON BIOTECHNOLOGY

The objectives of the national program on biotechnology are to formulate a national plan of action and policy; to promote the role of biotechnology in agriculture, health, and industry; and to encourage R&D in biotechnology and its application. The targets of the national program are:

- To increase the number of industries based on the application of biotechnology to produce goods and services
- To develop R&D capabilities in all fields of study related to biotechnology that could support a sustainable national bioindustry.

Meeting these targets requires application of a four-stage program for technological transformation in biotechnology:

- o Stage 1. Transfer of technology where the development of bioindustries requires the direct assistance of foreign technologies. At this stage, biotechnology is imported for immediate production of high-value added goods and services. This simultaneously provides opportunities to understand the design and techniques of the imported biotechnology.

- o Stage 2. Technological integration of biotechnology to develop a new design or blueprint for the development of a new biotechnology.

- o Stage 3. Technological development of the new biotechnology to afford a comparative superiority in bioindustry.

- o Stage 4. Basic research to support the future development of biotechnology.

Possible steps needed to develop biotechnology in Indonesia are: mobilization of the existing national capabilities in biotechnology, promotion of the utilization of existing biotechnological processes as well as R&D on new processes, and development of scientific and skilled manpower through special training and education.

In undertaking these steps, the following should be kept in mind:

- o The limitation of resources, including facilities, manpower, and funding, in each institution.

- o The purpose and function of each institution such as basic research, application, and education.

- o The weakness of the private sector which will likely be the consumer and marketer of products created by the institutes.

CENTER FOR RESEARCH AND DEVELOPMENT IN BIOTECHNOLOGY AT CIBINONG

Because development of the "new" biotechnology requires a large capital investment and substantial well-qualified manpower, it is more economical with the present situation in Indonesia to establish a new, well-equipped, and well-funded research and development center for biotechnology (the Center for Research and Development in Biotechnology at Cibinong) than support the existing R&D institutes under various government organizations. The objectives of the Center are to develop the national capability to conduct research and development in the "new" biotechnology and genetic engineering and to provide the sophisticated facilities needed for this undertaking. In the long run, it is expected that the biotechnological approach can be utilized to increase and promote the economic value of natural resources for food, feed, medicines, chemicals, and energy.

In developing the Center, the following guiding principles will be observed:

- The activities of the Center will be cross sectoral in character.
- The program of the Center will become the focal point of a national network in biotechnology.
- The Center's activities will produce information and products (prototypes) that are low volume/high value in character, with extensive market potential and usable for scaling-up studies.

The functions of the Center will be to:

- Develop and disseminate appropriate applications of modern biotechnology.
- Act as a clearinghouse in biotechnology.
- Offer its facility for internships and training in biotechnological research.
- Develop and perform the detailed planning of biotechnological manufacturing plants.

To realize its objectives, the Center will formulate selected research and development projects that support national programs in the fields of family planning, health care, food production, animal production, and the manufacture of pharmaceuticals and other biologically active compounds. Research will encompass microbiology, plant cell and tissue culture, genetic engineering, analysis, fermentation techniques, downstream processing, and lab-scale pilot studies.

The Center will be located on 200 hectares of government land at Cibinong which is situated between Jakarta and Bogor. Pharmaceutical and food processing factories, related research laboratories (agriculture, biology, health, animal husbandry), and universities are located nearby. The following facilities are planned for the future:

- Laboratory of applied microbiology and tissue culture
- Laboratory of biochemistry and molecular biology
- Processing laboratory
- Laboratory of limnology
- Supporting facilities (water treatment, greenhouses, workshop, staff housing).

During the first and second five-year development phases, the Center will be funded by the government of Indonesia and foreign assistance. Starting with the third five-year development phase, however, the Center is expected to be gradually able to finance its

activities through contract research and the provision of facilities as well as consultants in the field of biotechnology. It is expected that this ability will increase with time.

BIOTECHNOLOGY IN AGRICULTURE

Charles C. Muscoplat
Chairman, NRC Panel, and
President, Molecular Genetics, Inc.

Biotechnology will have a major impact on the future of world agriculture. Technologies such as embryo transfer and genetic engineering to produce new vaccines, drugs, antibiotics, and hormones for animal production; cell and tissue culture of important crop species; nitrogen fixation; and industrial fermentation will be key to increasing world agricultural productivity. Coupled with better farm management systems, better hygiene, conservation of natural resources, and development of new fertilizers and crop chemicals, these technologies can mean tremendous improvements in agricultural productivity.

The goals of these new technologies must be to increase agricultural productivity; to provide for a stable, high-quality, nutritious food supply; to protect international trade by safeguarding our livestock and crops from disease and toxic substances; and to preserve natural resources and safeguard the environment. Increased agricultural productivity will help feed the hungry and will improve the economics of agriculture-based countries by substituting technology for subsidies and preserving financial resources which can then be used to benefit the population in other ways.

The four technologies covered in this workshop are critically important to the advancement of world agriculture. Embryo transfer and techniques to improve animal production, plant cell and tissue culture, nitrogen fixation, and industrial fermentation can work together in a complementary fashion to improve agriculture. This paper briefly reviews each of these technologies and gives examples of how each technology can benefit world agriculture.

EMBRYO TRANSFER AND ANIMAL PRODUCTION

Perhaps one of the most exciting fields in biotechnology today is embryo transfer and animal production. Embryo transfer is becoming widely available, and in many parts of the world it is now a commercial reality. The advantage of the advances achieved recently in embryo transfer over classical breeding is the rapidity with which superior genetic traits can be established within a livestock population. Transfer of improved productivity genes into livestock germ plasm will

be achieved by the end of this decade. Once new livestock genotypes are established, embryo transfer is the only practical way to disseminate the new germ plasm quickly worldwide. It will then be essential that countries with intensive livestock production become skilled in the techniques of embryo transfer.

Techniques of molecular biology and genetic engineering are already a commercial reality and promise to be as important as plant cell and tissue culture, nitrogen fixation, and embryo transfer. Genetic engineering will allow the production of improved vaccines for infectious diseases such as foot-and-mouth disease and swine fever rinderpest. It will also soon provide an array of pharmaceutical products for increasing livestock productivity. Some examples are animal growth hormones for increased growth and milk production; fertility hormones for increased fecundity, ovulation, and conception as well as synchronization; and immune-modulating proteins such as interferons, interleukens, and related proteins necessary for disease resistance. Monoclonal antibodies will provide the tools needed for rapid diagnosis and detection of specific diseases as well as for specific passive antibody therapy. Another exciting use of the new technology will be the application of genetic engineering to aquaculture.

PLANT CELL AND TISSUE CULTURE

The process of culturing plant cells *in vitro* is becoming better understood and widely available. It involves the *in vitro* propagation of either plant embryonic or other plant organ system tissues. The main purpose of the technology is to introduce into agronomic plants altered genetic traits that provide some production advantage. Specifically, by exploiting somaclonal variation in plant cell culture, scientists can increase the genetic diversity of plants and widen the sources of germ plasm used for major crops. Currently, many crops are produced from very few or even single cultivars, thereby exposing producers to high risks that a single disease could destroy an entire crop species. Broadening the basis of genetic diversity will reduce risk as well as allow selection for improved traits.

Advanced techniques of plant cell culture can be used to alter specific single-gene traits such as selection for resistance to various crop chemicals, thereby giving the producer a wider choice of products and procedures for each type of agricultural operation, or increasing specific amino acid production to improve the nutritional quality of plants. One of the most exciting areas of investigation currently is the selection of plants resistant to viral, fungal, or even insect diseases. In the future, agricultural crops developed by tissue culture techniques may contain a combination of improved traits such as improved nutritional quality for both livestock and humans or tolerance of crop chemicals or toxic soil chemicals (the aluminum toxicity encountered in Brazil and other parts of the world, for example).

NITROGEN FIXATION

Most crops require increased nutritional support through the application of chemical fertilizers. For the most part, these chemicals consist of potassium, phosphorus, and nitrogen. Potassium and phosphorus are taken from the ground through mining, whereas nitrogen fertilizers are manufactured from natural gas utilizing atmospheric nitrogen. Fertilizers have been the single most important factor in increasing crop production. In combination with improved germ plasm, herbicides, insecticides, and soil conservation, nitrogen-based fertilizers have significantly boosted agricultural productivity worldwide.

Fertilizers add significant cost to crop production. In some parts of the world, nitrogen fertilizers are expensive, difficult to obtain, or simply unavailable for a variety of reasons. Theoretically, eliminating or reducing the need for nitrogen will improve both the economics and productivity of the important nonleguminous crops. Although the development of genetically engineered, nitrogen-fixing crops appears to be many years from being practical, such a development promises to be one of the most important advances in all of plant agriculture. Research is currently directed toward developing improved nitrogen-fixing bacteria, genetically altering plants, and developing plants better able to use conventional fertilizers.

INDUSTRIAL FERMENTATION

The fermentation process is vitally significant worldwide. Examples of industrial fermentation products of importance are single-cell proteins for nutritional supplementation, antibiotics, drugs, hormones, food products such as microbial rennens for cheese production, alcoholic beverages, and specialty chemicals. In addition, nearly all the genetically engineered products described above will be produced using the fermentation process. Thus, there is no doubt about the critical need to develop improved industrial fermentation processes.

In addition to the uses listed above, the creative use of fermentation techniques may also partially solve the important problems of agricultural waste. For example, although the large amounts of waste by-products associated with animal, crop, and food production may not be suitable for commercial use as they are, it may be possible with fermentation techniques to convert these products into either useful end products or valuable intermediates. The important challenges facing fermentation engineers are how to design both new microbes that facilitate appropriate bioconversions and the fermentation apparatus and its operation. It will also be important to address the difficult problems associated with materials handling in the bioconversion of agricultural wastes into useful end products.

CONCLUSION

Many challenges are facing agriculture today in the form of ever-increasing demands for agricultural production, diminishing natural resources, and concerns about the environment, transportation, international trade, and geopolitics. Farms are changing rapidly from single farmer operations to large industrial ones. The future will require complex management of scientific as well as financial and logistical support.

Each of the technologies described above can provide agriculture with a significant advantage. But achieving excellence in each of these areas will not be easy. It will require cooperation at all levels of government, industry, and academia worldwide. Because research is becoming increasingly expensive and difficult, it is becoming more difficult to purchase the necessary high-technology equipment, to build the proper facilities, and to train quality scientists. It is thus wise to form collaborative arrangements among countries, governments, industries, and universities and thereby take advantage of different areas of expertise found in each country and organization. Such a step would also reduce the financial cost of worldwide agricultural development, facilitate scientific exchange, and promote the acquisition of new knowledge. It would, in addition, build worldwide agricultural and technological alliances which will extend beyond agriculture and may serve as the basis of broader cooperation.

In the United States, cooperation is slowly developing among government, industry, and academic scientists. Regulatory agencies are becoming increasingly skilled at understanding and regulating products developed through new genetic technologies. Professional societies are beginning to facilitate greater scientific exchange among scientists on a global basis. It is important that this continue.

It is also important that an efficient patent system be developed to protect inventions arising from biotechnology. Such protection will ensure that industries invest research monies in continued product development and improvement.

In summary, the future of agriculture will be exciting. We must take care, however, to focus our efforts and financial resources carefully on a limited number of practical areas to avoid the dilution of effort that results in slower overall development.

THE BIOTECHNOLOGY INITIATIVE IN NORTH CAROLINA

Richard J. Patterson
President, North Carolina Biotechnology Center

The North Carolina Biotechnology Center is the first state-established center of its kind in the United States and, like that of several of its successors in other states, its mission is economic development from biotechnology. In pursuing its mission, the Center recognizes that biotechnology is expected over time to have a greater and greater economic impact in the United States and around the world. In North Carolina, the aim is to develop the Center's resources and capabilities so that the state will directly benefit from biotechnology.

HISTORY OF THE CENTER

The North Carolina Biotechnology Center was established in 1981 under the Governor's Board of Science and Technology. The idea behind this initiative was that a state investment in biotechnology would, in the long term, provide a major economic benefit to the state. Earlier state investments in other technologies established a favorable climate in which to start the Center. The first steps in its establishment were to assess thoughtfully research in biotechnology, look at where the biotechnology industry was and where it saw itself going in the future, examine the economy in North Carolina and its resource base, and establish the ability to target and to focus the Center's activities.

One of the first major undertakings was an inventory of researchers in biotechnology. This inventory characterized their expertise and identified the areas in which they were doing research. The first time this inventory was undertaken, it identified about 150 researchers in N.C. universities who were actively involved in biotechnological research. The second inventory, undertaken about a year and a half ago, identified more than 300 researchers in biotechnology or, it is estimated, about half of the manpower resource. Simultaneously, N.C. industries and companies that are either actively involved in biotechnology or likely to feel the impact of biotechnology were queried. This then identified the private sector element involved in biotechnology.

FUNDING

Funding for the Biotechnology Center has increased gradually. Initially, funding was about $200,000 a year from the Governor's Board of Science and Technology. In 1983, the Center received its first direct funding ($500,000) from the state legislature. Because the legislature was becoming increasingly interested in this investment in biotechnology, it established a legislative study committee to examine where biotechnology was today and to learn where those with expertise saw it going in the future. The study committee conducted almost two years of hearings, involving experts in research, business, and finance. As the committee listened to testimony, it heard about biomedicine, crop and animal agriculture, and forestry and marine industries--all of which are very important to the economy of North Carolina. This combination represents many opportunities, and the challenge then was determining what level of investment was necessary for the state to benefit. At the end of the two years of testimony, the study committee recommended that North Carolina invest almost $70 million in biotechnology over a five-year period. As a result, the budget of the Biotechnology Center for 1985-1986 is $6.5 million.

In 1984, the North Carolina Biotechnology Center was reorganized as a private corporation, giving it the flexibility to work with the private sector and with private and public universities and to pursue opportunities that were not possible when the Center was part of state government.

RESOURCES

The North Carolina Biotechnology Center is not building a research facility. Because the facilities of North Carolina research universities are extensive, the Center determined that the most effective way to marshal its resources and to have its desired impact was to invest in research activities in the universities rather than develop a separate activity that would possibly compete with the universities. The universities are key to the Center's efforts, and a major thrust of the Center is to increase their capabilities in biotechnology. This includes attracting new faculty. For example, North Carolina State University was recruiting a senior faculty member in plant molecular biology, which attracted a senior academician, Dr. William Thompson, from the Carnegie Institute in Stanford, California. Dr. Thompson left an attractive situation at Carnegie to come to North Carolina for scientific reasons. That is, North Carolina State University has an outstanding plant breeding capability, and he wanted to collaborate with plant breeders who understood crops as well as the traits on which a molecular biologist should be working.

PROGRAMS

Monoclonal Lymphocyte Technology Center

The Monoclonal Lymphocyte Technology Center is actually based in three universities: North Carolina State University, Duke University (private), and the University of North Carolina at Chapel Hill. Funding for the Center is provided by the National Science Foundation, sponsoring companies, and the North Carolina Biotechnology Center. Its present annual budget is about $675,000.

The Technology Center's objective is to undertake basic research that is relevant and of particular interest to industry. Thus, the sponsoring companies are critical in selecting research projects for the Center and in monitoring their progress. Over time the number of sponsoring companies will likely increase, and there will be a turnover in projects as the frontiers of science change. As the needs of industry change, the basic research of industrial interests will take on a different complexion as well.

Plant Molecular Biology Consortium

The Plant Molecular Biology Consortium is also based at the "Triangle Universities": Duke University, North Carolina State University, and the University of North Carolina at Chapel Hill. A major activity of the Consortium is a fellowship program for graduate and postgraduate training. Funding is provided by the Biotechnology Center and companies that sponsor the fellowship program. These fellowships are used to attract the very best people in the country to these universities for graduate and postgraduate training. The Consortium also sponsors seminars which bring this group together monthly.

The Biotechnology Center's competitive grants program is aimed at young scientists, or scientists moving into new fields, and at collaborative research--that is, collaborative research among university investigators and among university and industrial researchers. Since 1982, the Consortium has identified $16 of new research investment for particular research projects for each dollar of early investment. The resources of the entire state university system are drawn upon to conduct a peer review of research proposals for their scientific quality. This review process is important because it draws the Center much closer to the individual researchers in the fields that make up biotechnology.

Biomaterials Engineering Program

This program includes nine scientists at four different universities as well as one research institute funded by the Office of Naval Research. These individuals came together at a workshop sponsored by the Biotechnology Center and realized that they had

several shared interests. By putting these interests together, these scientists were able to attract a $650,000 three-year grant. They meet monthly to share the results of their research, and they have now written two new grant proposals to take their research to an even higher level.

Other Programs

Bioprocess engineering is critical to biotechnology. Because U.S. research institutions are lacking in bioprocess and biochemical engineering, many have increased their efforts to develop a greater capability for bioprocessing research. North Carolina universities are committed to bringing their capability up to a level considered a center of excellence. Since most products of biotechnology are the result of some type of engineered process, this expertise is critical to commercial success.

In the area of education-based activities, several state universities are now faced with a challenge; many do not have faculty qualified to teach their undergraduates or to conduct laboratories in molecular biology. Thus, the Biotechnology Center is working with these institutions to develop their educational capabilities in biotechnology.

Another Center educational program conducts training in the two-year community college system. One institution has a program for training technicians in biotechnology, chemistry, and biology. This college involved industry extensively in developing this curriculum, which was accepted by the community college system. In the fall of 1985, the first students started courses. This curriculum could be offered at any community college, particularly if a business needed trained personnel.

The Center is also addressing the need for better education in molecular biology in state primary and secondary schools, and it is supporting achievement of this goal through the science and math education initiatives being undertaken in North Carolina.

INVOLVEMENT OF INDUSTRY

One theme running throughout this discussion of the Center's activities is the involvement of industry. This involvement is very important in targeting the Center's programs, so that the activities undertaken are particularly relevant to the needs of industry and do not duplicate their efforts.

PART II

Working Group Reports

EMBRYO TRANSFER AND ANIMAL PRODUCTION

Use of Biotechnology and Genetic Engineering to Improve Animal Health and Agriculture

Charles C. Muscoplat
President, Molecular Genetics, Inc.
and
Anthony J. Faras
Professor, Department of Microbiology,
University of Minnesota Medical School

> Life is the evolution of molecular machines.... The beauty of something is not the atoms that go into it, but the way they are arranged.
>
> Carl Sagan

INTRODUCTION

Since prehistoric times, man has marveled at the influence of heredity. In his own offspring as well as those of domestic animals and fruits of the field, family resemblances have appeared and disappeared with mysterious predictability. As civilization progressed, the rudiments of genetic manipulation were learned and passed on from teacher to student. Tablets from ancient Babylonia show a sophisticated awareness of horse pedigrees, and carvings from a later date indicate the cross-pollination of date palms. Genetic engineering had begun.

Europe in the nineteenth century proved fertile ground for advances in understanding the genetic process. Jean-Baptiste Lamarck, a French scientist, suggested that an organism's characteristics were inherited from its parent cells. Half a century later, the Austrian monk Gregor Mendel discovered that heredity obeys precise laws of statistics. Mendel theorized that plant characteristics result from paired carriers of heredity. Today, these elements are known as genes. The contributions of Lamarck and Mendel were explored and expanded upon during the next hundred years.

DNA (deoxyribonucleic acid) was ultimately isolated as the carrier for heredity, and in 1953 scientists discovered its molecular structure. These revelations opened the door to an unprecedented explosion of scientific knowledge, the most recent of which includes the biotechnological developments of recombinant DNA technology or gene splicing (altering heredity by transplanting genes from one organism into another). Out of these scientific advances emerged today's burgeoning industry of genetic engineering--the directed manipulation of genetic materials to develop commercial products and processes.

Further advances in immunology have been responsible for the development of hybridoma technology in which highly specific antibody molecules, termed "monoclonal antibodies," have been responsible for an additional component of the new biotechnology.

The impact of these technologies has been far-reaching with the most rapid developments occurring in human pharmacology and agriculture. In fact, developments in the agricultural field are occurring so rapidly that genetically engineered products should be on the market before the end of the year. Animal production will gain the most benefits from these developments through a variety of approaches, including: (1) the use of genetically engineered efficacious vaccines and antitoxins to prevent infectious disease and thereby reduce animal losses, (2) the use of growth promotants to increase production of beef protein and milk, and (3) the use of nutritionally improved animal feed. The following sections describe the basic features of these new biotechnological developments and discuss the ways in which they have already contributed, or will contribute, to the improvement of animal health and production.

RECOMBINANT DNA AND HYBRIDOMA TECHNOLOGIES

Recombinant DNA Technology

Recombinant DNA technology, considered a modern-day form of genetic engineering, is not a single discipline in itself. Rather, it represents a fusing of ideas and techniques from biochemistry, molecular biology, genetics, and organic chemistry. It involves the restructure and editing of genetic information and the construction of microorganisms with new genetic information. Extremely powerful, this technology allows one to isolate genes from any source (viruses, bacteria, fungi, plants, animals), amplify isolated genes to unlimited quantities with economic benefits through fermentation, and finally, manipulate genes by mutating or rearranging their components for the development of hybrid or novel gene products.

If a single breakthrough in gene splicing were to be identified, it would be the identification and isolation of specialized enzymes, known as restriction endonucleases. These enzymes act as biological scissors able to cut chromosomes and DNA into unique pieces, and they enable the isolation of specific genes or gene fragments. Since restriction endonucleases make staggered breaks in DNA at sites exhibiting twofold rotational symmetry, the result is a piece of DNA with complementary cohesive ends which can then be, by virtue of these "sticky" ends, inserted into or recombined with another piece of DNA that has been cut by the same enzyme. Since there are well over a hundred different restriction endonucleases and since each enzyme recognizes specific, but for the most part different, sites on DNA molecules, these enzymes can be used to cut DNA into a variety of pieces containing one or more of the gene(s) of interest.

The basic recombinant DNA experiment is depicted in Figure 1. The essential ingredients of this technology include: (1) a DNA vector which generally represents the chromosome of either a plasmid which is autonomously replicating DNA molecules found in bacteria and yeast, or a virus which can infect bacteria or higher organisms (vectors must be able to replicate in living cells after foreign DNA is inserted into them); (2) a DNA fragment to be inserted into the vector; (3) a method of joining the inserted DNA to the vector; (4) a method of introducing the joined molecules (recombinants) into a host that can replicate them; and (5) a method of detecting those cells that carry the desired recombinant DNA molecule.

