PART I
The Technologies

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The Technologies

Introduction

This chapter reviews the scientific bases for the technologies discussed in this assessment. The most publicized and broadly applicable of these technologies is recombinant DNA (rDNA) technology, which includes gene cloning, and is explained first. The second technology discussed is monoclinal antibody (MAb), or hybridoma, technology. This technology, used to prepare complex molecules known as MAbs which can be used to recognize or bind a large variety of molecules, has an expanding number of applications. The last technology discussed, bioprocess technology, allows the scaling-up of a biological production process so that large quantities of a product can be made. Bioprocess technology is, in many respects, the most difficult and least understood of the technologies, so it receives a more intensive discussion in this chapter. Because of the lack in the United States of broadly applicable knowledge in bioprocess engineering, the section on bioprocess technology also ends with priorities for future research, giving a focus to where Federal research funds might best be spent.

Recombinant DNA technology

The development of rDNA technology-the joining of DNA from different organisms for a specific purpose—has allowed a greatly increased understanding of the genetic and molecular basis of life. This technology has also led to the founding of many industrial ventures that are addressing the production of numerous compounds ranging from pharmaceuticals to commodity chemicals. This section introduces some aspects of the scientific basis of rDNA technology, discusses methods that are used to construct rDNA, and notes several additional features of the commercial use of rDNA technology.

Structure and function of DNA

Throughout the spectrum of life, the traits characteristic of a given species are maintained and passed on to future generations, preserved simply and elegantly by the information system contained within DNA. DNA can be thought of as *a* library that contains the complete plan for an organism. If the plan were for a human, the library would contain 3,000 volumes of 1,000 pages each. Each page would represent one gene, or a unit of heredity, and be specified by 1,000 letters. As shown in figure 3, DNA, a double-stranded, helical molecule, is composed, in part, of four nucleotide bases—adenine (A), cwytosine (C), guanine (G), and thymine (T)-which are the letters of the chemical language. A gene is an ordered sequence of these letters, and each gene contains the information for the composition of a particular protein and the necessary signals for the production of that protein.

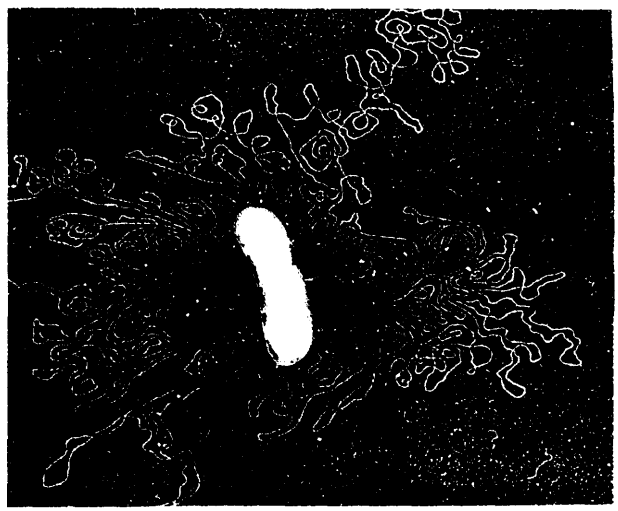


Photo credit: Science Photo LIbraty and Porton/LH **International**

The DNA of the bacterium Escherichia *co/i*

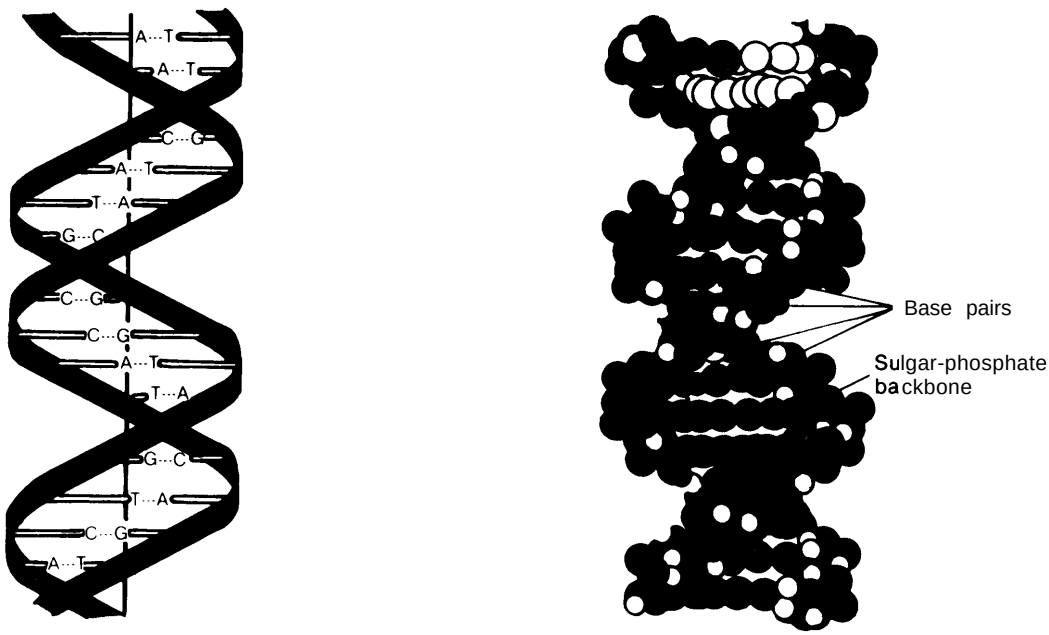
The mechanism by which DNA replicates is inherent in the structure of DNA itself. As can be seen from figure 3, the nucleotide bases are paired to form the rungs of the twisted DNA ladder. This pairing is absolutely specific: A always pairs with T and C always pairs with G. The pairing is accurate, but not very strong. Thus, in cell division, the DNA can "unzip" down the middle, leaving a series of unpaired bases on each chain. Each free chain can serve as a template for making a complementary chain, resulting in two identical DNA molecules, each a precise copy of the original molecule. Figure 4 illustrates the replication of DNA.

The DNA present in every cell of every living organism has the capacity to direct the functions of that cell, Gene expression, shown in figure 5, is the mechanism whereby the genetic directions in any particular cell are decoded and processed into the final functioning product, usually a protein. In the first step, called transcription, the DNA double helix is locally unzipped near the gene of interest, and an intermediate product, messenger RNA (mRNA), a single-stranded, linear sequence of nucleotide bases chemically very similar to DNA, is synthesized. The transcription process dictates the synthesis of mRNA that is complementary to the section of unzipped DNA in a manner that is somewhat similar to the replication of DNA. In the second step of gene expression, translation, the mRNA, after release from the DNA, becomes associated with the protein-synthesizing machinery of the cell, and the sequence of nucleotide bases in the mRNA is decoded and translated into a protein. The protein goes on to perform its particular function, and when the protein is no longer needed, the protein and the mRNA coding for that protein are degraded. This mechanism allows a cell to "fine tune" the quantity of its proteins while keeping its DNA in a very stable and intact form.

Proteins perform most of the necessary functions of a cell. By far the most diverse group of proteins is the enzymes, which are the proteins

t

Figure 3.–The Structure of DNA



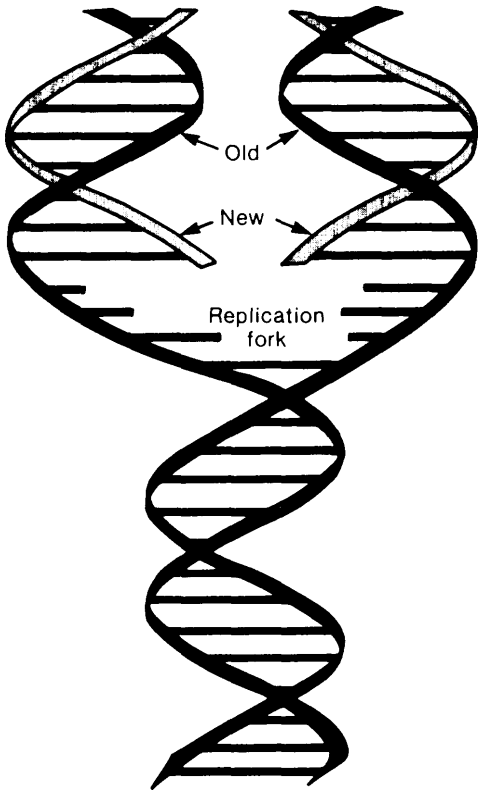
A schematic diagram of the DNA double helix.

A thr~dimensionai representation of the DNA doubie helix.

The DNA molecule is a doubie helix composed of two chains. The sugar-phosphate backbones twist around the outside, with the paired bases on the inside serving to hold the chains together.

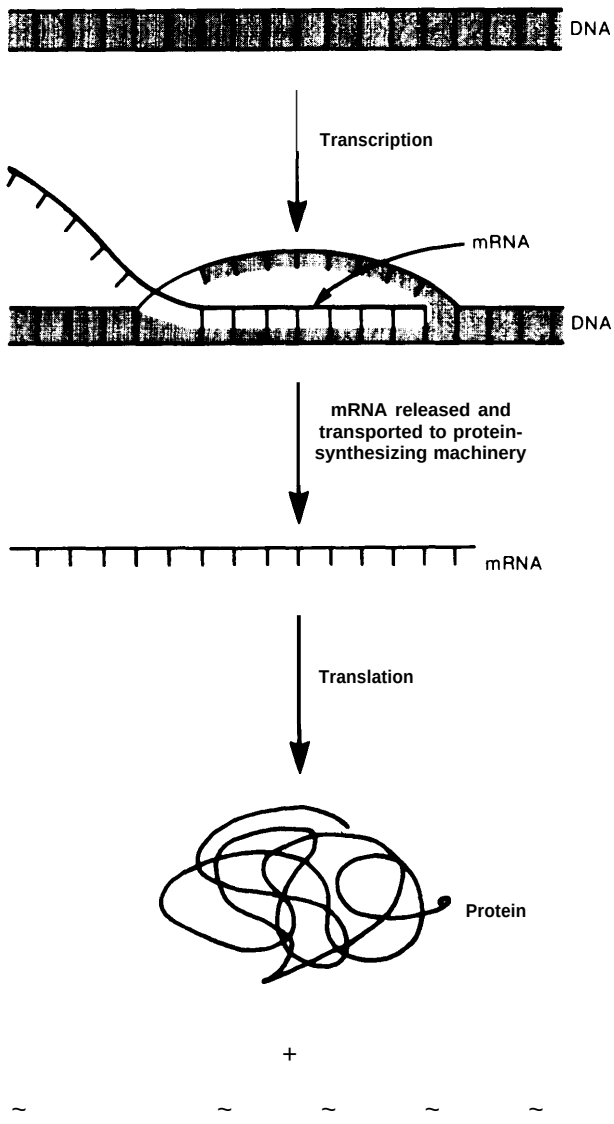
SOURCE: Office of Technology Assessment.

Figure 4.–The Replication of DNA



When DNA replicates, the original strands unwind and serve as templates for the building of new complementary strands. The daughter molecules are exact copies of the parent, with each having one of the parent strands

Figure 5.—Mechanism of Gene Expression



SOURCE: Office of Technology Assessment.

that catalyze biological reactions. Another group is the structural proteins, which are found, for instance, in cell membranes. Other proteins have regulatory functions; these include some hormones. Still others have highly specialized functions (hemoglobin, for example, carries oxygen from the lungs to the rest of the tissues).