Once the vector carrying the inserted foreign DNA molecule is placed into an organism such as bacteria or yeast, it will replicate to

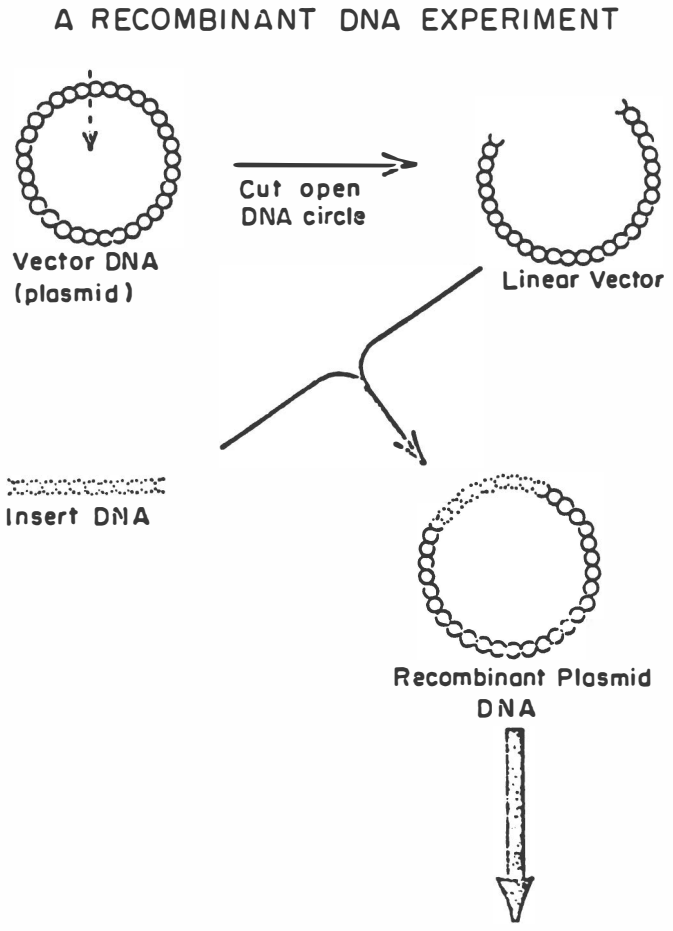

FIGURE 1 Basic recombinant DNA experiment.

make many copies of itself and the foreign gene insert, thereby providing an unlimited supply of the gene of interest. For the foreign gene insert to be expressed into protein in the bacterial cell, certain features important to the bacteria's biosynthetic machinery must be available to the gene (Figure 2). For example, appropriate recognition signals for both bacteria-mediated transcription (RNA production) and translation (protein production) must be present.

In addition to recombinant DNA techniques, two developments, both in organic chemistry, have greatly facilitated progress in genetic engineering. The first involves chemical methods to synthesize genes or gene fragments de novo in an effort to modify or alter genes. These methods enable the gene to be created chemically from knowledge of the sequence of the amino acids in the protein encoded for by the gene of interest. The second development involves chemical methods to synthesize peptides and small proteins de novo, allowing the generation of peptides containing active sites or antigenic determinants from knowledge of the nucleotide sequence of the gene. In an effort to make synthetic peptide vaccines, these developments have been employed recently to determine the specific regions of a viral protein molecule important in generating antibodies that neutralize an infectious virus.

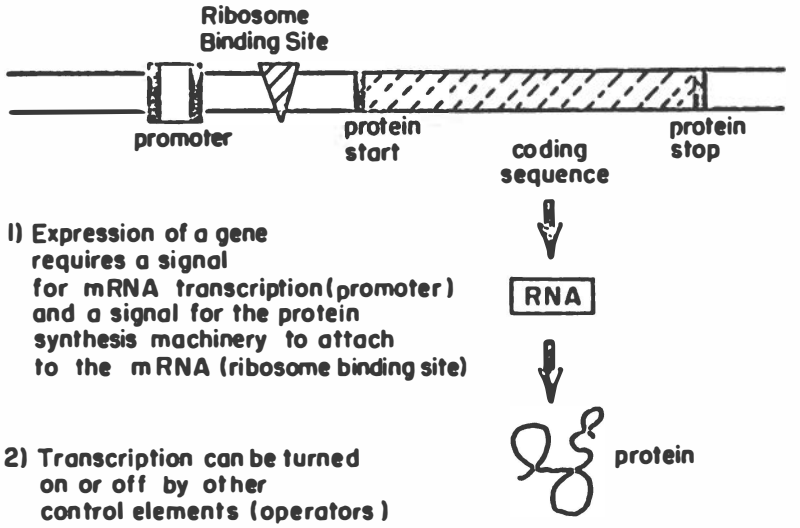

FIGURE 2 Typical bacterial gene expression.

Hybridoma Technology

The other major biotechnological development that will affect animal health and production is hybridoma technology. This technique results in the generation of monoclonal antibodies by cell fusion procedures. It will be useful in diagnosing specific diseases as well as therapeutically preventing and curing diseases affecting the morbidity and mortality of farm animals. Moreover, given their tremendous specificity these monoclonal antibodies will be useful in the purification of various genetically engineered products following fermentation in bacteria or yeast. This procedure involves fusing spleen cells from mice immunized with an antigen to which a monoclonal antibody is desired to mouse myeloma cells in culture. The fused cells are then screened for production of the specific monoclonal antibody with the labeled (radioactive or dye) antigen. The myeloma cells serve to immortalize the spleen cells so that they may be maintained indefinitely in cell culture. Special procedures are employed such as the use of myeloma cells requiring certain growth factors provided by the spleen cells fused over unfused myeloma cells. The monoclonal antibodies can then be obtained by harvesting the liquid medium from the cell cultures or by inoculating the fused cells into the peritoneum of mice and collecting the fluid present after ascites tumors have developed.

Vaccines and Antitoxins

One of the major and earliest ways in which recombinant DNA and hybridoma technologies will improve animal production is by providing the animal health care industry with efficacious vaccines and antitoxins that will reduce morbidity and mortality from infectious disease. Of the 45 million cattle born last year in the United States, approximately 10 percent died of infectious disease. Of the 94 million swine, up to 15 percent died of infectious disease. These losses occurred despite the use of conventional vaccines and large doses of antibiotics. Because antibiotics are an ineffective means of reducing the severity of diseases caused by viruses, many virus-induced diseases go unchecked.

Recombinant DNA procedures will enable the development of vaccines for infectious agents that grow poorly or not at all in cell culture, thereby obviating the availability problem of these agents. Moreover, a genetically engineered subunit vaccine will exhibit potency and efficacy, as well as safety, ease of manufacture, and economy of production. Immunologically, one must be able to administer the genetically engineered vaccine in a single dose and induce immunity of long duration. It must protect against all serotypes in a given geographic region and must not induce adverse reactions. In addition to these biological features, a genetically engineered vaccine will

exhibit several attractive manufacturing considerations including economy, long shelf life, lack of infectious virus, and stability at ambient temperatures.

The basic protocol for developing genetically engineered vaccine includes: (1) identification of the major surface antigen of the pathogenic organism of interest which will induce antibody capable of neutralizing or inactivating the infectious organisms; (2) identification of the surface antigen gene or its specific antigenic determinants; and (3) isolation and transfer of this gene into a plasmid vector capable of expressing large amounts of its product in fermentable organisms such as bacteria or yeast (Figure 3). Such methodologies have been employed to generate large amounts of vaccine proteins against bovine papillomavirus, porcine parvovirus, canine parvovirus, foot-and-mouth disease virus, and K-99 E. coli. Although results on the potency and efficacy of these vaccines are presently awaiting completion of preclinical, clinical, and field trials, preliminary tests on several of these genetically engineered subunit vaccines indicate that they are excellent immunogens that exhibit all of the favorable features predicted.

Monoclonal antibodies have also been generated for protection of newborn calves and swine against enteric collibacillosis, which is responsible for neonatal diarrhea or scours. Although both conventional and genetically engineered vaccines are available, the monoclonal antibody approach appears to be far superior to vaccination for two reasons. First, vaccination of the dam requires anticipating the problem, which may not be feasible, and, second, it requires maintaining breeding records since the vaccination must be given twice, at six and two weeks prior to birth. Because scours usually occurs within the first 24 hours of life with susceptibility to the disease being markedly reduced after 24 hours of life, and the action of the pathogenic bacteria is restricted to the intestine, oral administration of a protective monoclonal antibody to provide passive immunity within 24 hours of birth serves to protect newborns on farms where the disease is prevalent from developing the disease. In both preclinical and clinical testing to date, a monoclonal antibody against the K-99 strain of E. coli protected animals from lethal doses of challenge with the pathogenic strain of bacteria (Tables 1 and 2). The K-99 specific monoclonal antibody appears to be group specific and capable of reacting with the adhesive entity on the pilus of over a hundred strains of E. coli. Additional attributes of this monoclonal antibody include reproducible specificity and reduced costs. The effectiveness demonstrated to date by this reagent and its ease of administration indicate that it should be an extremely useful product for curtailing scours in calves. Similar monoclonal antibodies for two additional strains of pathogenic E. coli responsible for scours in newborn piglets (K-88, K-987) have also been developed and are presently in preclinical testing.

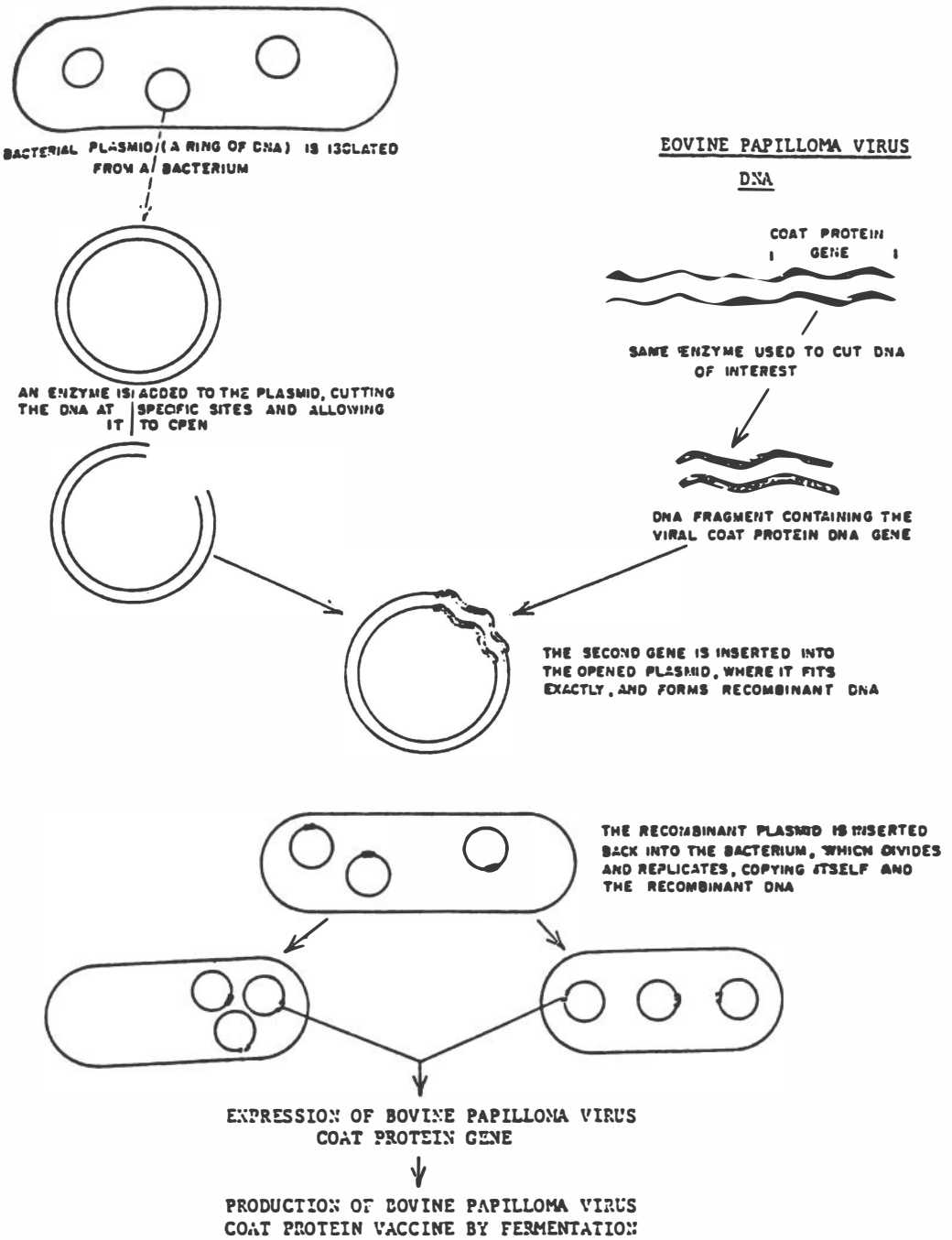

FIGURE 3 Developing a papillomavirus vaccine by recombinant DNA techniques.

TABLE 1 Protection of Newborn Pigs by the Oral Administration of a K-99 Specific Monoclonal Antibody

	Pigs	
Trial Group	Alive	Dead
Monoclonal antibody	11	3
Placebo	2	8

TABLE 2 Protection of Newborn Calves by the Oral Administration of a K-99 Specific Monoclonal Antibody

	Calves	
Trial Group*	Alive	Dead
Monoclonal antibody	9	1
Placebo	2	12

*Completed trials to date.

GROWTH PROMOTANTS

The development of natural growth hormones for livestock and poultry represents a major means of improving animal production, and genetic engineering techniques have made this development a reality for both logistical and economic reasons. Several groups have now cloned bovine growth hormone (bGH) to expression in bacteria and yeast.

The recombinant DNA procedures employed were similar to those utilized for cloning virus genes pertinent to subunit vaccine production. A mRNA species from pituitary gland enriched for bovine growth hormone nucleotide sequences is first reverse transcribed into DNA. This DNA copy is then inserted into plasmids for expression in bacteria and yeast. Because this cow bovine growth hormone gene lacks the required regulatory features necessary for these microorganisms to express this gene, some additional restructuring of the gene is required. One of these maneuvers results in the addition of an amino acid (methionine) at the beginning of the bovine growth hormone gene so that it differs slightly from naturally occurring bovine growth hormones. Despite the presence of this additional amino acid at the beginning of genetically engineered bGH, preliminary clinical studies have indicated that it is as effective as naturally occurring bGH in

the stimulation of milk production. For example, milk yields were increased by 10.3 percent for natural bGH over a six-day period of treatment and by 12.9 percent for recombinant bGH. Milk fat, lactose, and protein percentages, as well as feed intake, were not affected by the treatment. Feed efficiency (kg milk/kg feed) was improved by 9.5 percent and 15.2 percent for natural and recombinant bGH, respectively, and no adverse effects were observed based upon body temperature and somatic cell counts. Thus, recombinantly derived bGH enhanced milk production and improved feed efficiency in a manner similar to the biological responses observed with natural bGH.

Generally, engineered approaches to improving animal production appear to be directly applicable to hormones and other growth promotants where availability of the natural substance is limited and the costs of obtaining the natural hormone exceed reasonable marketing considerations. Further studies are required to determine the safety of recombinant bGH for both treated animals as well as the consumer of its milk. An additional growth promotant presently under development is porcine growth hormone.

FEED IMPROVEMENT

Corn, which is used as a major source of feed for animals, is an excellent source of energy but a poor source of protein. Poor protein quality is directly related to the deficiency of an essential amino acid such as lysine. In considering issues of protein quality, it is important to distinquish corn fed to hogs and poultry and that fed to dairy and beef animals. Because hogs and poultry have specific amino acid requirements not common to cattle and other ruminants, hog and poultry farmers must purchase additional protein to supplement a corn-based ration. An improvement in protein quality (that is, the relative amounts of essential amino acids) can have a pronounced effect on such factors as rate of gain and feed efficiency in hogs and poultry, which can in turn reduce the amount of supplement required to balance the ration.

Corn hybrids with high-quality protein are a unique example of a product that was developed using traditional plant breeding efforts and was successful in terms of protein quality and improved nutritional value but that failed because of unacceptable agronomic traits. In the early 1960s, a corn seed mutant (opaque-2) that exhibited an elevated lysine content was discovered. Since the altered amino acid composition resulted from a single gene with an easily identifiable outward appearance (opaque-2 kernels do not transmit light whereas normal kernels do), plant breeders and geneticists immediately began to introduce this gene into the agronomically important inbred lines. In general, conversion of these lines and the resulting hybrids led to improved lysine content and nutritional value as demonstrated by feeding studies. The downfall of opaque-2 in the United States came about as a consequence of depressed grain yields, poor seed quality, and greater susceptibility to insects and diseases. Thus, conventional plant breeding procedures have resulted in corn hybrids with improved lysine levels but depressed yields and low levels of grain quality and pest resistance.

Recombinant DNA technology is an important method for selection of specific desirable traits and exclusion of undesirable traits. In the case of corn, 50 percent of the bulk protein of the corn kernel is a storage protein known as zein. The amino acid composition of the zein storage protein is low in lysine and tryptophan, two essential amino acids for man and monogastric animals. Consequently, these proteins influence the nutritional quality of the corn kernel. Recombinant DNA procedures have been utilized to isolate the zein gene and determine its precise biochemical structure, and this has resulted in deduction of the primary structure of the zein storage protein. With the availability of this information, it is now possible, employing genetic engineering approaches, to alter the zein gene structure in an effort to increase its lysine content and therefore its nutritional quality. Once this has been accomplished, the high-lysine zein gene can be transferred back into corn cells and then corn plants containing a high-lysine storage protein regenerated.

Although the former task is still in the experimental stages, the latter task is not since tissue culture procedures capable of plant regeneration initiated from juvenile tissues of corn have already been developed. Stated simply, tissue culture is the process whereby large populations of cells are stimulated by nutritional and hormonal conditions to grow continuously in a defined laboratory environment. Shoot meristems develop in large numbers in these cultures. Under the proper conditions, these meristems develop rapidly into complete plants that produce seed at maturity. In fact, tissue culture corn regeneration technology has been useful in isolating mutants of corn that overproduce amino acid by virtue of the fact that these tissues randomly undergo spontaneous mutations in cell culture.

Recently, overproducer mutants from the aspartate biosynthetic pathway (responsible for the synthesis of lysine, threonine, methionine, and isoleucine) in which threonine is increased approximately a hundredfold have been isolated. This represents a 30-60 percent increase in the total threonine content of the kernel, an amount that would greatly improve the nutritional value of the grain if lysine and tryptophan were similarly increased. Analysis of the threonine overproducers indicates that they are inherited as dominant mutations and have the distinct advantage of creating very specific changes in the kernel (that is, selectively increasing the concentration of specific animo acids) without causing unwanted pleiotropic effects in other kernel or plant characteristics.

From this one example of crop development it is clear that genetic engineering approaches will represent a viable means of improving the nutritional quality of corn for feed. Many of the efforts to genetically engineer new strains of corn are directed at developing the appropriate vectors that will allow expression of genes such as zein at levels that will have a positive effect on the overall nutritional value of the seed. In a similar fashion, new strains of corn will be developed that offer resistance to disease, tolerance of herbicides, increased yields, and shorter maturation times.

CONCLUSIONS

Genetic engineering in agriculture will continue to be directed toward manipulation of microorganisms to produce animal vaccines, hormones, amino acids, and other chemicals or drugs with the ultimate aim of improving the quality, health, and production of farm animals. Genetically engineered products such as vaccines, antitoxins, growth promotants, and interferons will be introduced into the veterinary marketplace in the near future. Many of these products are now being tested in animals.

New and improved animal vaccines will be produced rapidly because they are needed badly and because the regulatory requirements for animal vaccines are not as lengthy as those for human pharmaceutical products. A number of vaccines produced by conventional technology are either expensive or unsafe, while others cannot even be made using this technology. Vaccines for calf scours, foot-and-mouth disease, feline leukemia, rabies, Rift Valley fever, and numerous other diseases are already under development.

Growth hormones for livestock and poultry will be major genetically engineered products. Many believe that genetically engineered growth hormones hold greater potential for agriculture than even vaccines. Certainly, new growth hormones that mimic natural growth hormones will replace the steroids and other growth promotants currently being used.

The feed industry will gain enormously from the developing genetic technology. Recombinant DNA will allow microorganisms to produce less expensive and more nutritious feed ingredients. Genetic engineering will eventually help increase crop yields, make possible more nutritious corn and other crops, and produce less expensive vitamins, amino acids, and single-cell protein.

Finally, there is the realm of antibiotics where, through recombinant technology, organisms that now produce in such low concentrations that it is not practical to recover the antibiotic can be altered to produce much larger quantities for the marketplace. Similarly, some antibiotics are produced naturally in environments so hostile that the antibiotic is rapidly destroyed. It is quite possible to utilize recombinant DNA technology to produce these antibiotics from transformed organisms in environments that would not have the destructive quality of the natural one. The amplification of productive capability through recombinant technology could also be utilized to increase considerably the concentration of existing antibiotics in culture media, thus decreasing their cost and expanding their availability.

In conclusion, it is evident that the industrialization of recombinant DNA technology can lead to useful products and processes. Because this is a basic methodology, the unforeseen applications may very well be more important than any of those that have been proposed so far. The underlying science of molecular biology and molecular genetics is dynamic, and it is reasonable to assume that new opportunities will be created as the depth of our scientific understanding increases. This new technology is surely no panacea.

On the other hand, it carries the realizable potential of contributing significantly to the solution of the most difficult problems facing animal health and production today.

Embryo Transfer and Animal Production in Indonesia

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INTRODUCTION

The application of embryo transfer and animal production technologies to increasing and improving animal products is already known in many countries. In Indonesia, however, this technology has not yet been developed in an integrated fashion and thus has not produced results that contribute meaningfully to the national development program. Although the need to develop biotechnology in Indonesia is not doubted, a number of constraints must be overcome before it can be developed successfully. Thus, to plan future needs and action the present state of these activities must be evaluated in terms of existing R&D programs, manpower, and problems.