The code by which genetic information is translated into proteins is the same for all organisms. Thus, because all organisms contain DNA and all organisms interpret that DNA in the same manner, all organisms, in essence, are related. It is this concept that forms the basis for the industrial use of DNA. In nearly every instance, a production process using rDNA technology depends on the expression of DNA from one species in another species. Only a universal genetic code would allow DNA to be used in this manner.

Despite the existence of a universal genetic code, regulatory signals indicating starts and stops of genes are known to vary among species. Thus, a gene removed from one organism and placed

in another will code for the same protein as it did in its native system, but its synthesis needs to be induced by the proper host signal. one of the great challenges of rDNA technology is to construct DNA molecules with signals that optimally control the expression of the gene in the new host .

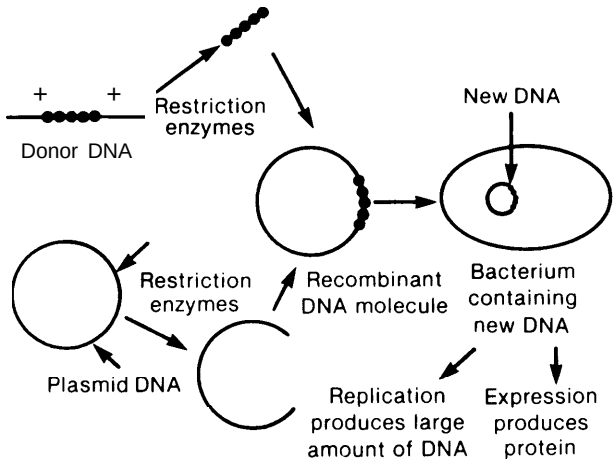
Preparing recombinant DNA

The amount of DNA present in each cell of a human (or most higher animals) is approximately 3 billion base pairs (2), and an average gene is about 1,000 base pairs, or about one millionth of the DNA. It is extremely difficult to study one gene in a million. Therefore, powerful tools have been developed to isolate genes of interest, place them in a foreign, simpler system, and replicate them many times to give a large amount of a single gene. The isolation of genes from higher organisms and their recombination in simple cells has already yielded a wealth of information, including insight into how genes determine the differences between different types of cells, how gene expression is regulated, and how genes may have evolved. For industrial uses, however, not only must the gene be cloned (reproduced), but that gene must also be expressed (the protein based on the gene be produced).

The basic technique of preparing rDNA is shown in figure 6. Preparations of restriction enzymes (enzymes that are made in certain bacteria and cut DNA at specific sites) are used to cut donor DNA (usually from a higher organism) into fragments, one of which contains the gene of interest, The resulting DNA fragments are then inserted into a DNA "vector," which is most often a plasmid. " Each plasmid vector will contain a different donor DNA fragment. These rDNA plasmids are introduced into host cells in a process called '(transformation." Once inside the host cells, the rDNA plasmids replicate many times, thus providing many copies of each donor DNA fragment. of the many bacteria transformed by plas - mids containing donor DNA, only a few will contain the DNA fragment of interest. The desired

"A plasmid is a circular, double-stranded piece of DNA which replicates in cells apart from the chromosome.

Figure 6.-Recombinant DNA: The Technique of Recombining Genes From One Species With Those of Another .



Restriction enzymes recognize certain sites along the DNA and can chemically cut the DNA at those sites. This makes it possible to remove selected genes from donor DNA molecules and insert them into plasmid DNA molecules to form the recombinant DNA. This recombinant DNA can then be cloned in its bacterial host and large amounts of a desired protein can be produced.

SOURCE Off Ice of Technology Assessment

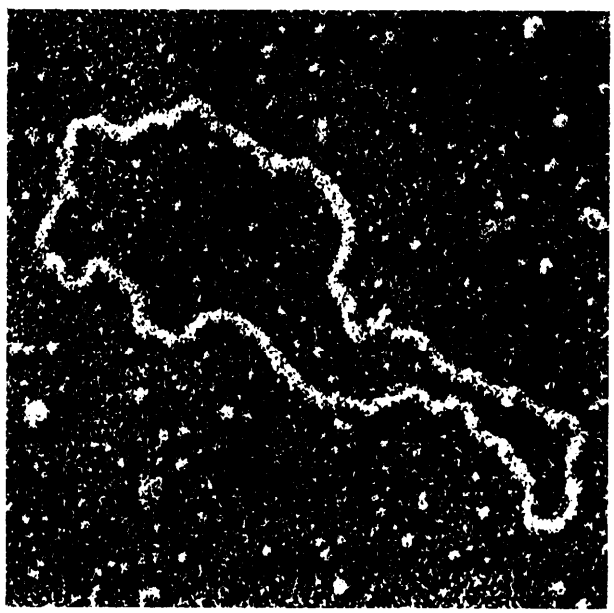


Photo credit: Science Photo Service and Porton/LH International

Bacterial plasmid

Photo credit: Science Photo Service and Po170rVLH International

Molecular biologist in laboratory

gene is located among the vast number of bacteria containing plasmids with a suitable probe. * Vectors other than plasmids can be used for cloning DNA. One method uses the DNA of viruses, and another uses cosmids, artificially constructed hybrids of plasmids and viruses. Another method uses transposable elements, fragments of DNA that can insert themselves into the host cell's chromosomes.

All rDNA methods require the following:

- a suitable vector that is taken up by the host, is capable of autonomous replication, and, during the process, replicates the segment of donor DNA faithfully;
- an adequate selection system for distinguishing among cells that have, or have not, received rDNA; and
- an appropriate probe for detecting the particular DNA sequence in question.