PRESENT STATE OF ACTIVITIES IN INDONESIA

Ongoing R&D Programs

The very limited R&D activities in embryo transfer and animal production technologies carried out to date in Indonesia have been scattered in various institutions and universities. Most of the activities worth mentioning have been conducted at Bogor Agricultural University (IPB) in embryo transfer. They have included work in the basic steps or procedures such as embryo preservation and embryo splitting. Embryo transfer is undertaken using the surgical method. For embryo transfers in diary cattle, the embryos were imported from the United States and the work was done mainly by foreign counterparts.

Training of domestic experts with the help of British experts has involved the use of Bali cattle. In this case, embryo transfer was carried out utilizing the nonsurgical method. On the whole, the pregnancy rate reached 45 percent, while the mortality rate of pregnant females was reported to be less than 2 percent. Using similar techniques, embryo transfer is being tried on buffaloes, but thus far no reports have been obtained on this experiment.

IPB is also conducting research in endocrinology, as well as assays of progesterone in relation to milk production. And in a very limited

way, research on monoclonal antibodies is also under way. As is the case for similar activities, however, the constraints commonly encountered in developing research and development in this field are a lack of equipment, material, and expertise.

Experiments on embryo preservation have been started by Airlangga University in Surabaya, but they are still in the initial stages and no results have been obtained.

Other universities do not have effective programs in embryo transfer and animal production biotechnologies. Without proper facilities and well-trained manpower, meaningful and focused research cannot be developed.

Constraints

Scientists engaged in the research activities described above are limited in number, qualifications, and availability. Many of the senior scientists with backgrounds in this kind of research are not being fully utilized for these research purposes; they spend most of their time on other activities. Thus, young scientists receive insufficient guidance. Without a full-time research effort, high-quality results cannot be achieved.

In the same way, a lack of defined research programs, resulting from the lack of qualified manpower, means that the development of facilities cannot be projected. The difficulties encountered in obtaining equipment and expensive materials in Indonesia are matched by the poor condition of existing facilities for research on embryo transfer and animal production biotechnologies. These circumstances are not conducive to research and development.

Finally, the lack of financial support is a major obstacle in procuring reference materials in this area. Reading material is becoming more expensive, especially since the users in this field are limited in number. No means of exchanging information within Indonesia exists, since no publication on the present research activities exists. All of these handicaps lead to limited communications among scientists engaged in this research, either within the country or abroad. Thus, Indonesian scientists working on embryo transfer and animal production biotechnologies are becoming increasingly isolated.

FUTURE PLANS

Future plans for the development of R&D on embryo transfer and animal production biotechnologies should focus on application of the latter. Moreover, attention must be given to developing manpower, building laboratories, supplying laboratory and supporting materials and scientific information, as well as establishing linkages within the country and overseas.

Manpower Development

Trained manpower is needed to work in the scientific disciplines required to develop and apply embryo transfer and animal production biotechnologies. These disciplines include reproductive physiology, endocrinology, breeding, genetics, animal nutrition, veterinary care, and embryology. The following approaches could be taken:

- Recruitment of young scientists from universities. A program has been started to send young graduates overseas to work in the kind of research of concern here, but this program needs better planning and coordination.

- Advanced training for senior scientists. The lack of communication among senior scientists and limited ongoing R&D activities in embryo transfer and animal production biotechnologies make it difficult to keep up with the progress being made in this field.

R&D Programs

A clear, well-defined R&D program in embryo transfer and animal production biotechnologies must be developed to support manpower planning and allocation. R&D activities in Indonesia would greatly benefit from joint programs with counterparts in developed countries such as the United States.

Based on the needs of Indonesia as well as the potential of achieving its goals, the following R&D programs in embryo transfer and animal production biotechnologies have been identified:

- Increasing through embryo transfer the number of animals, and thereby the amount of available animal protein

- Improving breeding stocks

- Producing supporting materials for animal production--for example, hormones, vaccines, and monoclonal antibodies.

Cooperative R&D activities between Indonesian and U.S. experts could be initiated by submitting, for example, requests by both parties to their respective appropriate government agencies. In this connection, the U.S. National Research Council could help solicit U.S. experts interested in cooperating with Indonesian scientists. On the other side, the Indonesian Ministry of State for Research and Technology could coordinate the implementation of a joint program in Indonesia.

Laboratories and Equipment

A plan for setting up laboratories and supplying needed equipment should be integrated with the R&D programs and efforts to apply them.

Communications

Media for scientific exchange are expected to be published by the Ministry of State for Research and Technology. Moreover, meetings between scientists working in embryo transfer and animal production biotechnologies should be encouraged while the centers of activities are being established.

PLANT CELL AND TISSUE CULTURE

One Perspective on Plant Cell and Tissue Culture

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Advances in plant cell and tissue culture are essential to realizing the potential of biotechnology in agriculture and forestry. Pioneer researchers using recombinant DNA technology look forward to being able to introduce complex multiple-gene characteristics into important crop and forestry species. Many research breakthroughs in recombinant DNA technology and plant cell and tissue culture must be accomplished before these dreams become reality, however. In the interim, applications of plant cell and tissue culture are benefiting agriculture and forestry in numerous ways, and they reveal a better understanding of this technique.

Early in this century, observations of plants differentiating from cultured tissue were first reported. Since then, research on plant cell and tissue culture has become increasingly sophisticated. One objective of these inquiries is to gain insight into developmental processes and to identify environmental factors and genetic components that influence these processes.

The ability to reproduce desirable species using plant cell and tissue culture techniques has resulted in their practical applications. Recent breakthroughs with recombinant DNA technology using bacteria has spawned tremendous interest in directly incorporating specific genes into plants using r-DNA techniques. The desire to manipulate plant cells like bacteria and produce genetically altered plants has made cell and tissue culture essential to applying r-DNA techniques to plants. The potential usefulness of plants manipulated in this way has stimulated major companies to establish substantial basic research teams and has stimulated small companies to capitalize on their aggressive pioneering research capabilities.

There has also been a steady increase in the applications of plant cell and tissue culture techniques for industrial uses. These applications are expected to result in economic benefits before those of recombinant DNA technology. This paper briefly reviews the techniques that make up plant cell and tissue culture technology and describes the advantages of and expectations about these techniques.

CLONAL PROPAGATION

Clonal propagation is a range of techniques that allow the manipulation of cells in culture so that plants develop and are propagated from the cells. Clonal propagation using meristems for micropropagation has been utilized extensively in horticultural, vegetable, and ornamental crops. Examples of commercially propagated plants--of which there is an ever-increasing list--are orchids, potatoes, and oil palm.

Clonal propagation techniques produce plants that are genetically and phenotypically uniform, a major advantage of these techniques. This form of asexual reproduction is especially useful when the genetically superior plants are heterozygous, because large numbers of genetically uniform plants can be produced. Another major advantage of clonal propagation techniques is that they produce disease-free plants. The useful tools of modern biotechnology include tests for disease, especially viruses and viroids, using monoclonal antibodies or DNA probes for specific disease agents. Depending on the particular agent, these antibodies and probes may be available commercially or from universities. Of course, conventional methodologies for disease testing can be used if appropriate immunoassays or probe assays are not available.

Clonal propagation of agronomic crops is important, but some problems must be solved before practical applications can be made. These problems arise from the fact that (1) many crops are difficult to regenerate in culture; (2) the economic value of a single plant is usually low; and (3) effective, well-developed breeding systems are already used for many agronomic crops. It is possible to use clonal propagation techniques to increase parental strains with superior genetic characteristics. The ability to increase parental strains rapidly can dramatically accelerate breeding programs, so that large amounts of seed can be produced more quickly. These applications are potentially important for agronomic crops.

PRODUCTION OF HAPLOIDS

Production of haploid plants by culturing pollen grains or other genetically appropriate material to develop homozygous inbred lines, especially as parental lines, has been widely investigated. Haploid plants are potentially useful in plant breeding programs. Possible special uses include recovery of male sterile plants, rapid production of inbred plants, and selection of inbred mutants. It is unclear to what extent haploid culture will increase in importance.

SOMOCLONAL VARIATION

In addition to their usefulness in producing plant uniformity, plant cell and tissue culture techniques can enhance genetic variation. The most widely described is somoclonal variation, which is

the spontaneous variability in plants produced in plant cell and tissue culture. This variation probably results in chromosomal and cytogenetic changes that occur during culture. Presently, this variability is largely evident in altered plant morphology such as leaf shape or flower color. Many regenerated plants are normal, however. Variability of plants may be significantly enhanced by using mutagens in tissue culture.

The extent to which somaclonal variation will be useful in crop breeding is unknown. The effort required to determine the genetic stability of this variation is extensive yet necessary for its use in crop breeding. Some plant breeders believe there is sufficient genetic variation in natural plant populations for crop breeding. Nevertheless, studies to understand variation generated in culture may provide new insights into plant genetics and cytogenetics. From this expanding base, unanticipated techniques in plant breeding and r-DNA might come about.

PROTOPLAST CULTURE

No discussion of plant cell and tissue culture techniques is complete without mentioning protoplast culture. Because this technique enables one to make single cells from plants and then manipulate these cells to differentiate into embryos, this stage represents an opportunity to work with plant cells as if they were bacteria, and it is the probable target for substantial research to incorporate r-DNA into the plant genome. There are limits to the culture of protoplasts and the propagation of plants from them. Although the number of species that can be reduced to protoplasts and then regenerated into plants is continually increasing, numerous crop species are not routinely reduced and regenerated. Often desirable genotypes from the species that can be successfully manipulated cannot be regenerated. For practical applications, it is extremely important to regenerate specific target genotypes regardless of the plant cell and tissue culture technique used.

INCORPORATION OF FOREIGN GENES

Many challenges lie between our present knowledge of how to incorporate foreign genes into plants and practical utility. The availability of appropriate vectors or methods for successfully transferring r-DNA constructs into plants is limited to a few systems and plant species. And only a limited number of genes have been adequately characterized for incorporation into r-DNA constructs. Essentially, these are single genes which can be readily screened in tissue culture. These genes are not for traits of major economic importance, however, although this is the objective of much industrial research.

Much uncertainty exists about the expression of genes after foreign DNA is incorporated into the plant genome. Assuming a gene is

expressed, the level of expression, the developmental time of expression, and tissue site of expression cannot be adequately predicted. In spite of these major challenges, companies in the United States are now field testing transformed plants and utilizing R&D to make "synthetic seeds," using the large number of embryos that can be generated from protoplasts and other appropriate cultured tissues.

AN APPROACH FOR INDONESIA

If a country wishes to apply plant cell and tissue culture techniques to industrial uses, the necessary research must be based on the system in which it will be used. For Indonesia, the required plans and objectives would be similar to those a company makes when determining its business plan. Since the primary target of most applications of plant cell and tissue culture techniques is crop improvement, it is essential to decide which plants have the highest priority. This decision relies on both a financial evaluation and a research consideration. If a specific crop--for example, oil palm--is important for international trade, are the prospects favorable for continued market strength? If it appears strong over an extended period, would a research investment pay off by providing continued favorable economic yields? The considerations for domestic plants can have similar and different components. For example, if a plant is needed for reforestation, its uses as firewood for cooking or as environmental protection become important factors that have little relevance in the international marketplace.

From the research perspective, the status of breeding programs and capabilities must be realistically assessed. Can plant cell and tissue culture of specific crops significantly improve breeding programs within a useful time frame? If all else is equal, techniques that are available today or tomorrow are substantially more useful than techniques that must be developed for several years before the results can be utilized in a practical breeding program. The ease with which desirable parental material and cultivars can be manipulated using plant cell and tissue culture techniques must be determined. If these manipulations cannot be done presently, can a research program be designed for successful propagation of these genotypes? Beyond plant cell and tissue culture techniques, what specific traits would significantly improve a crop? Can these traits be readily tested at an early developmental stage or it is necessary to screen large populations of plants in the field? Field testing at an early stage requires considerably more time and effort than early testing of cells in culture. For traits of highest value, however, the greater effort may be necessary and unequivocally justified.

When considering specific research objectives and crop needs within a broad perspective, it is important to assess realistically the competitive environment for research. What other laboratories worldwide are doing similar or related research? Although the initial response to learning that similar research is under way is often concern about competitiveness, from an adjusted perspective this

situation may actually confirm independently that one's research objectives are realistic although not original. Furthermore, additional research sites may present opportunities for collaboration. The biotechnology industry is permeated with almost every kind of joint venture imaginable in which the important needs of the participants are satisfied. In the use of plant cell and tissue culture for specific crops, there are opportunities for contract research and training. Universities worldwide are possible resources, and companies offer exploitable opportunities as well because of their expertise and their orientation toward practical goals.

CONCLUSION

Over the past five years, the business sector has invested heavily in plant biotechnology, including plant cell and tissue culture, and has employed outstanding researchers to formulate and implement their plans. This situation has many implications. One important implication is whether or not state-of-the-art research will continue to appear in the scientific literature. Many companies are publishing the results of their efforts. For the intellectual challenge these results provide, it is hoped that these companies and governments continue this enlightened publications policy.

The intellectual challenge of understanding the basic processes of biology has provided the foundation for industries known broadly as the biotechnology industry. Experts have predicted when key scientific hurdles will be crossed, and, characteristically, these predictions have overestimated the time required to make important breakthroughs. Who would have predicted in 1980 that companies would be field testing transformed plants in 1986? This observation is made to draw attention to the words "limits" and "challenges" which appear throughout the preceding pages. These limits could change dramatically, and it is important to detect the increased knowledge and to respond to new opportunities.

Present State of Plant Cell and Tissue Culture in Indonesia

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INTRODUCTION

The capability of plant cells to grow, propagate, and regenerate in vitro into whole plants presents a unique opportunity for agricultural development. The technique of plant cell and tissue culture was used at one time to study the totipotency of plant cells. Following the discovery of auxin and cytokinin in the late 1950s, however, widespread success was achieved in this field. Hundreds of plant species have been regenerated routinely in the laboratories of 69 countries. And today this technique has reached a state where it can be exploited for commercial benefits. For example, it is being employed successfully for such varied purposes as rapid clonal propagation and virus elimination; varietal development, genetic modification, and crop improvement; and production of secondary substances, independent of environmental factors.

Clonal Propagation

The most advanced applications of plant cell and tissue culture have been in rapid clonal propagation. A multiplication rate of a million times is not unusual with this technique. The first plant to be propagated through tissue culture was the orchid. In 1964, Morel estimated that as many as 4 million cymbidium could be produced in a year from a single shoot.

Success with orchids stimulated use of the technique in other crop species. Today, diverse ornamental crops, agronomic crops, estate crops, fruit crops, forest trees, and medicinal plants are also being produced clonally using tissue culture. Such plants are of uniform quality, grow faster, and mature earlier than the seed-propagated plants, and annuals have a longer life span. Clonal propagation is especially useful where a newly developed cultivar must be multiplied for commercial production. Indonesia will benefit from this technique in the production of uniform plant materials for export, industrial plantations, environmental rehabilitation/reforestation, agrotourism, and nucleus farm estates.

Crop Improvement

Food production can be increased either by expanding the amount of land cultivated or by increasing yield per hectare. Either way, however, presents problems. For example, cultivated land, which is becoming scarce, suffers from occasional increases in salinity and increases in the costs of energy needed for cultivation. Moreover, expansion of cultivated land can take place only in areas outside Java where the soils are susceptible to stress. These soils could, of course, be reclaimed by various cultural practices, but reclamation requires recurrent inputs. Use of tolerant cultivars may help minimize the use of soil amendments and fertilizers.

Efforts are now under way in Indonesia to use the technique of cell and tissue culture to develop plants that are less susceptible to stress. The soils outside Java are affected by excess or scarce supplies of water, high temperatures, and high salinity.

Secondary Metabolites

In the early 1950s, it was discovered that plant cells, like microorganisms, could be grown in liquid medium. Thus, plant tissue culture has provided an alternative to the cultivation of whole plants as important sources of many useful compounds, including drugs, flavorings, enzymes, essential oils, and food colorings. Just as it was found that some microorganisms could produce antibiotics, it was also found that some plant cells produce similar desirable compounds. It appears that the capacity for synthesis of specific compounds by the cell culture is retained.

Recognizing the possibilities, scientists have continued to develop this process. The first industrial application was achieved in the production of the pigment shikonin from Lithospermum erythiorhizon. In addition to producing natural compounds, plant cells can be used in a process called biotransformation. The most interesting and advanced biotransformation is the hydroxylation of digitoxin, a low-value by-product, into cardiac glucoside digoxin. Using this process, it should be possible to produce new compounds with pharmacological properties. Some cultures, however, produce metabolites that are much lower than those found in the whole plant, or they may produce chemicals that are structurally different from those in the whole plant.

Indonesia is rich in plant species used for traditional medicines. The traditional method of using a whole plant to produce these medicines (Jamu) has two limitations: (1) natural plant materials will eventually be exhausted, and (2) the concentration of active compounds may vary. The tissue culture technique should be able to overcome such limitations, however. In addition to traditional medicines, the tissue culture technique could be applied to producing other important chemicals for the pharmacological and food industries in Indonesia.

PRESENT STATE OF PLANT CELL AND TISSUE CULTURE R&D ACTIVITIES IN INDONESIA

Ongoing R&D Programs

The ongoing R&D program in plant cell and tissue culture covers *in vitro* clonal propagation, crop improvement, and production of secondary metabolites. Most of the research activities are in the field of clonal propagation and include a wide range of crops as well as

TABLE 1 Ongoing Research Activities in Plant Cell and Tissue Culture in Indonesia

Field of Study and Institution	Crop
Clonal propagation	
IPB	Melon, strawberry, asparagus, rattan, dipterocarp, *Santalum*, teak, *Costus*, cacao, banana, potato, carnation, citrus, petunia
ITB	Melon, strawberry, eucalypts, coffee
UGM	Orchids, teak, *Santalum*
BORIEC	Oil palm, cacao, coffee, coconut
MARIF	Asparagus, citrus
LBN	Orchids, tropical fruits
LEHRI	Potato
BP3G	Sugarcane
MARIHAT	Oil palm
SOCFINDO	Oil palm
Crop improvement	
IPB	Corn, tomato
ITB	Rice
UGM	Rice, corn
Secondary metabolites	
IPB	Garlic
ITB	*Solanum*, *Duboisia*, *Morinda*,
UGM	*Catharanthus*, *Pimpinella*,
UI	*Sonchus*

Note: IPB, Bogor Agricultural University; ITB, Bandung Institute of Technology; UGM, Gadjah Mada University; LBN, National Biological Institute; BORIEC, Bogor Research Institute of Estate Crops; MARIF, Malang Research Institute of Food Crops; LEHRI, Lembang Horticulture Research Institute; BP3G, Central Research Institute for Sugarcane; UI, University of Indonesia.

institutions. Research on crop improvement and production of secondary metabolites, on the other hand, covers only a few crops (Table 1).

Most of the research on clonal propagation has just reached the multiplication state; very little has reached the greenhouse or field planting state (for example, oil palm, banana, potato).

The crop improvement and secondary metabolites research activities are mostly in the initial stages--that is, production of calluses and plantlet regeneration. The aims of the crop improvement program are to produce crops with a high nutritive value and a tolerance of salinity, drought, toxicity, high temperatures, and disease. Research programs in secondary metabolites are aimed at producing pharmaceuticals and dyes.

Manpower

At present, about 29 degree holders are working in plant cell and tissue culture at various institutes (Table 2).

Facilities

Both the universities and research institutes have at least basic plant cell and tissue culture research facilities. The government and private institutes have better facilities than the universities, including facilities for large-scale production of plantlets.

TABLE 2 Distribution of Manpower, by Degree, at Universities and Government and Private Research Institutes

Institution and Specialization	Ph.D.	M.Sc.	B.Sc.
Universities			
Plant physiologist	5	3	
Plant breeder	2	1	
Biochemistry		2	
Agronomist/tissue culturist			3
Government research institutes			
Plant physiologist	1	4	
Agronomist/tissue culturist			6
Private research institutes			
Agronomist/plant culturist			2
TOTAL	8	10	11

Constraints

Researchers in the universities and research institutes are constrained in their work by a communications gap, lack of cooperation, and lack of information.

Universities and some of the research institutes are also faced with scarcities of important substances (for example, enzymes and plant growth regulators), sophisticated equipment, glassware, and other items needed for plant tissue culture work. On the other hand, some of the well-equipped private research institutes lack staff with higher degrees (M.Sc. or Ph.D.), thus leaving them unable to maximize the utilization of their sophisticated facilities.

FUTURE PLANS

R&D Programs

R&D programs must be established, with the following topics getting high priority:

- Rapid clonal propagation
  - -- Estate crops: oil palm, coconut, rubber, coffee, cacao, clove
  - -- Industrial crops: dipterocarp, rattan, sandalwood, teak, eucalypts
  - -- Horticultural crops: banana/plantain, melon, potato, strawberry, asparagus, garlic, pineapple, tropical fruits.
- Crop improvement
  - -- Food crops: rice and corn cultivars with a high nutritive value as well as a tolerance of salinity and heat
  - -- Forage legumes: high nutritive value
  - -- Horticultural crops: potato cultivars tolerant of high temperatures and salt concentrations as well as resistant to bacterial wilt; tomato cultivars tolerant of high salt concentrations and resistant to disease
  - -- Industrial crops: highly productive, disease-resistant sugarcane cultivars.
- Secondary metabolites

  The production of pharmaceuticals from the following plant species: Costus, Solanum, Morinda, Rauwolfia, Catharanthus, Pimpinella pruatjan, Stevia, Sonchus arvensis, garlic, Kampferia galanga.

Manpower

The various research institutes and universities presently require the following minimum number of university graduates: 30 doctorates, 14 masters of science, and 5 bachelors of science. Holders of Ph.D.s must be evenly distributed among the following fields: plant physiology, plant breeding, and plant biochemistry/molecular biology.

Study toward Ph.D. and M.Sc. degrees can be pursued either in-country (for example, at IPB, ITB, or UGM) or overseas (Japan, United States, Europe). Study toward advanced degrees in plant biochemistry and molecular biology should be taken overseas.

Periodic assessment of manpower needs at research institutes and universities is needed.

Facilities

Tissue culture laboratories in research institutes and universities should have at least the following facilities: preparation room, washing room, chemical storage room, sterile room, cold storage for media, and temperature-controlled culture room with illuminated shelves. In addition, acclimatization facilities, including a greenhouse with a misting and fogging installation to maintain high atmospheric humidity, should be provided.

Large-scale production units should be established as an extension of the existing research laboratories.