The most difficult part of the cloning process is isolating an appropriate probe. The genes that were first cloned were those that, in certain cells, produced large quantities of relatively pure mRNA. Since the mRNA was complementary to the gene of interest, the mRNA could be used as a probe. This method severely limited the number of genes that could be cloned, however, because most genes do not produce large amounts of mRNA. More recently, a different technique has been used that allows a much greater diversity of genes to be cloned. If the amino acid sequence of a protein is known, then, working backwards through the gene expression scheme, the nucleotide base sequence can be determined. Because of the advent of automated DNA synthesizers, a portion of DNA can be synthesized that is complementary to the gene. This piece of DNA can then be used as a probe. Thus, if enough of a particular protein can be isolated and sequenced, its corresponding gene can be cloned.

At present, rDNA is grown principally in simple micro-organisms such as bacteria and yeast. Yeasts, in addition to bacteria, are being used as hosts for rDNA cloning because they more closely resemble cells of higher organisms. Yeasts perform functions similar to those of higher eukaryotic cells. These functions include adding sugar groups to some proteins. For the function of many proteins, these sugar groups are essential. Recently, scientists have learned how to introduce ***novel*** genetic material into higher plants and animals. The special techniques that pertain to cloning DNA in plants are discussed in ***Chapter 6: Agriculture.***

Recombinant DNA technology in industrial processes

The commercial use of rDNA technology has several features in addition to those just discussed. In order to produce a product or improve a process, the cloned gene must be expressed to give a functional product. Since the signals that regulate gene expression vary from species to species, achieving the expression of a gene in a foreign cell may be difficult. The commercial development of biotechnology is highly dependent on the ability to achieve gene expression, for it is proteins (or their metabolizes) that either are the marketable products themselves or establish the cellular environment necessary for performing such practical tasks as degrading toxic wastes or increasing the efficiency of photosynthesis. To a large extent, the problem of gene expression has been addressed through the manipulation of the adjacent vector DNA so that it contains the host regulatory sequences. The cloned gene can

* ,4 probe is a sequence of DNA that has the same sequence as the desired gene and has been prepared in such a way that it can be identified after it base pairs with that gene,

then be "switched on" by using host-regulated controls (l). Moreover, it is possible to alter specifically the regulatory sequences so that the gene is expressed at higher levels or so that its expression is more readily controllable in an industrial situation (3).

The purification of a protein from an industrial bioprocess is greatly simplified if the protein is secreted from the cell into the growth medium. If the protein is secreted, it does not have to be purified away from all the other cellular components. It is possible to attach additional regulatory signals to the vector DNA that direct the cell to secrete the protein and, thus, simplify its purification. The successful development of methods to enhance gene expression and product function and secretion will undoubtedly enhance the commercial applicability of rDNA technology.

The computer-aided design of proteins is another technology that will expand the use of rDNA molecules industrially. In the past, enzymes were modified by mutagenizing the host cell and then selecting or screening for mutants that contained an altered enzyme. Now, through use of the techniques of X-ray crystallography, protein sequencing, and computer modeling, the amino acid sequence and three-dimensional structure of the protein can be determined and amino acid changes that should bring about altered enzyme properties can be selected. The DNA sequence of the cloned gene for an enzyme can then be modified to incorporate the amino acid changes. Specific gene modification is made possible because of technical advances resulting in rapid and inexpensive synthesis of small DNA segments that can be used to change specific base pairs in a DNA sequence. Near-term protein modification experiments could result in enzymes with increased temperature and pH stability. Longer term experiments could define the structure of active sites of enzymes to be used for specific catalytic functions.

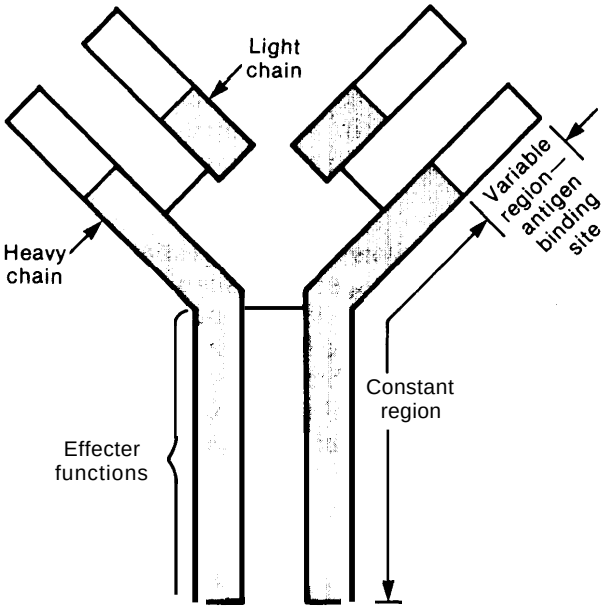
Monoclinal antibody technology

The production of antibodies in higher animals is one aspect of a complex series of events called the immune response. Specialized cells called B lymphocytes, present in the spleen, lymph nodes, and blood, recognize substances foreign to the body, or antigens, and respond by producing antibodies that specifically recognize and bind to those antigens. Any given B lymphocyte can recognize only one antigen. Thus, when a B lymphocyte meets and recognizes an antigen for the first time, the B lymphocyte is stimulated and becomes committed to producing a single type of antibody for the duration of its life. The end result of this aspect of an immune response is the antigen's removal from the body.

Antibodies bind to antigens and carry out their functions by virtue of the antibody's unique structure. All antibodies are comprised of four protein chains in a precise orientation, as shown in figure 7. One end of the antibody (the constant, or effecter region) is nearly identical among antibodies. This effecter region is associated with functions such as the secretion of antibodies from the B lymphocyte and "signaling" to the immune system after the antibody binds with the target antigen. The other end of the antibody, the variable region, contains the site that recognizes and binds to a particular antigen, and the structure of this end varies greatly from antibody to antibody to accommodate a wide range of antigens.

Apart from their natural functions in the protection of organisms via the immune response, antibodies have long been important tools for researchers and clinicians, who use an antibody's specificity to identify particular molecules or cells and to separate them from mixtures. Antibodies also have a major role in diagnosis of a wide variety of diseases. Antibodies that recognize known antigens are used to detect the presence and level of drugs, bacterial and viral products, hormones, and even other antibodies in sensitive assays of blood samples.

Figure 7.—Structure of an Antibody Molecule



SOURCE Off Ice of Technology Assessment.

The conventional method of producing antibodies for diagnostic, therapeutic, and investigational purposes is to inject an antigen into a laboratory animal and, after evoking an immune response, to collect antiserum (blood serum containing antibodies) from the animal. Although this method has been and continues to be widely used, there are several problems associated with conventional antibody technology, These include:

- minor contamination of the injected antigen with other molecules, so that the antiserum collected from the animal contains a mixture of antibodies against both the target antigen and the contaminating molecules;
- heterogeneous populations of antibodies with concomitant differences in activity, affinity for the antigen, and biological functions, especially when a number of different animals are used to prepare the antiserum; and
- the limited supply of quality antisera for any given purpose (10,28,32).

Since these difficulties are almost unavoidable in standard antibody preparations, the standardization of immunoassay and the accumulation of large amounts of reference antisera have been difficult. Such problems, although time-consuming and expensive, have not prevented the effec - tive use of antibodies as diagnostic, therapeutic, and investigational tools for both research scientists and clinicians, but the search for new methods for continual production of large amounts of pure antibodies has continued.

By what Cesar Milstein calls a "lucky circumstance," he and Georges Kohler began experimenting with the well-established technique of cell fusion in myeloma (antibody-producing tumor) cells adapted for cell culture. Milstein and Kohler fused myeloma cells with antibody-producing spleen B lymphocytes from mice that had been immunized with sheep red blood cells (SRBCS), and they found that some of the resulting hybrid cells, called hybridomas, secreted large amounts of homogeneous (monoclinal) antibodies directed against SRBCS (20)21). The myeloma parent cell conferred on the hybridoma the ability to grow permanently in cell culture and thus to support almost unlimited antibody production, while the B lymphocyte parent contributed the genes coding for the specific antibody against an SRBC antigen.

Photo credit: Scisnce Photo Service and PortodLH Inte?natlonal

Dr. Cesar Milstein, discoverer of monoclinal antibodies

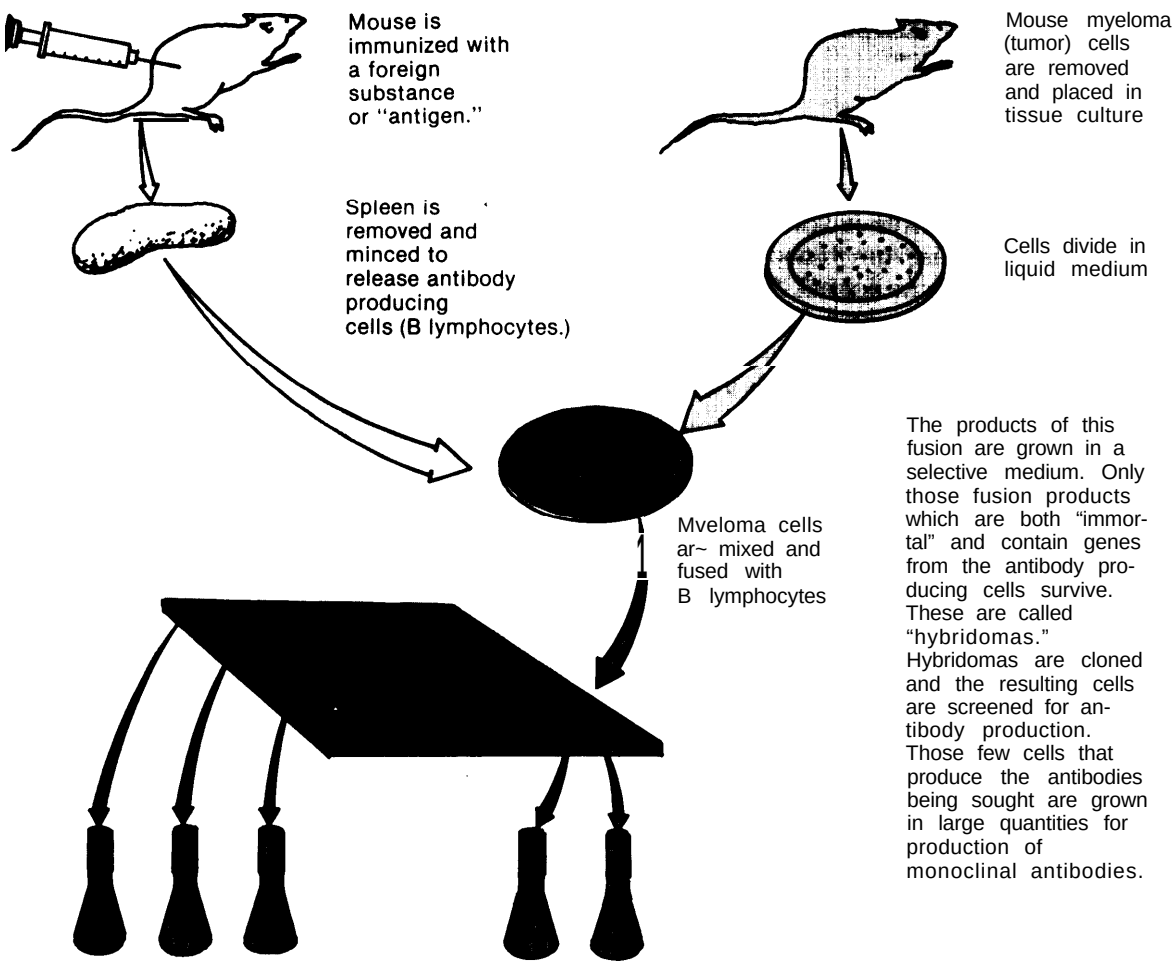
By using the method of hybridoma, or MAb, technology, it is now possible to "immortalize" individual antibody-producing cells by fusion with tissue culture-adapted myeloma tumor cells in the laboratory (4,5,8,13,22,25).