Mechanisms should also be established for obtaining chemicals, equipment, glassware, and other items needed for tissue culture work. Means of collecting indigenous plant species and effecting an international exchange should be provided as well. Finally, the relevant scientific journals and facilities for publication of research results should also be provided.

PLANT NITROGEN FIXATION

Technical Overview: The Biotechnology of Nitrogen Fixation

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Research in the area of nitrogen fixation has progressed rapidly the past few years. Some of the more significant advances are outlined in this paper, with emphasis on those findings that are of fundamental importance to the field or are relevant to practical problems.

FIXATION OF NITROGEN

One practical question has received considerable attention: Is it possible to transfer the genes for nitrogen fixation from rhizobia or other competent bacteria to agronomic plants such as corn? Alternatively, can corn or similar crop plants be genetically engineered to form symbiotic root nodules with rhizobia?

The development of biotechnology has been unexpectedly rapid with respect to solving the technical problems of transferring genes into crop plants and having these genes expressed appropriately. The transformation of monocots such as corn was achieved recently. In addition, genes transferred from one plant to another can be expressed in the proper tissue at the proper time. Findings such as these encourage one about the prospects of genetically engineering plants such as corn or wheat to fix their own nitrogen.

Other information, however, indicates that this goal will not be achieved in the next decade. It is becoming increasingly apparent that the molecular machinery required for nitrogen fixation is very complicated. At least 17 genes involved in this process have been identified to date, but it is not clear that these genes can be transferred en masse to other organisms and function effectively. Nothing nearly so difficult has ever been achieved. It has also become apparent that the establishment and maintenance of a symbiotic relationship with nitrogen-fixing bacteria are formidable and complex as well. At least 10 genes required for nodulation have been identified in rhizobia and at least 35 genes specific to nodules have been identified in host plants such as soybean and pea. While many of these "nodule-specific" genes may be present in nonhost plants such as corn, there is no present knowledge or assurance of this, nor is there any assurance that needed genes could be transferred to function in concert with those already present.

One very important conceptual advance in the area of nitrogen fixation is the glutamate exchange model. According to this model, the host supplies bacteroids within the nodule with the amino acid glutamate. The bacteria then remove the amino group and use the remaining carbon skeleton for both energy and synthesis of many compounds. Part of the energy obtained from host plant glutamate is used to reduce atmospheric nitrogen to ammonia. This ammonia, together with the ammonia stripped from the original glutamate, is passively excreted by the bacteroids and used by the plant, partly for synthesis of more glutamate and partly for synthesis of all the other nitrogen-containing compounds required by the plant. This model is consistent with a wide variety of metabolic studies. It is also attractive because it does not require either partner in the symbiosis to behave in an altruistic manner. This model will be of considerable importance in future attempts to improve the basic efficiency of nitrogen fixation.

PROCESS OF INFECTION AND NODULE FORMATION BY RHIZOBIA

There has been considerable clarification of the complex events that occur during nodule formation and the time course of these events during infection. Infections can develop through either root hairs or epidermal "cracks," depending on the host species. Infections through root hairs appear to require deformation of the root hair in a way that entraps the bacterium. Root hair deformation is induced by substances from the rhizobia, but the nature of these substances and their mode of action is not known. Root hair deformation in mature hairs involves the formation of branches, and appears to be distinct from deformation of emergent hairs. Emerging root hairs remain susceptible to deformation by rhizobia only during the period of elongation, which is a matter of a few hours. It has been shown in soybean and several other legumes that only a narrow band of root cells behind the growing root tip is susceptible to infection by rhizobia. This finding is important to the problem of competition between indigenous rhizobia and inoculated rhizobia. To compete effectively, inoculated rhizobia must be able to achieve and sustain good root colonization in the susceptible zones of the root system as it develops.

Great progress has been made in isolating and analyzing the various genes in rhizobia required for nodulation. One important class of nodulation genes is composed of the "common" nod genes, so-called because they appear to be present and functional in all rhizobia. It appears that these genes are responsible directly or indirectly for induction of root hair deformation and root cell division in the host. Other nod genes seem to be involved in host specificity and infection thread formation. The genes required for infection thread formation appear to code for enzymes involved in surface polysaccharide synthesis.

Although it is not clear what the gene products of the common nod genes do, it is known that substances present in the root exudate of the host plant stimulate the expression of the common nod genes. Similarly, the lectin from soybean root exudate stimulates

nodulation efficiency. Thus, we are beginning to learn something of the molecular communications between the symbionts. Such knowledge is crucial to the future manipulation of competitiveness, host range, and symbiotic compatibility.

Some progress has also been made in the molecular genetics of Azolla anabaena and in the host specificity of Frankia isolates. The use of Azolla for agriculture appears to be mainly limited by the intensive labor required for vegetative propagation. Work on controlled sporulation is in progress.

REGULATION OF NODULE FORMATION AND NITROGEN FIXATION

It is becoming increasingly evident that the legume/rhizobia symbiosis is a highly evolved and regulated association. Several recent investigations have shown that nodule formation and nitrogen fixation are subject to various forms of feedback regulation.

The addition of external fixed nitrogen such as nitrate, for example, leads to the rapid blockage of the earliest steps of infection and to the rapid inhibition of nitrogen fixation in previously established nodules. Initiation of the first few infections in a root has been found to inhibit the maturation--but not the initiation--of subsequent infections. It is clear that the host plant can block infection initiation or development at virtually any stage of development. It is also clear that the abortion of infections is a common event, indicating that the host optimizes nodule number. Nodulation on one side of a split root system strongly inhibits nodulation on the other side. The total number of nodules per plant remains remarkably constant despite many variations in exposure to rhizobia, again indicating homeostasis and optimization of nodule formation.

When irradiation or chemicals have been used to mutagenize legume seeds, mutant progeny that have altered symbiotic properties have been identified. In addition to mutants that formed few or no nodules, mutants capable of forming a great many nodules were also obtained. Such "hypernodulators" and "supernodulators" were encountered at frequencies of up to 0.2 percent. Characterization of a supernodulating mutant of soybean indicated that the total number of infections was increased severalfold, while at the same time the frequency of aborted infections decreased severalfold. It appears that supernodulation results from a defect in the feedback control mechanism that governs nodule number. Grafting experiments showed that the defect was in the shoot, not the root. It is possible that such mutants could be of considerable importance to agriculture in cases where increased nitrogen fixation is desired but increased growth is not, as with cover crops, for example. It is also important to be aware that artificially induced crop plant mutants can be isolated that have altered regulatory functioning. It may prove far simpler in many cases to manipulate regulatory functions (for example, time to flowering or number of fruits) through random mutation and selection than through genetic engineering.

COMPETITION FOR NODULE OCCUPANCY

A major practical problem for growers wishing to obtain maximum benefit from Rhizobium nitrogen fixation is that of getting inoculated rhizobia to generate enough nodules, particularly in the face of competition from native rhizobia already in the soil. Past research efforts have mainly concentrated on finding strains of rhizobia that nodulate and survive well in the soil. Recently, however, most attention has been given to finding hosts that are nodulated only by certain rhizobia, or rhizobia that are highly competitive on the roots but not in the soil.

Progress in this area has been relatively slow because the phenomena are complex. Some efforts have been made to identify traits that are crucial to good colonization and infection of host roots. There is some evidence that bacteria motility and chemotaxis are two crucial characters. Several laboratories are currently studying the role of bacterial attachment to roots, but no firm conclusions are yet possible. Rapidity of growth of rhizobia on host root exudates also appears to be an important capability, but the substances involved are not known.

The relative competitiveness of particular strains of rhizobia appears to depend on the genotype of the host, on the nature of the soil, and on the culture history of the bacteria. It has also been shown that nodule occupancy depends on the logarithm of the inoculum dosage, suggesting that some regulatory function may be involved.

Considerable work continues to be done in developing new formulations for coating seeds with inocula of rhizobia, although no major improvements appear to have been made over the standard peat-based slurries. It is of interest that many Bradyrhizobium strains and a few Rhizobium strains are able to survive for long periods of time and even multiply in distilled water suspensions. Thus, water may prove to an inexpensive and relatively selective medium for producing and storing inocula.

Biological Nitrogen Fixation in Indonesia

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INTRODUCTION

Despite a family planning program, it is predicted that Indonesia's population will reach 210.2 million by the year 2001. Adequate food supplies must, therefore, be given constant, serious attention.

Now that self-sufficiency has been reached in rice production, priority is being given to increasing the production of palawija (secondary) crops--corn, soybean, peanut, and mungbean. Soybean is one of the major crops (about 800,000 hectares are cultivated), but a large amount is still being imported. For example, an estimated 600,000 tons was imported in 1983, at a cost of more than US$170 million.

Efforts to increase soybean production include intensifying as well as expanding its cultivation. A massive 18.3 percent per annum expansion of production is planned through the current five-year national development plan (REPELITA IV). This can be achieved by enhancing production in existing areas using different or improved technologies, increasing the area of cultivation by rotation with lowland rice, and cropping new lands such as the transmigration areas. The latter has been made possible in part by implementation of a liming program for acid soils, which constitute a large part of the newly opened areas. The ability of soybean crops to obtain nitrogen through biological nitrogen fixation (BNF) without inoculation is not known. The available experimental data from some production systems, however, indicate the need for rhizobium inoculation.

Studies on the need for inoculation of cover crops--such as Calopogonium, Pueraria, Centrosema, and Mucuna--must also be conducted. This is especially important in relation to the government's efforts in estate expansion where it hopes to increase state income from the nonpetroleum sectors.

As long as rice, which is cultivated mostly in paddy fields, is still the main staple for the Indonesian people, studies of the Azolla-Anabaena symbiosis should also be given serious attention.

PRESENT STATE OF BNF ACTIVITIES IN INDONESIA

Ongoing R&D Programs

It is difficult to assess the ongoing R&D programs as the institutions involved in biological nitrogen fixation are scattered throughout the country with no regular communication among them. Most of the activities appear to be carried out at universities that, unfortunately, are constantly faced with inadequate financial support and research facilities. Thus, it has been difficult to maintain continuous research activities, let alone produce qualified research findings. This is one of the major reasons why Indonesian scientists are unable to participate in international scientific meetings; almost all of the agencies that provide funds for attending scientific meetings require the attendees to present scientific papers. Thus, this problem, as well as the lack of adequate library services, makes it almost impossible for most Indonesian scientists to keep abreast of current progress in this area, which contributes in turn to their inability to direct good research programs.

As for any field of biotechnology, the development of BNF requires the integrated use of different scientific disciplines, most of which have not been adequately established in Indonesia. These include biochemistry, microbiology, and molecular genetics. Thus, the curricula of those university departments allowing graduate students to carry out BNF research do not necessarily offer the scientific disciplines needed to equip students to carry out solid research in BNF.

Establishing communication among scientists has also been a constant problem. This problem includes the inadequate quantity and quality of scientific publications received and the inability of scientists to meet periodically as a result of lack of travel funds.

A national program on biological nitrogen fixation was established in 1981 under the coordination of the Indonesian Institute of Sciences. Financial constraints, however, meant that scientists invited to participate in the program were able to meet only three times during a five-year period (the last meeting held in 1984). The results of the program were limited to identification of institutions and scientists involved in BNF research and their research activities.

Applications

Since 1978, Gadjah Mada University (UGM) has furnished the government with rhizobium peat-based inoculants, mainly for soybeans and peanuts, with a production rate of two tons per week. Production is still on a pilot plant scale, and the methods of production are being continually investigated and improved. These inoculants are distributed by the government to farmers, but their use continues to require the close guidance of state extension workers. In addition to inoculants for soybeans and peanuts, inoculants for other legumes have been produced in smaller amounts, based on demand, by UGM, Bogor

Agricultural University (IPB), and the National Biological Institute (LBN), mainly for research purposes.

Manpower

About 20 qualified scientists, most of whom received their graduate degrees abroad, are involved in BNF research. Only a few, however, are full-time researchers. Well-trained technicians are not available at most institutions, and the curricula needed at various universities to support BNF research have not been adequately developed.

Facilities

Some institutions have adequate research facilities, while others do not. Lack of funds to maintain equipment is a general problem. Library facilities are also inadequate, especially the receipt of periodicals. These and the other problems described above constitute the constraints that prevent BNF research activities in Indonesia from flourishing. This in turn affects how widely BNF is applied to agricultural practices in Indonesia.

FUTURE PLANS

R&D Programs

A core group of scientists undertaking BNF research that represents the relevant disciplines must be organized. This group should meet periodically to exchange information on research progress, prospects, and constraints, and to identify relevant research topics and establish priorities. Annual scientific meetings for BNF should also be organized and travel funds provided for participants. Initiation of an Indonesian biotechnology newsletter would greatly help establish communications among the scientists.

Research on the rhizobium-legume symbiosis should focus on fast-growing tropical rhizobia for grain legumes such as soybean, peanut, mungbean, pigeon pea, and cover crops, and on the ecology of rhizobia. Compatible plant cultivars should be developed accordingly.

Studies on application of the *Azolla*-*Anabaena* symbiosis to rice cultivation in paddy fields should be conducted continually. Existing facilities at the best-equipped institutions should be developed to enable them to function as culture collection centers.

First priority should be given to a nationwide inoculation program that would enable farmers to benefit from BNF activities as quickly as possible. This would be followed by research to solve the more basic problems. If a nationwide inoculation program is encouraged, however, the inoculant production capacity in Indonesia should be greatly increased. The production capacity at UGM is only sufficient to inoculate 120,000 hectares annually; it would be difficult to increase

this capacity with the technology used currently. Accordingly, inoculant production should be opened to private enterprise interested in such a venture. In the interim, a quality standard for inoculant industries should be established as well as a government agency to monitor the quality of inoculants provided to farmers and to conduct rigorous testing of inoculant effectiveness. Regional production of inoculants should be considered to minimize transportation constraints that may shorten the shelf life of the inoculant.

Manpower Development

More well-trained technicians and scientists are required to develop a BNF research capability. Because it is important that the appropriate curricula are offered to prepare university graduates for carrying out solid research in BNF, rapid development of the following scientific disciplines is recommended: microbial physiology and molecular genetics, general and plant biochemistry, plant physiology and breeding, plant molecular genetics, and soil sciences. Each research center should have at least 15 qualified, full-time researchers among the eight disciplines (at least one Ph.D. per discipline) and 15-30 technicians (possibly S-0 graduates).

The existing curricula, especially in those university departments where students are usually enrolled to study BNF (for example, the department of soil sciences or agronomy), should be improved to include the scientific disciplines listed above. In the interim, to support the development of BNF in Indonesia it is strongly recommended that leading Indonesian universities soon create departments of microbiology. Several universities have sufficient qualified staff to offer at least an S-2 program in microbiology, but S-2 and S-3 candidates in disciplines such as biochemistry and molecular genetics may still have to study abroad. In addition, S-0 programs must be developed at some universities to produce qualified technicians and extension workers.

Facilities

A detailed list of equipment and facilities required for BNF research should be outlined as soon as a program is set up. Funds to maintain equipment should be made available as well as the relevant literature, especially periodicals.

To make appropriate specific recommendations, it is essential that an additional two meetings of the BNF workshop group be held, as well as at least one additional intergroup meeting with other individuals involved in the agriculture biotechnology centers.

BIOCONVERSION OF AGRICULTURAL BY-PRODUCTS

The Business of Indonesian Biomass

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NATURE OF BIOMASS

Biomass comprises collectible, plant-derived materials that are abundant, inexpensive, and potentially convertible to feedstock chemicals by fermentation or chemical processes. It is found as starch in Indonesian corn, rice, potatoes, cassava, sago palm, and other agricultural products. It is also found as monomeric sugars or soluble oligomers in cassava syrup, molasses, and raw sugar juice. Biomass also occurs as lignocellulose in the form of wood chips, crop residues, forest and mill residues, urban refuse, and animal manures. Of these materials, wood chips, rice straw, and cassava and its derived materials (starch and syrup) are probably the most important sources currently.

Chemically, almost all biomass, regardless of its source, contains about 45 percent oxygen on a moisture- and ash-free basis (Browning, 1963; Wenzl, 1970) and 50 percent moisture as collected (Table 1). Thus, biomass makes a poor fuel. At 50 percent moisture, materials such as sugarcane bagasse have a net heating value as received of only about 6,800 Btu/lb (dry basis) or about half that of bituminous coal (Paturau, 1969). Cellulosic biomass is therefore a poor choice as an energy source, unless it is a waste material that must be disposed of at least cost. Biomass as starch or lignocellulose, however, has potential as a feedstock for oxychemicals that retain the oxygenated nature of the basis CH_2O structure.

It would be much more difficult economically to attempt to squeeze H_2O out of CH_2O. For example, in dehydrating ethanol to ethylene, the molecular weight is reduced from 46 to 28. This means that water worth approximately $1.80 per gallon as ethanol is discarded. Such a situation is obviously poor economics. In the early 1980s, one might have, in fact, considered doing this because of the fantastic rate of increase in the cost of ethylene. Now, however, with the softness of that market the economics of producing olefins from ethanol looks dim for the foreseeable future (O'Sullivan, 1984).

Three general conclusions can be drawn from these considerations. First, if one wishes to provide energy, or aromatics, or methane/syngas, it is best to buy a coal pile. Second, if one is, however, looking for oxychemicals that maintain the oxygenated nature of the glucose molecule, biomass should be seriously considered. Finally, if olefins

TABLE 1 Biomass Elemental Analysis (Weight Percent)

	Pine Wood	Bagasse	Corn Cobs	Urban Refuse	Feedlot Manure	Giant Kelp
Ash (d.b.)	0.5	2.0	1.0	14.0	24.0	39.0
Moisture	50.0	50.0	7.0	18.0	70.0	90.0
Ash-free dry solids						
Carbon	52.0	47.0	44.0	48.0	46.0	45.0
Oxygen	41.0	46.0	48.0	45.0	43.0	46.0
Hydrogen	6.0	6.0	8.0	6.0	7.0	6.0
Sulfur	0	<0.1	<0.1	0.2	0.5	0.6
Nitrogen	0.1	0.5	0.6	0.6	3.3	2.0

are needed, biomass might be a possible source. A very hard and critical, albeit long-term, look at such apparent opportunities is required, however.

MARKET POTENTIAL

In broad terms, the market potential for biomass-based chemicals applies to either specialty chemicals or oxychemical feedstocks. In considering specialties, a company might decide to produce new products such as glucosides from cassava syrup for the merchant market. Conversely, a company might consider further market opportunities for the products it currently manufactures.

On the other hand, companies in oxychemical feedstocks--that is, bulk commodity chemicals--would probably prefer to approach further marketing opportunities from the point of view of captive use rather than that of entering the merchant market. Captive use would involve changing from an expensive, fossil-based process to a more inexpensive, renewable-based process.

Price is obviously a strong determinant. As shown in Figure 1, price is inversely related to market volume. Specialty chemicals and pharmaceuticals command high prices but a low volume. This paper concentrates on the relatively low-cost, high-volume products labeled "primary petrochemicals" in Figure 1.

The markets for "bioproducts"--products that might be produced from renewable materials--are listed in Table 2 (Busche, 1983b). Overall values are shown for world sales, except the value of commodity organic solvents and acids which is shown only for the United States. Commodity oxychemicals have a U.S. market volume of about $14 billion. For the rest of the products listed, sales are an order of magnitude lower. At the bottom of the list are some of the newer products of biotechnology, genes and whole cells, for which markets have yet to

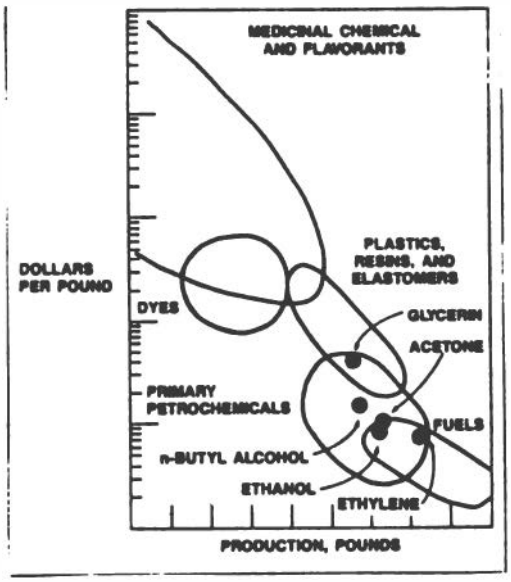

FIGURE 1 Specialty chemicals and pharmaceuticals.

develop. The materials in the middle of the table have explosive markets that are now opening for products such as polypeptides and new hormones produced by recombinant methods.

TABLE 2 Bioproduct Markets

Product	Current World Sales (US$ millions)
Organic solvents and acids[a]	14,180 (U.S.)
Amino acids	1,700
Antibiotics	1,625
Vitamins	667
Industrial enzymes	440
Steroids and alkaloids	380
Polypeptides and hormones	260
Nucleotides, nucleosides	160
Medicinal enzymes	155
Biopolymers	--
Polysaccharide gums	100
Genes > IMM MW[b]	--
Cells	--

[a]Current and potential applications.
[b]One million molecular weight

POTENTIAL FOR OXYCHEMICALS

It seems unreasonable to expect that biomass could compete effectively, even in the long run, with fossil feedstocks in the production of aromatics, particularly since coal is essentially aromatic in nature. Possible exceptions might be the aromatics that could be produced from lignin residues by hydrogenation and hydrodealkylation.

Depending on relative economics, ethanol from biomass might someday, but probably not before the year 2020, compete with heavy hydrocarbon feedstocks as a raw material for ethylene and butadiene. World War II processes for accomplishing this are already being introduced into Brazil's ethanol-based energy economy. For the free market economy of the United States, however, these dehydration processes suffer from inherently poor raw material stoichiometry, expressed earlier in the maxim: "Don't remove H_2O from CH_2O."

Hence, it seems more reasonable to expect that as fossil fuels become more expensive, both cellulose and starch could become increasingly important as cheap raw materials for oxychemicals that retain the oxygenated nature of the glucose monomer units of biomass. Against the background of the present synthetic organic chemicals industry, the 16 top oxychemicals listed in Table 3 have been, are being, or could be produced from renewable materials rather than fossil materials. All except adipic acid, 1,4-butanediol and methylethylketone are primary feedstocks in the sense that they can be produced directly from biosugar.