Preparing monoclinal antibodies

The method used to prepare MAbs is summarized in figure 8. The purified antigen of choice is injected into a mouse, and a few weeks later, the spleen of the mouse is removed, The B lymphocytes (antibody-producing cells) are isolated from the spleen and fused with myeloma cells. The resulting cells are placed in a cell culture medium that allows only the hybridomas to grow. The many hybridomas that result are cloned, and each clone is tested for the production of the antibody desired. A particular hybridoma clone either may be established in an in vitro culture system or may be injected into mice, where the hybridoma grows in abdominal cavity fluid (ascites) from which the antibodies are readily collected.

This method allows the preparation of large quantities of highly specific MAbs against almost any available antigen. The antibodies produced by MAb technology are homogeneous, and their production is predictable and repeatable, as compared to polyclonal antibodies produced with conventional immunological methods.

Figure 8.—Preparation of Monoclinal Antibodies



SOURCE: Off Ice of Technology Assessment, adapted from Y. Baakln, "In Search of the Magic Bullet," *Technology f7ev/ew,* pp. 19-23.

Photo credit: Sctence Photo Service and Por@n/LH international

Scanning electron micrograph of human hybridoma cells

Photo credit: Science Photo Library and PortmlLH International

One step in the isolation of hybridomas

Despite the great promise of MAbs, there are several persistent technical problems to be considered:

- obtaining MAbs against certain weak antigens (antigens that do not produce a large immune response) remains difficult (11,24);
- homogeneous antibodies cannot perform some functions such as forming a precipitate with other antigen-antibody complexes, a necessary function for some diagnostic assays;
- low frequency of fusion is a continuing prob-1em in the preparation of hybridomas, as is the stability of the hybridomas and antibodies (14); and
- some MAbs are sensitive to small changes in pH, temperature, freezing and thawing, and can be inactivated during purification.

Many of these problems are being alleviated or solved as research with MAbs progresses.

Another problem being addressed is the development of hybridomas for specific species. Some suitable myeloma cell lines exist for mice, rats, and humans (12,20,27), but a wider variety of human cell lines and cell lines for other species are needed if wider applications of MAb technology are to be made. Hybridomas are often made with cells from two different species, but these fusions regularly result in the preferential loss of the spleen B lymphocyte chromosomes, resulting in an absence of antibody production (24). For therapeutic applications, it is desirable to treat people with human antibodies to avoid allergic reactions and other problems of antibody cross-reactivity. Thus, MAbs from a human myeloma/human spleen cell fusion are needed. Several investigators have reported the development of human myelomas that are suitable for

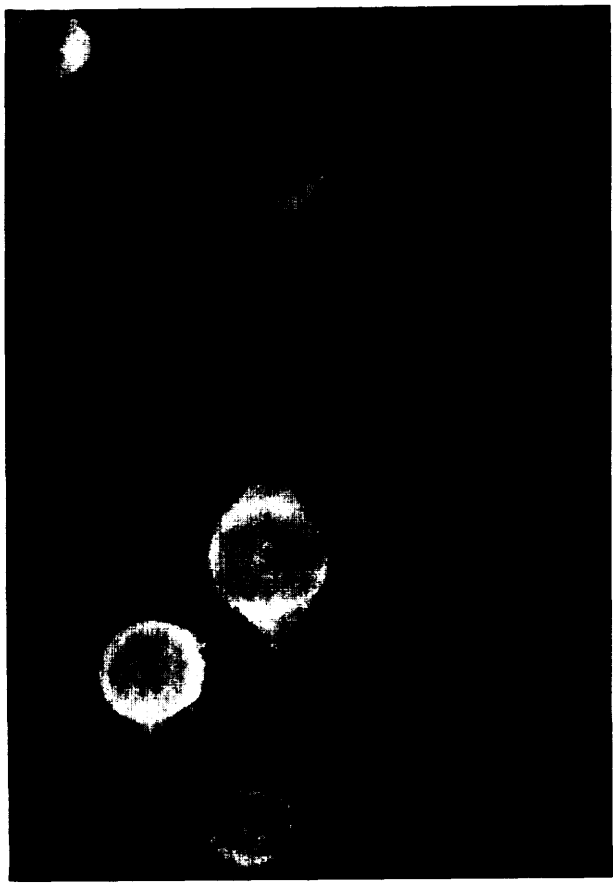


Photo credit: Science Photo L/brary and PortonILH International

In vitro identification of specific cells using fluorescently labeled monoclinal antibodies

hybridoma preparation (9,17,23,27). Successful fusions apparently result from using these cell lines (6).