Ethanol is shown in Table 3 in terms of both its current use as a solvent and its potential use as a feedstock for ethylene and butadiene and as an octane enhancer in gasoline. Ethanol commands a $9 billion potential out of a total of $14 billion in current sales for oxychemicals. Other materials of interest have lesser potential.

Certainly, the United States and the world already have an ample production capacity to supply current needs for such feedstocks, as shown in Table 4 (Stanford Research Institute, 1981). Over the next five years alone, however, the need for a considerable increase in U.S. or world production capacity is indicated for many feedstocks:

	Percent Increase, United States	Percent Increase, World
Ethylene	+14	+31
Butadiene	+30	+26
Ethylene glycol	+26	+33

For such expansions, oxychemical plants based on renewable materials would need to compete on a grass-roots basis with new fossil-based plants. Overall, nevertheless, the long-term, inexorable pressure of rising OPEC oil prices will generate the need for alternative sources of fuels and chemicals.

TABLE 3 Oxychemical Markets for Innovations in Biotechnology

Oxychemical	Current U.S. Value ($ millions)
Ethanol	
Ethylene	6,790
Butadiene	1,320
Octane enhancer	560
Industrial	380
Subtotal	9,050
Ethylene glycol	1,260
Adipic acid	1,030
Acetic acid	620
Isopropanol	500
Acetone	460
Acrylic acid	360
Glycerol	250
1,4-Butanediol	240
Propylene glycol	220
Methylethylketone	210
n-Butanol	200
Citric acid	190
Sorbitol	90
Propionic acid	35
Fumaric acid	25
TOTAL OXYCHEMICALS	14,180

Source: U.S. International Trade Commission, Washington, D.C.

MARKETING POTENTIAL

Marketing potential relates to where a company stands in the marketplace, keeping in mind whether its products are produced for captive use or are intended for the merchant market. Each company must judge for itself where its position lies or could be developed in that scheme of things.

PRODUCT POSITION

In terms of commodity feedstocks, there is no product position. Because these are all well-known materials, proprietary product patents

TABLE 4 Production Capacity, 1980 (Million Annual Pounds)

	United States	Western Europe	Japan	World
Ethylene	36,300	36,000	12,700	104,500
Ethylene glycol	5,380	2,970	1,370	12,220
Butadiene	4,020	4,600	1,620	12,530
Acetic acid	3,540	2,540	1,450	8,520
Acetone	3,290	2,390	620	6,890
Isopropanol	2,800	--	--	--
Ethanol	2,510	1,240	260	17,860[a]
Adipic acid	1,910	2,350	150	4,930
Propylene glycol	870	820	15	1,920
Methylethylketone	870	570	140	1,800
n-Butanol	800	--	--	--
1,4-Butanediol (and THF)	380	220	20	620
Glycerol	340	470	120	1,010

[a]Includes 5,930 million lb, USSR; 5,580 million lb, Brazil.

have long since expired. Companies entering these markets will need to develop both a manufacturing position and a raw materials position to be successfully involved.

RAW MATERIALS SUPPLY

As a product of solar energy, biomass depends on land dedicated to useful photosynthesis. For example, of the total 2.3 billion acres of the United States, 380 million acres (17 percent) are devoted to crops, 720 million acres (32 percent) to forest and woodland, and 680 million acres (30 percent) to pasture or grazing land (USDA, 1981).

Of all Indonesian crops, cassava is the primary source of starch because of its ample supply potential and low cost relative to other sources of starch or sugar. Lignocellulosic crop residues are also abundant, but commercial collection systems are limited and need to be developed to exploit this potential resource. Lignocellulose is the structural material of plants.

More complex than starch, it is a composite of three polymers (see Table 5). In wheat straw and hardwoods these comprise: 42 percent cellulose, a linear polymer of glucose that occurs as microfibrils; 35 percent hemicellulose, an amorphous-branched copolymer composed mainly of xylose; and 22 percent lignin, a cross-linked polymer of substituted phenylpropane units (Browning, 1963; Wenzl, 1970).

TABLE 5 Composition of Cellulosic Materials (Percent Dry, Extractive-Free)

	Soft-wood	Hard-wood	Wheat Straw	Bagasse	Corn Stover
Alpha-cellulose	43.8	42.4	42.4	38.7	42.8
Hemicellulose	26.5	35.6	33.5	39.0	42.0
Lignin	29.5	21.7	22.5	20.6	14.0
Ash	0.2	0.3	1.6	1.7	1.2
Polysaccharides					
C_6	56.6	51.1	45.8	46.2	49.0
C_5	6.9	18.2	24.6	27.0	25.6
TOTAL	63.5	69.3	70.4	73.2	74.6

In the United States, for example, only 8 million annual dry tons of crop residues--such as sugarcane bagasse, cotton gin trash, and rice hulls--are collected annually at certain processing sites (see Table 6). About 105 million annual dry tons of corn stalks and 180 million annual dry tons of cereal straw are available and could be collected if the demand warranted it. The additional potential supply of 525 million dry tons of other agricultural residues is too diffuse to be collected economically or must be retained on the land to maintain the soil.

TABLE 6 U.S. Cellulosics Potential--Cropland Resources (Million Annual Dry Tons)

Cellulosic Material	Collected Supply	Collectible Reserve	Potential Resource
Corn stover	--	105	212
Cereal straw	--	180	180
Soybean residues	--	25	50
Bagasse, gin trash, rice hulls	8	--	8
Other crops	--	--	360
TOTAL CROPLAND	8	310	810

Note: 1977-1979 crop data.

Similarly, the annual growth of the American forests could provide an economically collectible supply of 270 million dry tons of lignocellulosic biomass (see Table 7).

This amount is the net of mortality and commercial removals from a commercial inventory of 25 billion tons of standing tree stems. Eastern hardwoods, which are less important to the pulp and paper industry than the stronger fibered conifers, are primary target sources (Browning, 1963; USDA Forest Service, 1974).

Urban solid waste is the ultimate end for paper and board products. This source might supply another 30 million tons from the 32 largest urban centers. The supply from each of these would exceed the 400,000 annual dry tons needed for a cellulose-based chemicals plant (Wahlgren and Ellis, 1978; U.S. Environmental Protection Agency, 1974; Drobney et al., 1971). The heterogeneity of these materials, however, raises safety and process problems in downstream operations.

Geographical distribution of biomass must also be considered. In contrast to coal, which occurs in a three-dimensional sense as thick seams in many strip-mined areas, biomass occurs as a two-dimensional, diffuse supply, spread over the land from which it is derived. Most corn and wheat, and their residues, are found in the heartland of the United States.

TABLE 7 U.S. Cellulosics Potential--Forest Resources (Million Annual Dry Tons)

	Collected Supply	Collectible Reserve	Potential Resource
Net annual growth[a]	--	270	450
Logging residues	--	105	145
Process residues and wastes			
Pulp mills	3	38	46
Saw mills (excl. chips)	13	13	26
Paper and board mills	--	12	13
Fuel wood	3	--	3
Urban solid wastes	41	12	77
TOTAL FOREST	60	450	760

[a]Net of mortality and removals from a commercial inventory of 25 billion tons.

TABLE 8 U.S. Cellulosics Potential--Grassland Resources (Million Annual Dry Tons)

	Collected Supply	Collectible Reserve	Potential Resource
Cattle	5	4	237
Hogs	--	--	11
Broilers	--	--	6
Chickens	--	2	4
Sheep	--	--	2
TOTAL GRASSLAND	5	6	260

Note: 1977 data.

Mainly animal manure, the "grassland" cellulose resource is too diffuse (Table 8), except for a few large feedlots, to be a source of lignocellulose for chemicals (Niessen and Alsobrook, 1972; Veirs, 1971). A similar biomass inventory should be made in Indonesia.

The timber resource in Indonesia is vast (USDA, 1975). Hardwoods are generally less desired than conifers by the pulp and paper industry because they have short, weak fibers. Hardwoods are, however, preferred for chemical use since they contain less lignin and tacky dirt-collecting extractibles. Presumably, they could be used without seriously competing with the pulp and paper industry for raw materials.

How do these raw material supplies compare with the total potential need for chemicals? Table 9 shows the biomass needed to produce various oxychemicals. With the exception of the olefins, ethylene, and butadiene, the entire U.S. supply of oxychemical feedstocks could be produced from only 11 percent of the current corn supply. This is about the amount of material that corn refiners presently use annually. Likewise, only about 3 percent of the available excess cellulose supply would be required to do the same job. There is obviously ample raw material available, provided a business system can be developed to get biomass to the right place at the right price. It is assumed that the same situation applies to Indonesia, but this should be confirmed.

COMPETITIVE PRICE ADVANTAGE

Competitive price advantage is the bottom line for commodity chemical businesses. A system for producing feedstocks from renewable resources is shown in Figure 2. Many options are available.

TABLE 9 Oxychemicals from Renewable Resources

Oxychemical	1979 U.S. Production (million lb)	Percent of Corn Crop[a]	Percent of Cellulosic Biomass[b]
Ethanol			
Ethylene	29,200	36.50	9.50
Butadiene	3,600	5.30	1.40
Industrial	1,310	0.90	0.20
Ethylene glycol	4,600	1.60	0.40
Acetic acid	3,300	1.30	0.30
Acetone	2,500	2.70	0.60
Isopropanol	1,970	2.10	0.50
Adipic acid	1,800	1.40	0.30
n-Butanol	560	0.60	0.20
Propylene glycol	550	0.20	0.05
Glycerol	370	0.10	0.03
Butanediol & THF	300	0.30	0.10
Sorbitol	130	0.04	0.01
TOTAL EXCLUDING OLEFINS		11.00	3.00

[a] 7.4 billion bushels, 1979.
[b] 770 million dry tons, collectible supply.

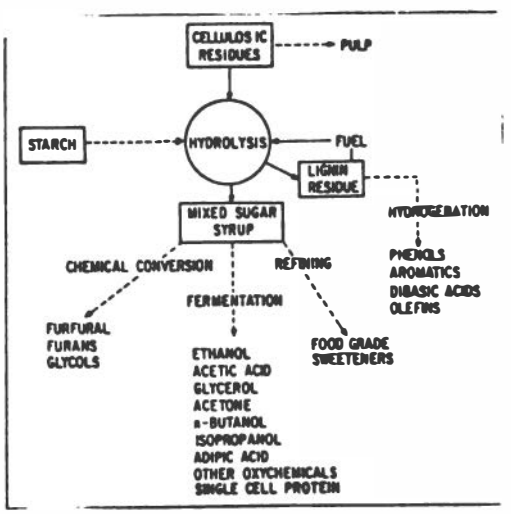

FIGURE 2 Feedstocks from renewable resources.

Biorefinery Model

Starting with cassava, the starch could be hydrolyzed to a sugar syrup, which would then be refined and further processed to provide food-grade sweeteners such as high-fructose syrup. Otherwise, the syrup could be fermented to ethanol which is also being done today. Other fermentation products could be produced similarly if the price were right.

Alternatively, cheaper cellulosic materials could be hydrolyzed and the biosugar processed in a similar manner. The lignin residue produced could be used as a fuel in the plant (this is currently done with bagasse in raw sugar factories), or it could be hydrogenated in the same way that coal can be hydrogenated to produce phenols and aromatics (Parkhurst et al., 1980). In fact, lignin, the geological precursor of coal, might be considered the ultimate source of aromatic chemicals.

In the hydrolysis of cellulosic materials, the biosugar product is a mixture of sugars, principally glucose and xylose in the case of hardwoods. These might be separated and further processed. For example, xylose might be used to produce xylitol by fermentation, or it could be chemically converted to ethylene glycol (Clark, 1958; Larcher, 1934; Tanikella, 1983), or furfural and furans as the Quaker Oats Company presently does (Harris, 1977; Duffey and Wells, 1955).

To evaluate the competitive advantages of any scheme developed from the "biorefinery" overview, one needs to examine the relative cost of raw materials and the conversion costs of the process alternatives.

Cost of Raw Materials

The cost of raw materials is certainly a big share of the total cost. For example, Table 10 shows the costs of producing ethanol from corn in a 25-million gallon batch-process plant operating in 1980. At that time, plant investment was roughly a dollar a gallon. It is higher now, and more recent data are probably available. These data make the point, however, that corn, net of grains credit, costs $.60 per gallon out of a $.97 per gallon mill cost and a $1.42 per gallon cost-plus-30 percent pretax return. Consequently, the cost of raw materials is of paramount importance.

Corn versus Fossil Materials

U.S. costs for various feedstocks over the past decade are shown in Figure 3. Each is shown on a common basis of cents per pound, although the more usual units for each are also given. The prices of all fossil materials--gas, oil, and ethylene derived from these--rose sharply over the 1970s. On the other hand, the price of corn rose much more slowly, representing the basis for any hope of switching from fossil materials to renewable materials.

What will happen to prices in the future? The recession of the early 1980s made everyone's timetable obsolete. Clearly, the industry misread the effects of conservation and recession; each year the

TABLE 10 Economics of Ethanol from Corn (1980 Dollars)

Basis	
25 million gal/yr 190° alcohol	
Batch process	
2.86 gal/bu	
Investment: $29 million	
Cost ($/gal)	
Corn @ $3.00/bu	$1.05
Grains credit @ $140/t	(.46)
Steam--alcohol recovery	.10
--grains recovery	.06
Other conversion costs	.23
Mill costs	.97
Cost plus 30 percent pretax	$1.42

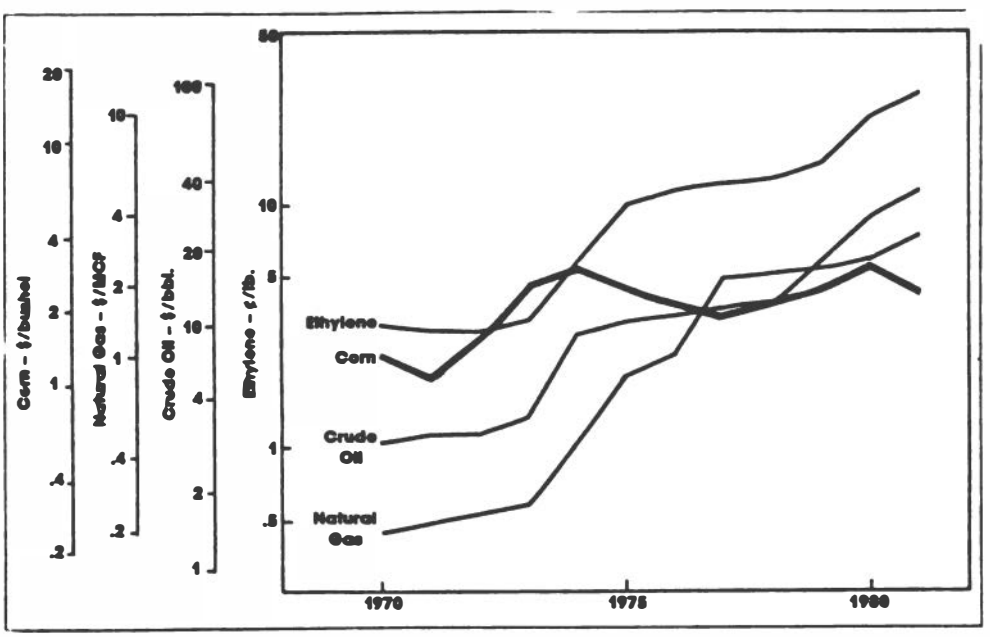

FIGURE 3 Feedstock prices.

projected demand dropped. Now, demand and price are fairly flat and are expected to stay flat over the next few years.

Now that the recession is over, what will happen to the price of oil over the next decade? Conoco, Inc. has projected that the price of crude oil will fluctuate between $15-$20 a barrel through 1990. During the 1990s as the supply-demand balance tightens, prices will begin to move up rapidly reaching $40-$50 a barrel by the year 2000.

Hydrolysis of Polysaccharides

Two options are available for converting starch or lignocellulose to chemical products: (1) convert directly, or (2) hydrolyze to the corresponding monomeric sugar for use as an intermediate feedstock. If technically feasible, the use of the polysaccharide directly would be preferred in most cases (Wang et al., 1981). Programs at the Massachusetts Institute of Technology and University of California at Berkeley have centered on this possibility; however, most fermentations take place more readily using a monomeric sugar feedstock. Moreover, it may be preferred in certain cases to have a large common supply of sugar feeding a number of smaller fermentation operations as part of a "biorefinery" complex.

Cellulosic biomass at $25-$35 per dry ton is far cheaper than corn at $110 per dry ton (Goldstein et al., 1978; Arola and Miyata, 1981). It is very difficult, however, for most naturally occurring organisms to hydrolyze cellulose because of the intractable nature of the cellulose crystallite. Thus, a trade-off occurs between the low cost of raw materials and the high investment needed for hydrolysis equipment.

TABLE 11 Costs of Pretreated Wood Chips (Dollars per Dry Ton): 1980, 1985, 1990

	1980	1985	1990
Hardwood chips (40-mile haul)	25.75	36.80	51.54
Cost plus 30% PTROI ($/dry ton)			
Chips	28.36	40.48	56.59
Receiving and grinding	6.70	9.92	14.42
Acid pretreatment	26.66	48.64	74.00
TOTAL	61.72	99.04	145.11
$/lb equiv. sugar[a]	.044	.071	.104

[a]At 90 percent molar yield of polysaccharides (70 percent dry basis).

Note: PTROI = pretax return on investment.

Various pretreatment processes have been under study to improve hydrolyzability in a direct one-step bioprocess for converting lignocellulose. In the mild acid pretreatment process, wood chips or other sources of lignocellulose are acidified to hydrolyze and recover the hemicellulose sugars while opening up the structure of the alpha-cellulose to enzyme attack (Krappert et al., 1981). The cost of the pretreatment, however, increases the cost of the wood chips from $35 to over $90 per dry ton, as shown in Table 11. This results in loss of much of the cost margin between wood and corn.

In a two-stage process, cornstarch or lignocellulose is first hydrolyzed to the corresponding sugars before converting to the final product in a subsequent operation. The large wet corn milling industry now provides a commercial supply of hydrolyzed cornstarch (Janke and Koppel, 1980). For 1985, it was estimated that corn syrups could be produced commercially from corn at $3.40 per bushel at a cost of about $.12 per pound of sugar (estimated from the data of C. R. Keim. 1980. Industrial and Engineering Chemistry Product Research and Development 19:4, and of other wet milling industry sources). This price placed a competitive cost ceiling on the market value of lignocellulose-based "biosugars." In addition, the "residues" from corn wet milling are high-value oil and protein feeds, while markets for lignin, the residue from cellulose hydrolysis, have yet to be developed.

Cellulose hydrolysis does not presently appear to be economically competitive with starch hydrolysis as a source of sugar. Processes using concentrated acids to catalyze the hydrolysis of cellulose have not been successful commercially because of the need to recover and recycle the acid. The dilute acid process reduces acid-associated costs to about $.003 per pound of sugar, produced at a lignocellulose cost of $.03 per pound sugar (1985 dollars); however, power costs are high. Plant investment amounts to $.18 per annual pound, which is also too high for the process to compete in its present form with corn hydrolysis.

The acid hydrolysis of cellulose is hardly new. Bergius's Reinau Process was based on the use of supersaturated hydrochloric acid as described earlier in German Patent No. 11836, issued in 1880. This process was used until the end of World War II. Concentrated sulfuric acid was the basis for the process piloted by the U.S. Department of Agriculture's (USDA) Northern Regional Research Laboratory in 1945 (Dunning and Lathrop, 1945) and by the Japanese at Hokkaido in the 1950s (Takubo et al., 1960). Neither process was commercialized because of the problem of recovering and recycling the acid.

Concurrently, the use of dilute sulfuric acid at higher temperatures was introduced by Scholler in a plant at Tornesch, Germany, in the 1930s (Luers, 1930, 1932). This batch process used less acid, but yields were poorer than with the concentrated acid processes. During World War II, the American War Production Board assigned further development of the process as a source of ethyl alcohol to USDA's Forest Products Laboratory. The continuous "Madison" process that resulted was incorporated in a pilot plant built by the Tennessee Valley Authority (TVA) at Wilson Dam (Gilbert et al., 1952), and in a larger plant built in Springfield, Oregon, that processed

300 tons of wood waste daily (Harris et al., 1945, 1946). None of the acid hydrolysis plants survived peacetime economies, except those that continued to operate in the USSR.

Interest in acid hydrolysis as a route to alternative energy sources revived in the 1970s as a result of the world oil situation. Dilute acid systems appear farthest along in development. Dilute 0.4 wt% sulfuric acid is being used at moderate temperatures--for example, 170°C/5 min in a mild prehydrolysis pretreatment for recovering heat-sensitive hemicellulose sugars prior to applying highertemperatures, and 270°/5 sec for hydrolyzing alpha-cellulose. The two-step process has been explored on a small scale in a number of laboratories, notably USDA's Forest Products Laboratory (Zerbe, 1982), and that of Dartmouth College (Grethlein, 1978). New York University has operated an advanced pilot system that used a Werner-Pfleiderer twin-screw extruder/reactor to control temperature and residence time to the limits critical to the yields of this process (Rugg, 1982). Finally, the Georgia Institute of Technology and TVA designed integrated pilot plants that were also based on dilute acid technology (O'Neil, 1980).

Research on new versions of concentrated acid hydrolysis has lagged behind that on dilute acid processes. Michigan State University made progress in using gaseous or liquid hydrogen fluoride to break down alpha-cellulose at low temperatures without subsequently degrading the sugars. However, considerable reversion of monomer sugars to oligomers occurs while increasing the temperature to recover the hydrogen fluoride. This requires a mild posthydrolysis of oligomers by dilute sulfuric acid. As a result, the investment in this process approximates that needed for the two-stage dilute acid process (Hardt et al., 1982). Other concentrated acid projects do not seem to be as firmly developed. This includes Purdue University's use of methanol to extract and recycle concentrated sulfuric acid from the acid-impregnated biomass and North Carolina State University's evaluation of superconcentrated 15N hydrochloric acid in a variation of the Bergius process (Goldstein et al., 1983).

A biological approach to cellulose hydrolysis involves the use of cellulolytic enzymes such as those produced by the fungus Trichoderma reesei (Mandels, 1981). This process had been under development by the U.S. Army Natick Laboratory since World War II. More recently, at least 15 other laboratories around the world have instituted similar projects. The enzyme used is produced extracellularly in a separate fermentation process and then transferred to the hydrolysis section as a supernatant liquid after filtering off the cells. Development of hypercellulolytic mutants at Natick Laboratories, Rutgers, and Cetus Corporation increased the productivity of this step tenfold (Allen and Andreotti, 1982; Montenecourt et al., 1981). Two problems remain, however: (1) the need to pretreat the cellulose feed to improve accessibility of the substrate to enzyme attack, and (2) inhibition of the enzyme by the product glucose and its dimerous cellobiose. Dartmouth College has shown that dilute acid prehydrolysis is an effective pretreatment (Krappert et al., 1980, 1981), but this step adds $.03 per pound to the cost of the sugar produced (1980 dollars).

The Iotech (Foody, 1980) and Stake (Bender, 1979) steam explosion pretreatments, as well as the Colorado State University (Dale and Moreira, 1982) liquid ammonia freeze-explosion technique, may prove to be more cost-effective.

The economics of some of these hydrolysis processes are compared in Table 12. Even at best, these approaches appear to provide only a trade-off between the new, untried sources of glucose and corn syrup, a well-established source. Few in industry would be seriously interested in introducing such new processes unless a considerable apparent cost savings could be developed to justify the risk involved.

Conversion Costs

Conversion costs usually include labor-related costs, utility costs, and capital-related costs. Capital-related costs also must include some idea of how much return on investment is expected by the company electing to enter a new business. Thus, capital investment for direct plant equipment, as well as for allocated utility investment and working capital, is usually the dominant factor affecting total cost. The process yield also has a strong effect on the resulting cost of raw materials.

It is rather difficult to generalize about conversion cost differences between fermentation and petrochemical processes. Synthetic processes are usuallly operated in a continuous mode on a large scale to attain the economics associated with such large-scale operations. Fermentations are more often operated in a batch mode. Although fermenters are relatively cheap per unit volume compared with high-pressure synthesis reactors, large volumes are usually needed and fermentation plants are operated with multiple units. Thus, as design capacity increases the attendant decrease in investment perunit of production flattens out at a relatively low scale.

Yields can be high or low in either synthetic processes or fermentation processes, making it again difficult to generalize.

TABLE 12 Biomass Costs (Dollars per Dry Pound): 1980, 1985, 1990

	1980	1985	1990
Corn stover	$.015	$.021	$.031
Whole tree wood chips	.013	.018	.026
Pretreated wood chips	.031	.050	.072
Biosugar ex lignocellulosics			
Enzyme/acid pretreat	.080	.129	.193
Concentrated acid/recycle	.081	.123	.181
Dilute acid/extrusion	.088	.140	.209
Concentrated acid/once-through	.126	.187	.268
Corn syrup (as glucose)	.084	.104	.137

Similarly, although fermenters operate at low temperatures and at low unit energy demands, the product is generally contained in a very dilute aqueous beer. As a result, product recovery operations can involve some energy-intensive processes such as distillation.

Fermentation Costs

Of all the fermentation parameters, product concentration has the greatest effect on conversion cost. Batch time, or dilution rate for continuous operation, is second in importance. The effect of each on cost is shown in Figure 4. Concentration is of primary importance because its reciprocal (gallons per pound) is a measure of the size of the fermentation and recovery/purification plants needed to produce a unit of product. Dilution rate affects only fermenter volume, but since fermenter investment is usually a large proportion of total plant investment, the effect on cost can also be large. Figure 5 shows the importance of increasing concentration to above 100 g/l. This level is seldom realized in practice because of product inhibition of the organism.

The advantage of continuous operation is evident. A batch mode usually requires about 12 hours to turn a fermenter around at the end of a run. This means that even with a hypothetical zero fermentation time for a batch operation, the effective "dilution rate" would reach a maximum of 0.08 hr^{-1} (that is, the reciprocal of 12 hours total cycle time). The convergence of the curves at an "infinite" dilution rate simply means that at high rates the effect of fermenter investment becomes less important than that of other investment items.

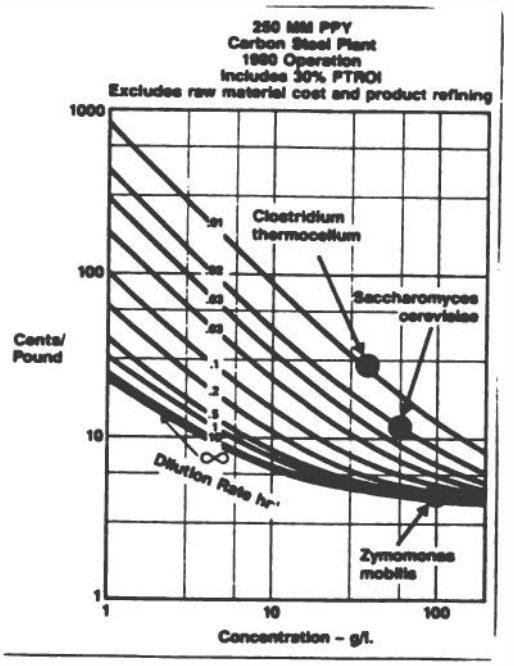

FIGURE 4 Fermentation conversion costs.

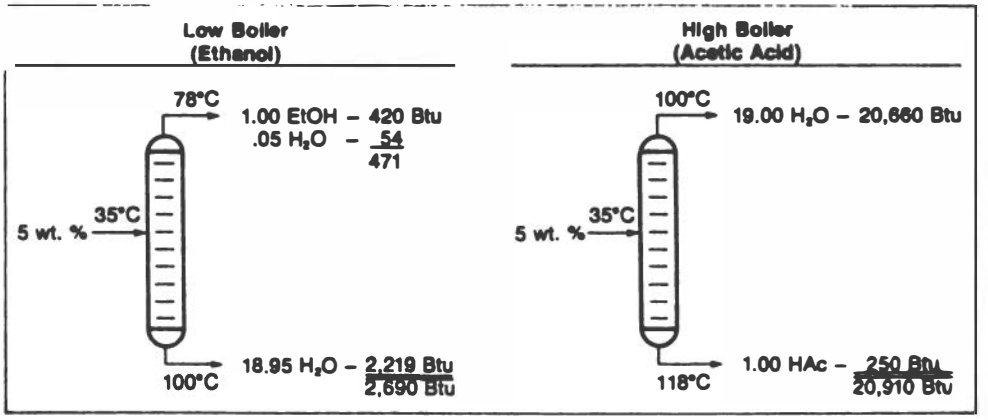

FIGURE 5 Simplistic distillation heat balances.

Figure 5 also compares the conversion costs of three alternative routes to ethanol. The $.12 per pound ($.75 per gallon) cost for the Saccharomyces cerevisiae yeast system represents the conventional batch fermentation of corn sugar in a distillery at a batch time of about 54 hours for a product concentration of 60 g/l as limited by product inhibition (Novack et al., 1981; Maiorella et al., 1983). A more efficient system for corn sugar based on the bacterium Zymomonas mobilis operates at a higher 100 g/l concentration because of lower product inhibition, and at a higher effective dilution rate of 0.7 hr^{-1} (Rogers et al., 1982). These two improvements reduce the conversion costs to $.05 per pound ($.28 per gallon). In contrast to these two corn-based processes, MIT has been developing a process using the thermophyllic bacterium Clostridium thermocellum to convert lignocellulosics directly to a mixture of 32 g/l ethanol plus 7 g/l acetic acid at a total batch time of 100 hours (Cooney et al., 1983). The longer time and lower concentration result in a higher $.29 per pound ($1.80 per gallon) conversion cost. It is hoped that this process can be improved further to reduce cost. Because in this case the raw material cost is lower than that of the corn-based processes, the conversion cost can be higher and still effect a break-even position.

Product Recovery Costs

In addition to the need for an improved fermentation process, a corollary need exists for new energy-efficient processes to recover the products from dilute aqueous solution.

Recovering products from fermentation broths invariably involves separating the product from a dilute (usually under 10 wt% but more generally 1-5 wt%) aqueous solution. The magnitude of this problem and the approach taken to solve it depend on whether the product has a boiling point below or above that of water, occurs as a salt, or is a precipitate. Low-boiling organic solvents are relatively easy to separate from water by distillation because of an usually high boiling point and volatility differences. Distillation is the present

separation method of choice in these cases, particularly where the heatcan be supplied by low-pressure steam and refrigeration is not required to condense the overhead vapors (Null, 1980).

Since the solvent is boiled away from water, the energy expended is a simple function of its latent heat of vaporization--for example, about 2,300 Btu/gal for ethanol (Perry et al., 1963). As shown in Figure 5, the heat lost to water is mainly that required in sensible heat to heat water to its boiling point. About 70 percent of this can be recovered by heat exchange with the feed. For a 5 percent ethanol broth leaving a fermenter at 35°C, this heat amounts to about 14,000 Btu/gal or 2,220 Btu/lb of ethanol produced. In contrast, to recover a high-boiling solvent like acetic acid from water by distillation, the water must be boiled away from the product. In the case of a 5 wt% solution of acetic acid in water, this heat energy amounts to at least 19 times the latent heat of water, or about 21,000 Btu/lb of acid.

Low-Boiling Solvents

Ethanol serves as a good example of a low-boiling solvent of current national interest.

It also exhibits a minimum-boiling azeotrope with water. In the actual recovery of ethanol from fermentation broths, considerably more energy is required than indicated in the above simplistic example, the result of the need for a high reflux ratio to reach concentrations approaching the 95 percent azeotrope in the "pinch region" of the vapor-liquid equilibrium diagram. Indeed, outmoded beverage alcohol plants have reported overall process energy needs as high as 150,000 Btu/gal (Remirez, 1980). Over recent years, much attention has been given to improving the recovery of anhydrous ethanol for use in gasohol. In newer energy-efficient fermentation plants, total plant energy demand has been reduced to as low as 30,000-50,000 Btu/gal. Most of this is for the recovery operation.

Various other recovery methods are also being introduced, including vapor recompression distillation, multiple-effect distillation, supercritical extraction, azeotropic distillation, vacuum distillation, extractive distillation, and sorption (Busche, 1983a). A summary of the energy demands for some such processes for recovering low-boilers is shown in Table 13. Some form of distillation combined with vapor recompression or cascaded pressure staging appears to be the current method of choice for producing a product at a concentration up to the azeotrope. Such systems have been amply demonstrated in commercial practice. Each new case, however, should be evaluated on its own merits. The energy savings adaptations require additional heat exchanges, compressors, and so on, compared with conventional distillation. In some cases, particularly for small plants, adding such equipment investment may not be justified by the value of the energy saved.

If anhydrous alcohol is needed, the new sorption processes for removing water from the azeotrope might be considered over the incumbent azeotropic distillation process. Appraisal of these processes, as well as of other new approaches such as supercritical extraction at the pilot and demonstration levels, should be continued.

TABLE 13 Energy Demands for Recovering Ethanol from Aqueous Solution

Process	Concentration (wt%) Initial %	Final %	Energy Form	Energy Demand (Btu/gal) Actual	Equiv. Steam
Simple distillation	10	95	Steam	18,000	18,000
Multiple effect distillation	10	95	Steam	7-10,000	7-10,000
Supercritical extraction	10	91	Elec.	2,850	8,600
Vapor recompression distillation	10	95	Elec.	1,930	5,800
Azeotropic distillation	95	100	Steam	9,400	9,400
Adsorption-water	95	100	Steam	2,000	2,000
Vacuum dehydration	10	100	Steam	37,000	37,000
Adsorption-ethanol	10	100	Elec. & steam	13,000	31,300
Simple dist. and azeo. dist.	10	100	Steam	27,400	27,400
Vapor recomp. dist. Absorption	10	100	Elec. & steam	3,930	7,800

[a]At 33 percent steam-to-electricity conversion efficiency.

High-Boiling Compounds

As indicated earlier, the cost of distilling water from a higher boiling product is prohibitively expensive. For example, acetic acid and water are relatively close in volatility. To recover glacial acid from a 1.5 wt% aqueous solution by simple distillation, as shown in Figure 6, would require a column operated at a very high 2.8 reflux ratio, at a steam load of 275,000 Btu/lb of acid recovered (E. L. Mongan, Jr.,

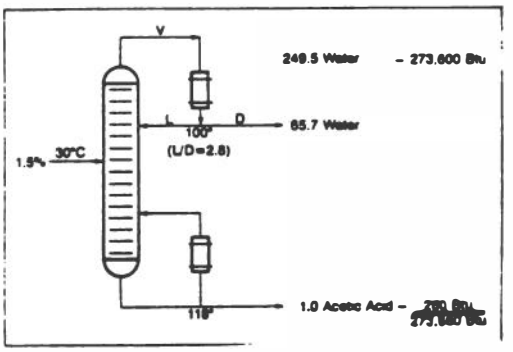

FIGURE 6 Recovery of acetic acid by simple distillation.

Engineering Department, E. I. du Pont de Nemours & Co., Inc., personal communication, 1981). Thus, some other approach such as solvent extraction needs to be considered in this case.

Solvent extraction combined with azeotropic distillation for dilute feeds has been used for many years to recover acetic acid in manufacturing cellulose acetate, vinyl acetate, and other products (Jones, 1967; Hanson, 1979). Acetic acid could be produced from glucose either directly using Clostridium thermoaceticum or indirectly by way of ethanol using the older two-step vinegar process with Acetobacter acedti or Acetobacter suboxidan (Busche, 1984).

In either case, if extraction were used to recover the product, the process would have to be operated at low pH to result in a product that acts as an extractable free-acid rather than a salt. The acid could then be recovered by the extraction process shown in Figure 7. In the case of a plant that was scaled to produce 250 million annual pounds of

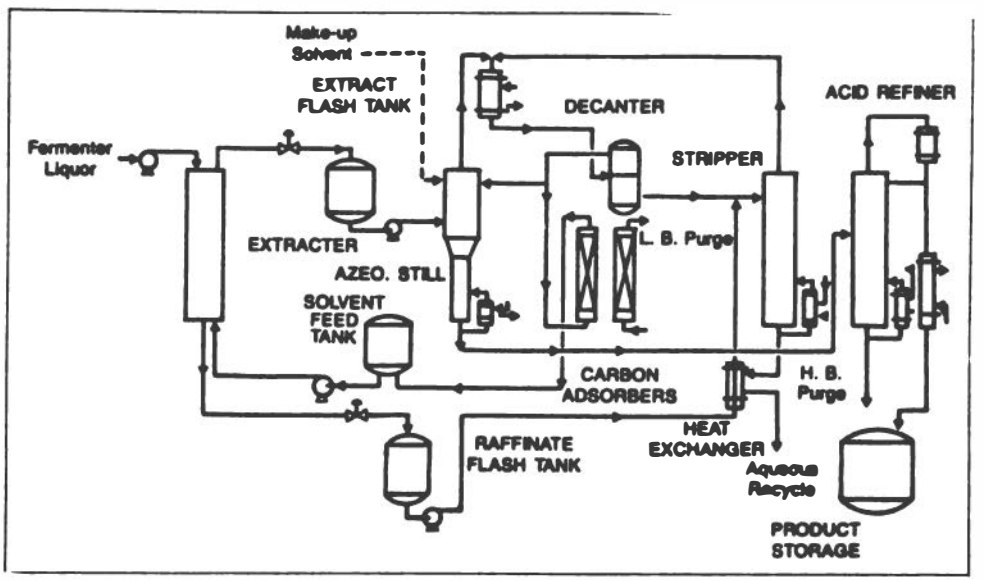

FIGURE 7 Acetic acid recovery via solvent extraction.

glacial acetic acid and that was at the mid-point of construction in mid-1982, the direct investment in the recovery section would have amounted to $57 million. Product recovery costs are shown in Figure 8. Reductions in cost could be realized with increases in product concentration. The concentrations shown range from the low 10-20 g/l concentrations expected for the Clostridium thermoaceticum system (Schwart and Keller, 1982) to the 55-120 g/l concentrations demonstrated by Wang for the Acetobacter suboxidans system on ethanol (Wang et al., 1978).

The energy demands for recovering acetic acid by various processes are summarized in Table 14. At the moment, solvent extraction appears to be the process of proven choice. Recovering free acid from salt solutions is much more difficult. To this end, continued development of the electrodialysis process is recommended. Membranes that have improved structural integrity and anti-fouling characteristics need to be developed along with the process itself.

In summary, of the commercially demonstrated recovery processes, distillation appears most suitable for low boilers, while solvent extraction appears well suited for high boilers. A number of new approaches such as supercritcal extraction, molecular sieve adsorption, and membrane separation hold promise for further development.

FUTURE NEEDS

In 1977, it became cheaper for the first time in 27 years to produce ethanol from starch instead of from ethylene. Notwithstanding the present softness in the crude oil market as the cost of petroleum rises again, it can be expected that at some future time the

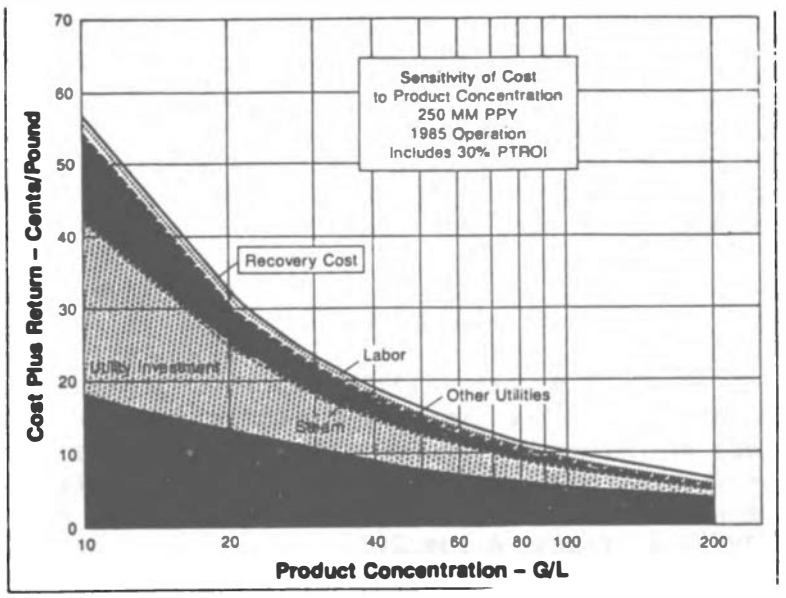

FIGURE 8 Product recovery costs.

TABLE 14 Energy Demands for Recovering Acetic Acid from Aqueous Solution

Process	Concentration (wt%) Feed Form	Initial	Final	Energy Demand (Btu/lb) Energy Form	Actual	Equiv. Steam[a]
Simple distillation	Acid	1.5	100	Steam	274,000	274,000
Melt crystallization	Acid	1.0	100	Elec.	7,500	22,000
Solvent extraction	Acid	2.0	100	Steam	11,000	11,000
Acidification/ extraction	Salt	1.5	100	Elec. & Steam	57,800	79,400
Vapor recompression evaporation	Salt	1.0	55	Elec.	10,400	31,200
Electrodialysis	Salt	1.0	100	Elec.	2,400	7,200

[a]At 33 percent steam-to-electricity conversion efficiency.

fermentation of renewable material to produce other feedstocks or specialty chemicals will become viable once again. To foster this, the concomitant development of new fermentation systems and new recovery processes appears critical to establishing a cost-competitive fermentation industry.

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Bioconversion of Agricultural By-products in Indonesia

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INTRODUCTION

Biotechnology, an integrated activity of a number of scientific disciplines, has long been well known in Third World countries, including Indonesia. The government of Indonesia has decided that the development of biotechnology should be given high priority, with emphasis on health care, agriculture, and industry. In the health care sector, the application of biotechnology is expected to facilitate the production of antibiotics, human vaccines, monoclonal antibodies, interferons, hormones, vitamins, proteins, and diagnostics. In the agricultural sector, the application of biotechnology may benefit the production of animal feed, breeding of new plant cultivars and animal breeds, production of the traditional fermented food and beverages, and utilization of agricultural by-products. In the industrial sector, biotechnology will greatly help in the production of biomass from agricultural by-products, production of high-value chemical compounds as well as solvents, and treatment of industrial and municipal wastes.

PRESENT UTILIZATION OF AGRICULTURAL BY-PRODUCTS

Current R&D Programs

R&D programs are now under way at various Indonesian universities and government and private research institutes. Because each institution usually has its own program, there is little coordination among institutions and thus considerable duplication of effort.

Most research is aimed at improving existing technologies for the utilization of agricultural products or by-products. Examples include improving fermentation technology for manufacturing the traditional fermented foods and beverages from pulses and starch-rich food crops, improving the ensiling process of lignocellulosic wastes and trash fish for producing animal feed, and improving the fermentation process for producing alcoholic drinks from rejected fruits.

A number of state-run and private factories use the fermentation process to manufacture such products as ethanol, citric acid,

monosodium glutamate, single-cell protein, and the traditional fermented foods and beverages. Since before World War II, cane sugar refineries have produced ethanol as a side product, utilizing molasses as the raw material. The plant recently opened by the Agency for the Assessment and Application of Technology (BPPT) in Lampung, however, is designed solely for ethanol production, utilizing cassava as the feedstock. All of the monosodium glutamate sold in Indonesia is produced by privately owned large foreign companies or their subsidiaries. The same is true of the plants in Lampung that produce citric acid from tapioca wastes. In contrast, most of the plants that manufacture the traditional fermented foods are small and lack sophisticated technology, but they are owned by Indonesians.

Manpower

Because Indonesian universities do not offer degrees in the disciplines that support biotechnology--for example, microbiology, biochemistry, genetics, and biochemical engineering--the number of scientists who have received training in one of the various disciplines of industrial biotechnology is very small. The situation is even worse when counting only those individuals who devote full time to their scientific specialization; most are engaged in administrative activities. Trained technicians are also scarce. Here, too, the situation is not improving because the number of schools for training these technicians is still limited. Furthermore, graduates usually prefer to work in the private sector rather than in government research institutions.

Facilities

In most cases, the R&D facilities of government institutions are better than those of universities. They are usually not in good condition, however, because of a lack of funds for maintenance. As privately owned facilities do not have budget constraints, they are in much better shape.

A lack of library and documentation facilities also limits the proper development of biotechnology in Indonesia. The few that exist are usually not well supplied with the current biotechnology literature or research results.

FUTURE PLANS

R&D Programs

The amount of by-products and waste generated annually by the agricultural sector is approximately three to five times the product itself. What can therefore be done with this biomass to give it economic value?