Monoclinal antibodies and recombinant DNA technology

The combination of MAb technology and rDNA technology offers intriguing possibilities for further technological exploration. Recombinant DNA techniques could be used to produce portions of antibody molecules in bacteria to circumvent some of the problems (e.g., hybridoma instability) associated with MAb production in mice or tissue culture. Additionally, these MAbs would be free of impurities, such as viruses, found in animal cells and possibly could be produced in large amounts with a concurrent savings in cost.

The first cloning and expression of a complete antibody molecule in a bacterial system was announced recently by the U.S. firm Genentech and the City of Hope Medical Center and Research Institute (19). The protein chains were expressed separately in bacteria and reconstituted by the researchers. The pharmaceutical applications of bacterially produced antibody genes will be limited. Antibody molecules must be modified by the cell to function in most diagnostic and therapeutic applications. Bacteria do not perform the modifications necessary for proper function. However, it maybe possible to clone the antibody genes in a cellular system such as yeast where the proper modifications can be made.

The production of MAbs in rDNA systems may prove useful for making reagents used in industrial applications where only the antigen-binding function may be necessary. With genes cloned for the antigen-binding regions of the antibody, portions of MAbs may be produced more economically in bacterial rDNA systems than in a large-scale mouse ascites or cell culture protocols.

Large-scale production of monoclinal antibodies

Although MAbs can be produced by several methods, manufacturers primarily use mouse ascites to produce the modest amounts of MAbs needed to service current diagnostic and research markets. As applications for MAbs to human therapy are developed, the need for larger quantities of MAbs (free from mousederived contaminants that might cause allergic reactions) may encourage a switch to the use of large-scale cell culture to produce MAbs. If MAbs are to be used in industrial applications (e.g., in the purification of proteins), production methods will be needed to produce even larger quantities of antibodies, In these cases, efficient cell culture or microbial bioprocess techniques will probably be necessary to provide enough antibodies to fill these needs.

Improved, more controllable cell culture systems will be needed for the production of MAbs in the future. A crucial need for large-scale cell culture is either the isolation of hybridoma cell lines that attach to surfaces or the use of techniques for immobilizing cells on a solid matrix.

Immobilized cells could be grown in large quantities in culture; the MAbs secreted from the cells could then be routinely collected from the medium. Immobilized cell methods may prove valuable for large-scale MAb production. Such methods are already used industrially, for example, in growing cells that produce polio virus for subsequent vaccine production (26,31).

Damon Biotech Corp. (U. S.) has recently introduced the technique of microencapsulation to MAb technology (7). This method uses a porous carbohydrate capsule to surround the hybridoma cells and to retain the antibodies while allowing the circulation of nutrients and metabolic wastes. After several days in culture, the encapsulated colonies are harvested and washed to remove the growth medium, the capsules are opened, and the antibodies are separated from the cells. According to Damon Biotech, 40 to 50 percent (by weight) of the harvested medium is made up of MAbs. The company claims the microencapsulation method for producing MAbs is significantly less expensive than the ascites method, provides a high concentration of antibodies, and does not require the maintenance of animals (18).

Industrial uses for monoclinal antibodies

Because of their unique properties of homogeneity, specificity, and affinity, MAbs can be used effectively in downstream purification systems for molecules, especially proteins, A MAb purification system relies on the binding of a target molecule to a MAb immobilized on a solid support such as a bead, The beads are packed in a column, and a mixture containing the target molecule is passed through the column. The MAb binds the molecule while the impurities wash through the column. Then the binding is reversed, and the target molecule is released and collected from the column.

Before MAb-based purification systems can be used in large-scale, several practical and technical factors must be optimized. These include cost, purification of the antibody itself, and elution of the desired product after purification by the antibody. Elution requires the use of an antibody of somewhat lower affinity than one would use for diagnostic or therapeutic applications so that the binding can be reversed easily.

Various important proteins, including alpha-fetoprotein and leukocyte interferon, are now purified using MAbs (29,30). MAb purification systems may be used in the future to purify a vast number of compounds, particularly substances present in small amounts.

A simple extension of the procedure just described involves using MAbs to bind unique surface proteins and, with them, the cells to which they are attached. This permits separation of cells with surface proteins of interest and is carried out by passing the cells over a suitable matrix to which the antibodies have been bound. In another procedure, fluorescence-activated cell sorting, cells are mixed with fluorescently labeled MAbs, and the mixture is passed through a special instrument called a flow cytometer, which responds to the fluorescent marker and sorts the cells into labeled populations at rates of 50,000 cells per minute (15,16). So far, fluorescence-activated cell sorting has been used mostly for research purposes, but as the method is improved, it may be employed in a range of clinical applications.

Conclusion

Many fields of biological research are being affected by MAb technology. Researchers now use MAbs to study problems in endocrinology, biochemistry, cell biology, physiology, parasitology, and many other fields, because the products of MAb technology are easily standardized and reproduced. Furthermore, many diagnostic, therapeutic, and industrial uses for MAbs are becoming apparent, and, as outlined in subsequent chapters of this report, several U.S. and foreign firms are developing these applications. Industrial purification applications of MAbs and the widespread advantages of MAb technology in preparing pure and easily standardized antibodies offer substantial benefits in industrial, research, and clinical laboratories. Recombinant DNA and MAb technologies can complement each other, because rDNA technology can lead to the production of new compounds, and MAbs can aid in their identification and purification.

Bioprocess technology*

Bioprocesses are systems in which complete living cells or their components (e.g., enzymes, chloroplasts, etc.) are used to effect desired physical or chemical changes.** Since the dawn of civilization, bioprocesses have been used to produce alcoholic beverages and fermented foods. Until the 19th century, alcoholic fermentation and baker's yeast production were carried out in the home or as local cottage industries. As industrialization occurred, both these bioprocesses moved into factories.