Considering the many components that make up these by-products, it seems wise to classify the latter according to those components that could be developed further:

- Starch and cellulose: tapioca, cornstarch, sago palm starch, rice straw and hulls, sugarcane bagasse, wood chips, palm kernels
- Sugars: molasses
- Oil or lipids: palm oil press cake, coconut press cake, soybean oil press cake
- Protein: trash fish and shrimp, slaughterhouse wastes.

Based on these components, the kinds of products that could result, and the market potential of these products, it is then possible to devise the appropriate R&D programs. Programs that seek to produce the following appear to be feasible and profitable:

- Single-cell protein and biomass for animal feed
- Industrial enzymes, especially hydrolases
- Antibiotics--tetracycline, penicillin, erythromycin, kanamycin
- Steroid drugs
- Vitamin B_{12} through fermentation
- Fish protein from fish scraps
- Supercritical extraction-based products such as vitamin A/B carotene (crude palm oil), coconut oil, soy oil, and decaffeinated coffee and caffein from coffee.

In implementing the above programs, maximal utilization should be made of existing facilities in universities and government institutes. Where the required facilities are not available, it is advisable to locate them in the new R&D center for biotechnology now under construction at Cibinong.

It is imperative that the private sector and state enterprises be encouraged to embark on ventures in bioindustries in cooperation with government R&D institutes and universities. If necessary, joint ventures with an overseas partner can be undertaken. An example of cooperation among various government R&D institutes, universities, and industry in Indonesia is shown in Figure 1, while a scheme of cooperation between Indonesian R&D institutes and foreign counterparts is presented in Figure 2.

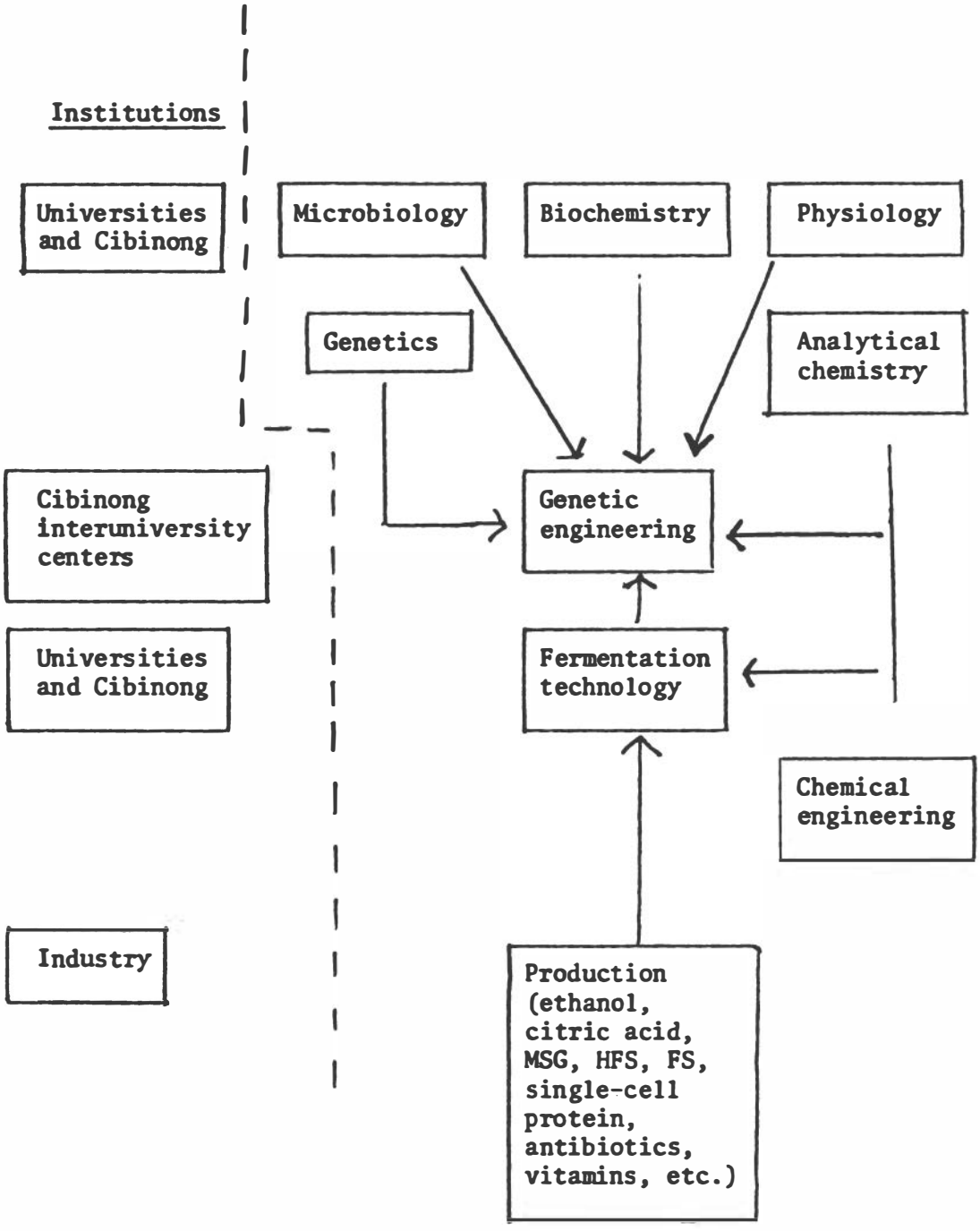

FIGURE 1 Scheme of cooperation among various government R&D institutes, universities, and industry.

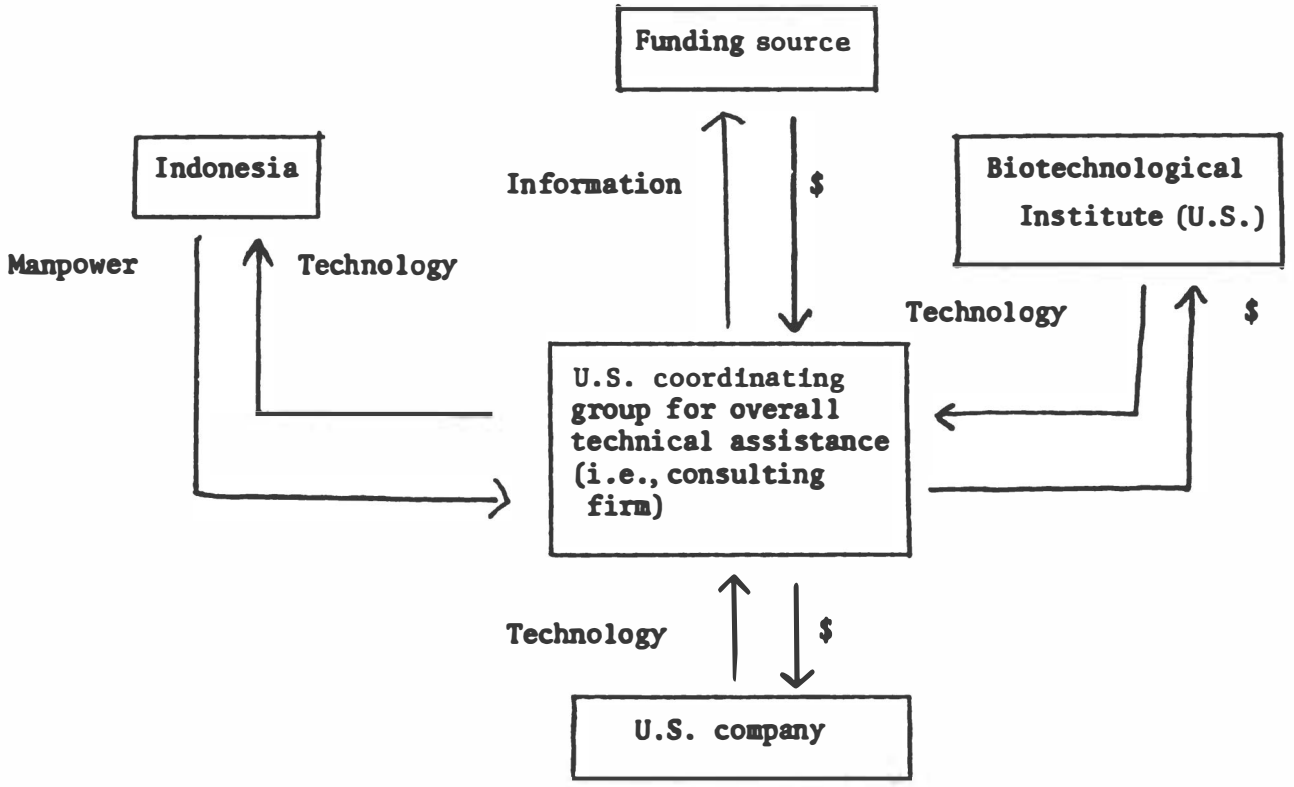

FIGURE 2 Scheme of cooperation between Indonesian R&D institutes and foreign counterparts.

PART III

Conclusions and Recommendations

GENERAL RECOMMENDATIONS OF THE NRC PANEL

Charles C. Muscoplat
Chairman, NRC Panel

The intensive sessions and field trips held with the Indonesian team members and their leaders during this five-day workshop gave the U.S. National Research Council (NRC) panel an excellent opportunity to observe firsthand the capabilities and ongoing projects in the agricultural biotechnology area. This included a stop in central Sumatra where the panel visited a palm oil estate to observe their research activities, as well as a palm oil factory. Visits were also made to Bogor Agricultural University, the Food Research Institute in Bogor, and several dairy farms. The latter included a dairy in a small village as well as experimental research farms such as Tapos.

These visits allowed the U.S. panelists to meet with their Indonesian colleagues undertaking research in the four areas of biotechnology that were considered by this workshop: embryo transfer and animal production, plant cell and tissue culture, plant nitrogen fixation, and bioconversion of agricultural by-products. In general, the NRC panel found these individuals to be highly motivated, industrious, tenacious, and, within the limits of their training, very capable. Indeed, the work that these scientists are currently undertaking has provided an excellent training ground for sharpening individual skills.

Present capabilities--though limited by the inadequate number of personnel, levels of training, research materials, and laboratory facilities--are nonetheless sufficient to undertake important projects and make significant contributions to the progress of the Indonesian economy. To maximize this potential, however, a number of aspects need considerable strengthening. The general recommendations of the NRC panel are listed below. These recommendations are supported by the observations and findings of the working groups, whose specific recommendations follow.

1. In the area of applied agricultural research, a priority-setting mechanism involving both scientists and policymakers should be established to allocate resources correctly. The criteria for setting priorities might include: nature of the problem, economic impact, human impact, technical feasibility within desired time frame, available scientific physical and human resources, environmental factors, and international or national factors. The objectives selected for each priority should be limited, and significant effort should be focused on each

priority to ensure success. The panel noted that some of the working groups tended to attempt too many diverse projects, thereby diluting efforts to nonproductive levels.

2. Given the relative lack of equipment, laboratory space, and operating capital in the areas the team visited, the panel strongly suggests establishing incentives for collaboration among laboratories to solve common problems. Such incentives should provide for intellectual collaboration, as well as sharing of equipment, supplies, and even facilities. Research grants or equipment can be provided to those groups demonstrating the strongest collaborative program. Research at universities and educational institutions should compete equally with research in other agencies of the government such as the Ministry of Agriculture. The panel believes that over the long term Indonesia must be self-sufficient in providing for training of human intellectual resources. Thus, it is important that agricultural educational institutions receive a significant opportunity for either greater direct financial support or access to facilities, equipment, and human resources via collaboration with other government agencies or the private sector.

3. Because it understands the difficulty of traveling to foreign scientific meetings and the scarcity of literature, the panel recommends that a program of organized scientific exchange be established within Indonesia to provide a forum for collaboration and communication. Such a program may resemble the beginnings of a scientific society. The panel noted that many of the individuals attending the workshop had never had the occasion to meet together except under informal circumstances. The cost of establishing such a scientific interaction would not be great, but the benefits would be substantial.

4. The panel was concerned that the various scholarship programs are burdened with lengthy administrative procedures which can discourage young scientists. Every attempt should be made to streamline the granting of scholarships to deserving students for training abroad. In addition, funds should be sought to support these young scientists and their research while their papers are being processed. We understand that often these individuals cannot afford to wait until the scholarships are awarded. Thus, their services are lost to the research community.

5. **A task force should be established to initiate a grants program through the Indonesian National Research Council and to solicit monies such as PL 480 funds for research and collaboration in Indonesia.**

SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS OF THE WORKING GROUPS

EMBRYO TRANSFER AND ANIMAL PRODUCTION

1. Establish priority-setting mechanisms and reasonable, quantitative goals for an embryo transfer and animal production program.

2. Inventory current skills and needs to facilitate making recommendations for future training.

3. Establish by the end of calendar year 1986 an embryo transfer and animal production working group consisting of relevant disciplines. This working group will promote embryo transfer technologies and related biotechnologies as a step toward establishing an inventory, promoting conservation, and improving germ plasm. The germ plasm should then be made available to the end users. This group must meet at least two to three times each year to discuss priorities, results, and manpower allocations. It should propose future manpower needs and training areas and establish collaboration or joint research programs with other countries. The working group should also be responsible for establishing a center to disseminate information on the progress and priorities of research in this area. Such an information center could also promote the sharing of resources and facilities and help establish appropriate collaboration.

5. Establish a national center for embryo transfer and animal production.

PLANT CELL AND TISSUE CULTURE

1. Establish a mechanism for determining which research and crops should have highest priority and for monitoring research progress.

2. Before research is begun, determine who will use the research results and establish relationships that facilitate the transfer and use of results.

3. Provide support and infrastructure to promote research collaboration among universities, government laboratories, and the private sector. Benefits would include rapid progress and minimal duplication. Support is needed in the form of annual professional meetings held in-country, travel funds to permit participation in meetings, and receipt of scientific journals.

4. Hold meetings of the Indonesian plant cell and tissue culture working group at least once each year to review research results, establish new objectives in this area, and review manpower needs.

5. Continually assess manpower needs so that appropriate training both in Indonesia and abroad is provided in a timely manner.

6. Promote increased cooperation between Indonesian and U.S. experts to advise on graduate research, conduct short-term training programs, and exchange ideas and observations.

7. Invite experts from U.S. companies to assist in preparations for large-scale plant production.

8. Explore the possibility of obtaining research grants for work in plant cell and tissue culture and crop improvement. U.S. experts could be involved in the review of proposals and the progress of funded research.

9. Establish criteria for evaluating projects such as the following:

   - Name of crop
   - Total value of crop: Monetary value per hectare? Calorie content? Protein content? For animal or human consumption? Export commodity or for domestic use?
   - Objectives (e.g., plans to breed for drought resistance)?
   - Amount of time needed for research to accomplish objective?
   - Resources needed to initiate and accomplish objectives (e.g., personnel, equipment, supplies)?
   - Which researchers, government organizations, or private enterprises will use the results of successful research?
   - Is R&D being undertaken elsewhere, either in-country or abroad?

PLANT NITROGEN FIXATION

1. Obtain the two most crucial kinds of government support needed to develop and sustain qualified researchers:

   - Two- to three-year grants to cover the cost of "housekeeping" operations (i.e., ordinary chemicals, glassware, and minor equipment). The lack of such grants currently prevents researchers, particularly at universities, from making reasonably productive use of their training and abilities.

- o Funding for a national scientific and technical library. The present informational problems are extremely serious, but they could be solved rather inexpensively through establishment of a single national technical library that provides rapid mail distribution of photocopies of title pages of incoming materials and free copies of entire chapters or articles when requested.

2. Establish a government agency to monitor the quality of microbial inoculants provided to farmers and to conduct rigorous tests of inoculant effectiveness. In addition, initiate a system for distributing quality-controlled inoculum to the estates and farms that need it.

3. Consider regional production of inoculant to minimize transportation constraints that might shorten its shelf life.

4. Open inoculant production to private enterprise interested in the venture.

5. Develop existing facilities at the best-equipped institutions so that they can function as culture collection centers.

6. Organize a core group representing the different disciplines supporting biological nitrogen fixation. The group should meet periodically to exchange scientific information (research progress and prospects, constraints), identify relevant research, and establish priorities. In addition, a scientific meeting on nitrogen fixation should be held annually and funds made available for all researchers in this area to attend.

7. Emphasize those areas of plant nitrogen fixation that would have an immediate impact. For example:

   - o Development of microbial inoculants for agriculture includes the question of biocontrol of plant pests and disease and the area of mycorrhizal symbiosis. Given the heavy independence of Indonesia on trees, it would be quite simple and extremely cost-effective to inoculate every seedling with an appropriate mycorrhizal fungus. This step could provide direct economic benefits in addition to those of tissue culture cloning quickly and inexpensively.

   - o Plant pests and diseases routinely cause 15-30 percent losses in crop yields in most countries. Because chemical methods for pest and disease control require expensive imports, the development of inexpensive, locally grown microorganisms for use as biocontrol agents would be a clearly desirable research project.

8. For adequate support of future research and development in biological nitrogen fixation and agricultural microbiology, rapid development of the following scientific disciplines is needed: microbial physiology and molecular genetics, general and plant biochemistry, plant physiology, breeding, and plant molecular genetics and soil sciences.

9. To strengthen current scientific cadres, include departments of microbiology and biochemistry in the leading Indonesian universities. In addition, improve the existing curricula for the undergraduate training for scientists and to produce qualified technicians.

10. Arrange further Indonesia-U.S. cooperation in biological nitrogen fixation through joint cooperative research which could be of mutual benefit and executed in either the United States or Indonesia. This cooperation would involve scientists from both countries, as well as the assistance of U.S. experts. These experts would function as research counterparts; review, evaluate, or direct research; and conduct short courses on specific laboratory techniques.

BIOCONVERSION OF AGRICULTURAL BY-PRODUCTS

1. Establish a clearinghouse to exchange and provide information on the bioconversion of agricultural by-products.

2. Undertake training of a substantial number of scientists in the various disciplines underlying biotechnology to meet the manpower requirements of the R&D programs as well as the production activities. Qualified technicians must be trained as well. Training in some of these disciplines could be provided by some of the leading universities in Indonesia, but training in molecular biology, biochemistry, and biochemical engineering, for example, must be sought overseas. Priority should be given to training bachelor degree-level problem solvers, particularly chemical engineers who can design and operate pilot facilities and commercial plants. An estimate of the minimal manpower requirements for developing programs in single-cell protein, enzymes, antibiotics, steroid compounds, and vitamins is shown in Table 1.

3. In the meantime, mobilize the available experts to start work on programs that will eventually expand when more manpower and facilities are made available. An on-the-job training program should be initiated in the overall framework of technology transfer.

4. Equip existing research laboratories, government institutes, and universities with the basic instruments needed to conduct R&D

Scientist/	Single-cell protein [a]			Enzymes [a]			Antibiotics Steroids [a]			Vitamin B_{12} [a]			Vitamin A [b] CO_{12}		
Specialist	Expl.	Dev.	Com.	Expl.	Dev.	Com.	Expl.	Dev.	Com.	Expl.	Dev.	Com.	Expl.	Dev.	Com.
Microbiologist	3(4)	1		2(4)	1		3(4)	1		2(4)	1		1(2)	1	1
Economist	1(1)			3(3)	3		3(3)	1		3(1)	1		1(1)	1	1
Organic chemist	1(1)			3(2)	1		2(1)	1		3(2)	1		1(1)	1	1
Chemist engineer	2(2)	3(8)	2(16)	1(2)	3(8)	3(16)	1(2)	3(8)	3(16)	1(1)	3(8)	3(16)	3(2)	4(8)	3(16)
Molecular geneticist	1			2	-		2	-		2	-		-	-	-
Pharmacist	-			-	-		2(2)	2(6)	3(16)	1(2)	2(6)	3(16)	-	-	-
Veterinarian / animal husbandry	1(2)			-	-		1	1		1	1		2	1	1
Medical doctor				-	-		1	1		1	1		-	-	-

Note: ( )= technician.
a = fermentation process.
b = extraction process.

TABLE 1

Minimal Manpower Requirements for Developing R&D Programs in Single-cell Protein, Enzymes, Antibiotics, Steroid Compounds, and Vitamins

programs in biotechnology. When expensive equipment is required, it should be placed at a central location. This could include the equipment needed for pretreatment, product recovery, and a complete fermentation pilot plant.

5. Improve the existing bioindustries--such as those for the production of alcohol, citric acid, and monosodium glutamate--in terms of process technology and strain improvement.

6. Formulate a plan for developing the strategic bioindustries that would enhance national resilience in the fields of food and animal feed, health, and essential basic chemicals. An example of integrated utilization of straw or other cellulosic by-products to produce useful materials through biotechnology is shown in Figure 1.

7. Establish rules and regulations for genetic manipulation.

8. At least once each year hold a meeting of the working group on the bioconversion of agricultural by-products to discuss priorities and results. Individuals working together on a specific project should meet at least four times a year.

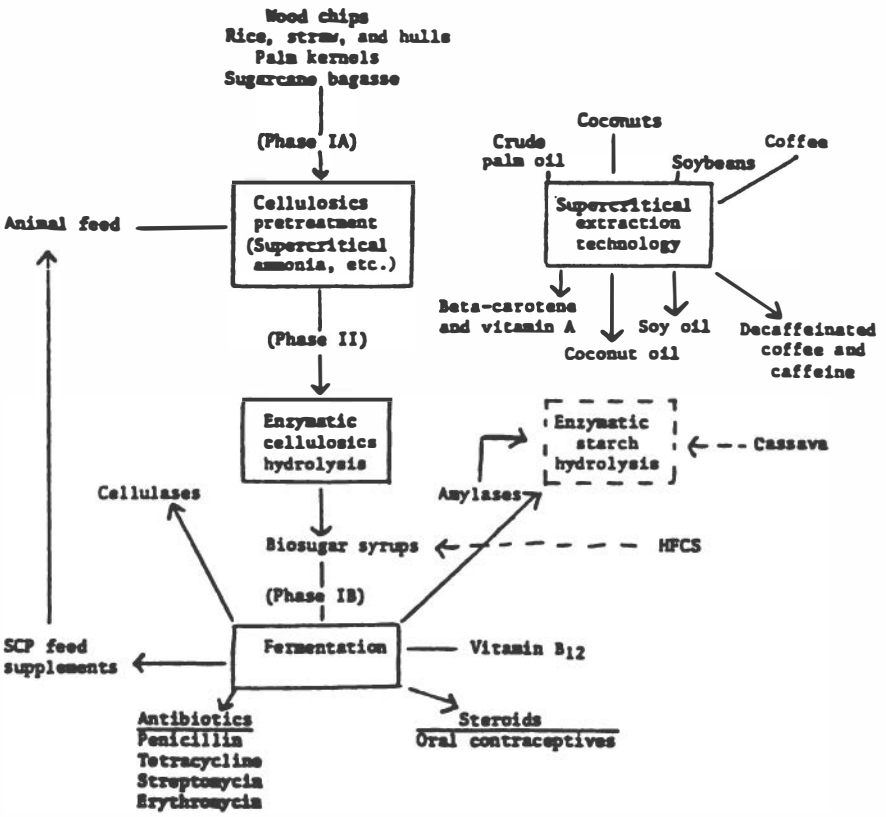

FIGURE 1 Scheme showing the production of chemicals from agricultural by-products.

APPENDIXES

APPENDIX A

Report of the Biotechnology Steering Committee

A. M. Satari, Vice Chairman

Deputy Chairman for Basic and Applied Sciences,
Agency for the Assessment and Application of Technology

It is both a pleasure and an honor for me, as chairman of the workshop steering committee, to extend a sincere welcome to all of you honoring us with your presence at the opening of this Workshop on Biotechnology in Agriculture. Our warm greetings and appreciation go especially to the participants from the United States as well as from Indonesia who will share their invaluable knowledge, experiences, and ideas during our two-day discussions.

This workshop is one of many cooperative activities held by the U.S. National Research Council and the Indonesian National Research Council since the Symposium on Potential Indonesia-U.S. Collaboration in Science and Technology was held in Washington, D.C., in October 1983. One of the priorities recommended at that symposium was a cooperative program in biotechnology and related fields.

This is the second workshop held on this subject; the Workshop on Marine Algae Biotechnology was held December 11-13, 1985. At that meeting, matters related to the cultivation, processing, and marketing of marine algae were discussed, and joint recommendations were made to the Indonesian government.

The present workshop will focus on four topics:

1. Animal breeding with emphasis on embryo transfer
2. Plant cell and tissue culture
3. Plant nitrogen fixation
4. Bioconversion of agricultural by-products.

The discussions will begin in plenary session, and meetings of the working groups will follow. It is expected that the discussion of each topic will include R&D programs, the application of biotechnology and its priorities, feasible U.S.-Indonesia cooperation in its implementation, and education and training.

Members of the Workshop Steering Committee were Didin S. Sastrapradja, Chairman; Haryanto Dhanutirto; A. A. Loedin; K. H. Kho; Setiati D. Sastrapradja; Susono Saono; A. M. Satari; Sediono M. P. Tjondronegoro; Charles C. Muscoplat; and Rose Bannigan.

We invited about 50 persons to participate in this workshop--six participants from the United States and 44 from Indonesia. Preworkshop visits have also been organized, permitting the participants to observe the existing facilities and obtain firsthand information from scientists in the respective fields. Visits were made to Medan on March 10 to observe the activities at Marihat Research Station, to Bogor on March 11 to meet scientists at Bogor Agricultural University and to look at their experimental research activities, and to Tapos on March 12 to visit the cattle breeding experiment station. Even though these activities represent only a few of those being developed in Indonesia, we hope that participants obtained an overview of the efforts being made to apply biotechnological methods in this country.

The steering committee is fully aware of the long road ahead before Indonesia can make optimal use of the newly discovered methods of biotechnology engineering. It is therefore our sincere hope that this workshop makes a significant contribution to speeding up biotechnological development in Indonesia by suggesting concrete and feasible recommendations for actions.

Finally, allow me to take this opportunity to officially extend our gratitude and appreciation to the U.S. National Research Council for their support, to the U.S. and Indonesian participants for their active participation, to the Office of the Minister of State for Research and Technology, to the Agency for the Assessment and Application of Technology, to the secretary and his staff of the Indonesian National Research Council, to the organizing committee, and to all those who in one way and another have made this workshop possible.

APPENDIX B

Opening Remarks

Richard A. Cobb
Chief, Office of Agriculture and Rural Development,
USAID Mission in Indonesia

The U.S. Agency for International Development [USAID] is particularly pleased to support this cooperative program of the Indonesian National Research Council and the U.S. National Research Council. We believe that the first workshop held last December on the biotechnology of marine algae was a success, and we look forward to the outcome of this workshop. USAID has been supporting agricultural research in Indonesia for the past 10 years, and it will continue projects on food crops and agricultural research through the next decade.

This discussion of biotechnology in agricultural research is very timely, because biotechnology will likely become the foundation of a new revolution in agricultural research and development through the remainder of the century. Looking back to the 1960s, the "green revolution" paved the way for substantial increases in crop production. It was, however, limited to a few crops and showed the greatest yield increases in irrigated areas. The effects of the green revolution, which relied on the traditional plant breeding techniques, were largely achieved by the mid-1970s. Over the past 10 years, there have been additional attempts to apply the agricultural practices built around high-yielding varieties in the small holder sector. Special attention has been given to research and extension through research on farming systems. Some necessary and important work is taking place, but the strides forward in farming research have been very modest to date.

Biotechnology, particularly the work in molecular biology that led to the techniques of plant cell and tissue culture, offers some remarkable possibilities. For example, tissue culture is becoming increasingly important as a way of increasing the speed and efficiency of germ plasm evaluation. It can expand vastly the geographic sphere of adaptable cereal grain varieties, particularly in areas where soil or rainfall is marginal. In addition, biotechnology is applicable to any living organism and opens up an entire range of crops that traditional breeding cannot accommodate. Clearly, biotechnological advances in agriculture offer possibilities that surpass what has been possible from the green revolution and farming systems research.

There are, however, some implications of biotechnological research and application that differ significantly from those of the green

revolution. The most important of these has to do with the respective roles of private capital and public agencies.

The major advances in the life sciences are now on the verge of being commercialized. Four important areas of technological change that will affect global agriculture--plant genetic manipulation, industrial tissue culture, genetically engineered animal products, and use of genetically manipulated microorganisms to produce or displace agricultural products--have occurred as a result of heavy investment by agricultural genetic engineering firms in several countries. In particular, major investments have been made by petrochemical and pharmaceutical companies in seed-related technologies, cloning of disease-resistant potatoes, soybean and cotton breeding, animal production, tomatoes, tobacco, and forest products. In comparison with green revolution technologies, the instrumentation, facilities, and, most important, the personnel required for biotechnological R&D and production are relatively expensive. This, combined with the fact that there is a broad range of areas over which biotechnology appears to be commercially exploitable, means that the private sector--transnational corporations--will have a large role in both the development and production of biotechnologies in the future. At the same time, it is likely that the international agricultural research centers, which were the centerpiece of the green revolution, will have a diminished role. Fiscal austerity has limited the ability of international centers to expand beyond their conventional plant breeding programs. Thus, biotechnology, particularly cell and tissue culture, is essentially transferring agricultural activities to the factory.

What are the implications for Indonesia? The effect of biotechnology on agricultural production could be profound. Transnational pharmaceutical and chemical companies, genetic research firms, and university laboratories are pursuing the development of bioengineered plant crop varieties across the spectrum of world crops. Research presently under way in the following areas has direct relevance to Indonesia: varietal improvement; achievement of nitrogen fixation in nonleguminous crops; enhancement of photosynthetic activity; manipulation of growth regulators; improved stress tolerance to drought, salinity, acidity, and other soil conditions; and pest and pathogen resistance. The development of varieties that use water more efficiently will enable marginal areas to become more productive without recourse to expensive irrigation. Achievement of nitrogen fixation in rice or maize could greatly reduce subsidies for fertilizer. Pest-resistance characteristics would lower the cost of chemical inputs. These possibilities present far-reaching consequences for the development of agriculture in the eastern islands of Indonesia. In addition, forest species presently being genetically engineered for rapid growth would have important implications for soil stabilization and erosion control problems in many upland areas of the country.

The use of biotechnology presents some challenges, however. I will mention three. First, the transition from science to commercialization will have to be made in Indonesia as elsewhere. Investments in biotechnology are being stimulated by demand in the marketplace.

The relationship between the consumer and biotechnological development will therefore have to be clearly drawn and understood. This means linkages with commercial expertise for market analysis, training, and attention to quality control and reliable levels of output. Recommendations were made in these areas following the marine algae workshop in December. Specific recommendations and follow-up actions from this workshop will also be important.

Second, over the long term there are questions of equity. The development of biotechnology is market driven. Thus, corporations are investing heavily because they can sell the products of technologies. Indonesia will have to deal with the question of how the benefits of biotechnology, both employment opportunities and the application of the science, can be spread as widely as possible to help the unskilled workers and small-scale farmers in less-advantaged areas of the country.

Third, there is a question of the proper role of the Indonesian public sector--namely, the agricultural research community--in biotechnological research. Many institutions are involved: BPPT [Agency for the Assessment and Application of Technology], LIPI [Indonesian Institute of Sciences], AARD [Agency for Agricultural Research and Development], and the universities. How should the role of these organizations change? What investments are necessary? What actions need to be taken by the government to assure that public technology transfer programs meet the agricultural production goals of the country?

We look forward not only to the report of this workshop but also to the application of its deliberations. Please accept my best wishes for a very successful workshop.

APPENDIX C

Keynote Address

Doddy A. Tisna Amidjaja
Vice-Chairman,
Indonesian National Research Council

Allow me to first convey a message of deep regret from the chairman of the Indonesian National Research Council, Dr. B. J. Habibie, who also serves as our minister of state for research and technology, for not being able to be with us today. He had to attend to urgent matters in Europe. He expressed, however, his very keen interest in the topics and issues to be discussed, and he would appreciate being informed in detail about the outcome of the discussions.

On his behalf and as part of my function as vice-chairman of the Indonesian National Research Council, I have the privilege and pleasure of joining the previous speakers in extending our heartfelt welcome to all of you honoring us with your presence at the opening of this Workshop on Biotechnology in Agriculture. My warm greetings and appreciation especially go to our U.S. guests and eminent scholars, who will share their invaluable experience, knowledge, wisdom, and ideas with their Indonesian counterparts during this two-day meeting.

As reported by the chairman of the steering committee, the organization of this workshop is within the framework of a long-standing cooperative arrangement between the U.S. National Research Council and Indonesian scientific institutions and constitutes one of a series of its endeavors. Thus, it is probably beneficial that we recall at this juncture the earlier cooperative scientific endeavors between the U.S. National Research Council and Indonesia, with the hope that this refreshed awareness of our past fruitful meetings will evoke in the coming two-day scientific dialogues a heightened vivacity.

In the spirit of the planned and well-programmed national development of Indonesia's new order, we consider the 1968 Workshop on Food, sponsored by the U.S. National Research Council and the Indonesian Institute of Sciences, an important milestone in this long-standing scientific relationship. The findings of that workshop played an important role as a source of reference in the formulation of a food policy for Indonesia's first five-year development plan (REPELITA I, 1969-1974). In later years, workshops on industrial research, natural resources, rural productivity, etc., were followed up by many activities.

I would like to mention especially the November 1982 Panel Discussions on Science and Technology Planning and Forecasting for Indonesia: Special Emphasis on Manpower Development. These

discussions were jointly sponsored by the Indonesian Ministry of State for Research and Technology, the Indonesian Institute of Sciences [LIPI], and the Board on Science and Technology for International Development of the U.S. National Research Council. These panel discussions produced important recommendations on biotechnology and agro-industry which should be considered by this workshop as a baseline in formulating realistic development strategies in view of the current prevailing economic climate of the country. This climate exerts a strong influence on the financial and budgetary policies for development programs in the current REPELITA, with an extended recovery time likely to reach a "takeoff" condition that will accelerate implementation of development programs in the following REPELITAs.

In August 1983, the Workshop on Planning, Monitoring, and Evaluation of Research and Technology in Indonesia was held, followed in October 1983 by a symposium on potential Indonesia-U.S. collaboration in science and technology. The most recent workshop, as reported by the steering committee, addressed marine algae biotechnology and was held in December 1985. I also had the honor of opening that meeting.

The continuing growth of Indonesia during these last two decades has undeniably meant more welfare and progress for the country as a whole. Some dominant factors, however, that sustain the livelihood of society--such as population increases and the rational management and utilization of natural resources--are not yet fully under control, because the prerequisites and infrastructure have not yet been fully met.

Indonesia's future development will depend heavily on its ability to use science and technology effectively, especially in the utilization of Indonesia's endowments of natural as well as human resources. To quote Dr. Habibie: "Our natural and human resources have to undergo value-added processes, to be turned into high-value economic commodities and highly competent productive professionals and skilled citizens."

It is true that Indonesia, through constant intensive and extensive efforts and many years of planning and hard work on the agronomic as well as organizational aspects, has finally in this fourth five-year development plan achieved self-sufficiency in rice production. Its stability has not been proven, however, and there are still many factors prevailing in all phases of the agricultural process that must be improved and established. Indeed, the current five-year development plan (1984-1989) continues to stress the agricultural sector, emphasizing industries to process raw materials. It also promotes industries that produce machinery for agricultural and agro-industries and for light and heavy industries. In the meantime, the price of oil is dropping. Thus, more emphasis has been placed on increasing the production of non-oil commodities, especially for export purposes, which requires that they be competitive and of high quality. The use of national products is also being urged.

The rapid development of biotechnology in the developed countries has demonstrated a considerable impact on different industries. At the same time, while certain nuclei exist in several Indonesian scientific

institutions, the Indonesian government has issued directives that biotechnology should be developed to sustain agro-industries, health care and pharmaceuticals, chemicals, and biomass conversion.

In fact, biotechnology was used early in Indonesia's civilization in, for example, the production of kecap (soya sauce), tempe, and brem (Balinese wine). The products urgently needed now from biotechnology, however, range from enzymes, pharmaceuticals, and certain chemicals to biomaterials such as frozen embryos, which are for the most part imported. We are aware that Indonesia has enough raw materials to fulfill her own needs only if we can master biotechnology. It is understood, however, that certain prerequisites and the infrastructure for its development must be established to realize this wish.

At present, research and development activities aimed at some aspects of biotechnology are growing in research institutions, universities, and certain laboratories of private industries. A limited research staff with specialization in related fields of science that sustain biotechnology--biochemistry, microbiology, genetics, and embryology, for example--is available. Furthermore, certain techniques have been mastered in several laboratories, such as tissue culture and certain sophisticated biochemical procedures and biological manipulation. These institutions are also attempting to send potential staff abroad for training in selected techniques or for academic studies in the field of biotechnology.

The Indonesian government is making efforts to stimulate the growth of research and development and the application of biotechnology in Indonesia. An interdepartmental committee has been established by the minister of state for research and technology to formulate a strategy for the development of biotechnology in Indonesia. The construction of integrated facilities has been initiated in Cibinong. To support this planned Center for Biotechnology, LIPI is sending a number of staff abroad for training courses and academic studies in various disciplines that will support this program on biotechnology in the future.

Indeed, the watchword in biotechnological development is well-functioning, effective coordination among institutions, especially universities, national research laboratories, and industries. Programs should be established not only in the institution-building phase (that is, manpower and facility development), but for all the operations as well. Moreover, in addition to the common facilities of the biotechnology center, networks of laboratories and research groups should be established among the interuniversity centers and national laboratories. The recommendation made at the above-mentioned 1982 panel discussions regarding manpower training, the establishment of a Crop/Agro-biotechnology Industrial Center (CABIC), and a food resource development plan should be stressed further.

It is our sincere hope that this workshop will make concrete, feasible recommendations for the immediate application of biotechnological development to agriculture in Indonesia. In this respect, the steering committee has chosen four topics that we think are the most important in this area. The recommendations made will, it is hoped, include program priorities and manpower development needs as well as follow-up Indonesia-U.S. cooperation in these fields. Even

though the world economy is at present not too promising, we must plan realistically in our joint effort for contingencies by having available rational alternatives.

I would like to take this opportunity to join the chairman of the steering committee in extending our gratitude and appreciation to the government of the United States, the government of Indonesia, the U.S. National Research Council, the Office of the Minister of State for Research and Technology, the Agency for the Assessment and Application of Technology, the steering and the organizing committees, and all those who made this workshop possible. I would also like to thank the speakers, the session's chairpeople, and the participants for taking part in this workshop.

APPENDIX B

Workshop Agenda

Thursday, March 13

Morning

Opening Ceremony

Report of the Steering Committee
A. M. Satari
Deputy Chairman for Basic and Applied Sciences, BPPT

Remarks
Richard A. Cobb
Chief, Office of Agriculture and Rural Development, USAID Mission in Indonesia

Keynote Address
Doddy A. Tisna Amidjaja
Vice-Chairman, Indonesian National Research Council

Coffee

Overview

National Policy on Biotechnology in Indonesia
Didin S. Sastrapradja, Assistant II Minister for Research and Technology/Chairman, National Committee on Biotechnology

Biotechnology in Agriculture
Charles C. Muscoplat, Chairman, NRC Panel, and President, Molecular Genetics, Inc.

The Biotechnology Initiative in North Carolina
Richard J. Patterson, President, North Carolina Biotechnology Center

Lunch

Afternoon — **Group Discussions**

Embryo Transfer and Animal Production
Mozes Tulihere, Chairperson
Mr. Sunartono Adisumarto, Rapporteur

Plant Cell and Tissue Culture
Gustaaf A. Wattimena, Chairperson
Mrs. Livy Winata Gunawan, Rapporteur

Plant Nitrogen Fixation
Goeswono Soepardi, Chairperson
Ratna Siri Hadioetomo, Rapporteur

Bioconversion of Agricultural By-products
Indrawati Gandjar, Chairperson
Saraswati, Rapporteur

Friday, March 14

Morning — **Continuation of Working Group Meetings**

Lunch

Afternoon — **Plenary Session**

Summary of Recommendations and Conclusions
Chairpersons of each Working Group

Comments by Chairman of the NRC Panel
Charles C. Muscoplat

Closing Remarks
A. M. Satari

APPENDIX E

Workshop Participants

STEERING COMMITTEE

A. M. Satari, Chairman
Didin S. Sastrapradja
A. A. Loedin
Kho Kian Hoo
Sediono M. P. Tjondronegoro
Setijati D. Sastrapradja
Susono Saono
Haryanto Dhanutirto
Charles C. Muscoplat
Rose Bannigan

U.S. NATIONAL RESEARCH COUNCIL PANEL

Charles C. Muscoplat, Chairman
Wolfgang D. Bauer
Robert M. Busche
Anthony J. Faras
Richard J. Patterson
Rose Bannigan, Staff Officer

WORKING GROUPS

Embryo Transfer and Animal Production

Chairperson:	Mozes Tulihere (IPB)
Rapporteur:	Sunartono Adisumarto (LBN-LIPI)
Participants:	A. A. Loedin (Dep. Kesehatan)
	Djokowuryo S. (IPB)
	Yuhara Sukra (Debdikbud)
	Harimurti (IPB)
	Sri Sudarwati (ITB)
	Komang (Unair)
	Ida Kusumah (BPPT)
	Yan Nari (R&D Agriculture)

	Lien Sutasurya (ITB)
	Reviany Widjayahusumah (IPB)
U.S. Panelists:	Anthony J. Faras
	Charles C. Muscoplat

Plant Cell and Tissue Culture

Chairperson:	Gustaaf A. Watimena (IPB)
Rapporteur:	Livy Winata Gunawan (IPB)
Participants:	E. Noerhadi (ITB)
	Moeso Soeryowinoto (UGM)
	Setiati D. Sastrapradja (LBN-LIPI)
	Usep Sutisna (LBN-LIPI)
	Thardi (R&D Agriculture)
	Gale Ginting (PTP VII)
U.S. Panelist:	Richard J. Patterson

Plant Nitrogen Fixation

Chairperson:	Goeswono Soepardi (IPB)
Rapporteur:	Ratna Siri Hadioetomo (IPB)
Participants:	Yoedoro Soedarsobno (UGM)
	Ibrahim Manwan (R&D Agriculture)
	Susono Saono (LBN-LIPI)
	Soetarjo Brotonegoro (Marif)
	M. Ismunadji (R&D Agriculture)
	Jutono (UGM)
U.S. Panelist:	Wolfgang D. Bauer

Bioconversion of Agricultural By-products

Chairperson:	Indrawati Gandjar (BPPT)
Rapporteur:	Saraswati (BPPT)
Participants:	A. M. Satari (BPPT)
	Muhammad Wirahadikusumah (ITB)
	Triadi Basuki (LBN-LIPI)
	Purwo Arbianto (ITB)
	Ibrahim S. (ITB)
	Sumpeno Putro (R&D Agriculture)
U.S. Panelist:	Robert M. Busche

Mobile Members:	Didin S. Sastrapradja (LIPI/Ristek)
	Sediono M. P. Tjondronegoro (DRN/Ristek)
	Kho Kian Hoo (BPPT/Ristek)
	Haryanto Dhanutirto (BPPT/Ristek)
	Rose Bannigan (U.S. NRC)

General Susono Saono
Rapporteurs: Charles C. Muscoplat

ORGANIZING COMMITTEE

Chairman: Haryanto Dhanutirto (BPPT/Ristek)
Secretary: Jana Anggadiredja (BPPT/Ristek)
Members: Rachmaniar Rachmat (BPPT)
Moch Mochtar (Ristek)
Sawedi (BPPT)
Dadang A. Permadi (Ristek)
Titi Marpaung Bc. Hk. (LIPI)
Sri Wahyuni Sh. (LIPI)
Sutarjo (Ristek)
Ratna Wulan (BPPT/DRN)
Abdul Firman (BPPT)
R. Sukmaya (BPPT)
Indang Wahyurini (Ristek)
Asti Suryani (Ristek)
Budi Minerva (Ristek)
Notulis: Nelson Simanungkalit (BPPT)
Daya Pamudji (BPPT)
Hasni Muchtar (BPPT)
Puspo Wardoyo (BPPT)
Patna Chandra (BPPT)
Poyaningsih (BPPT)
Sadjuga (BPPT)
Donowati (BPPT)

OTHERS

Iman Suripto
Desmond O'Riordan
Edi Setianto
Richard A. Cobb
Doddy A. Tisna Amidjaja
Jusdy Achmad
Rum Husen
Mochtar Machful
Wardiman Djojonegoro