Although other minor products made with bioprocesses were added over the years, bioprocesses did not become significant in the overall spectrum of chemical technology in the United States until the introduction of commercial acetone and butanol production during and after World War I. Somewhat later, large-scale microbial production of citric acid was introduced, and by the beginning of World War II, the U.S. bioprocess industry was thriving, with solvent alcohols and related low molecular weight compounds comprising the bulk of bioprocess production, The rapid growth of the petrochemical industry during World War H caused the displacement of microbial production of industrial solvents, however, and by 1950, microbial production of such solvents (including nonbeverage alcohol) had virtually disappeared in the United States.

This contraction of bioprocess manufacturing might have been the death-knell for old biotechnology had it not been for the introduction of, and the proliferation of markets for, antibiotics during the 1940's. The unique qualifications of biological processes for the synthesis of complex molecules such as antibiotics rapidly became apparent. Microbial production of a number of vitamins and enzymes was initiated at about this time, although only on a small scale. Thus, in the decade from 1940 to 1950, there occurred a complete transformation of industrial bioprocesses. Production of high-volume, low-value-added industrial chemicals (e.g., acetone, butanol) by anaerobic processes employing primarily yeasts and bacteria was largely replaced by more modest-scale production of high-value-added products (e.g., pharmaceuticals, vitamins, enzymes) made by highly aerobic processes in a variety of less familiar bacteria (e.g., the actinomycetes) and some fungi (see table 1). These aerobic processes are generally quite vulnerable to contamination by other micro-organisms and require much closer control of process conditions. Such aerobic processes continue to be used in industry today.

The advent of new biotechnology has sparked renewed interest in the industrial use of bioprocesses. The discussion that follows examines the dependence of new biotechnology, including rDNA and MAb technology, upon bioprocess technologies. Two aspects of the interrelationship between new genetic technologies and bioprocess technologies are emphasized:

- the engineering problems unique to genetically modified organisms, and
- the ways in which genetically modified organisms or parts of organisms maybe used to enhance the efficiency and usefulness of bioprocesses.

In order to be viable in any specific industrial context, bioprocesses must offer advantages over

Table I.-Volume and Value of Biotechnology Products

Category	Examples
High volume, low value . . .	Methane, ethanol, animal feed, waste treatment
High volume, intermediate value	Amino and organic acids, food products, polymers
Low volume, high value . . .	Pharmaceuticals, enzymes, vitamins

SOURCE: Off Ice of Technology Assessment, adapted from A. T. Bull, G. Holt, and M. D. Lilly, B/otechno/ogy: *International Trends and Perspectives* (Paris: Organisation for Economic CoOperation and Development, 19s2).

• This section is based largely on a contract report prepared for the Office of Technology Assessment by Elmer Gaden, University of Virginia. The information in that report was extensively reviewed and added to by James Bailey, California Institute of Technology; Harvey Blanch, University of California, Berkeley; and Charles Cooney, Massachusetts Institute of Technology.

•*The term bioprocess is used here in preference to the more familiar term "fermentation" because it more correctly identifies the broad range of techniques discussed. A fermentation process, though often used to denote any bioprocess, strictly speaking refers only to an anaerobic bioprocess.

competing methods of production. In most cases, bioprocesses will be used industrially because they are the only practical way in which a desired product can be formed. Biological processes may be desirable:

. in the formation of complex molecular structures such as antibiotics and proteins where there is no practical alternative,
. in the exclusive production of one specific form of an isomeric compound,
- because microa-ganisms may efficiently execute many sequential reactions, and
- because bioconversions may give high yields.

Examples of the categories of current uses of bioprocesses are the folIowing:

- production of cell matter ((’biomass” itself) (e.g., baker’s yeast, single-cell protein);
- production of celI components (e.g., enzymes, nucleic acids);
- production of metabolizes (chemical products of metabolic activity), including both primary metabolizes (e.g., ethanol, lactic acid) and secondary metabolizes (e.g., antibiotics);
- catalysis of specific, single-substrate conversions (e.g., glucose to fructose, penicillin to 6-aminopenicillanic acid); and
- catalysis of multiple-substrate conversions (e.g., biological waste treatment).

Bioprocesses may offer the following advantages over conventional chemical processes:

- milder reaction conditions (temperature, pressure, and pH);
- use of renewable (biomass) resources as raw materials for organic chemical manufacture, providing both the carbon skeletons and the energy required for synthesis;
- less hazardous operation and reduced environmental impact;
- greater specificity of catalytic reaction;
- less expensive or more readily available raw materials;
- less complex manufacturing facilities, requiring smaller capital investments;
- improved process efficiencies (e.g., higher yields, reduced energy consumption); and
- the use of rDNA technology to develop new processes.

Some of the conceivable disadvantages of bioprocesses, on the other hand, are the following:

- the generation of complex product mixtures requiring extensive separation and purification, especially when using complex substrates as raw materials (e.g., lignocellulose);
- problems arising from the relatively dilute aqueous environments in which bioprocesses function [e.g., the problem of low reactant concentrations and, hence, low reaction rates; * the need to provide and handle large volumes of process water and to dispose of equivalent volumes of high biological oxygen demand wastes; complex and frequently energy intensive recovery methods for removing small amounts of products from large volumes of water);
- the susceptibility of most bioprocess systems to contamination by foreign organisms, and, in some cases, the need to contain the primary organism so as not to contaminate the surroundings;
- an inherent variability of biological processes due to such factors as genetic instability and raw material variability; and
- for rDNA systems, the need to contain the organisms and sterilize the waste streams, an energy-intensive process.

Solutions to some of these problems through the use of biotechnology may make bioprocesses more competitive with conventional chemical syntheses. Genetic intervention may be used in some instances to modify micro~rganisms so that they produce larger amounts of a product, grow in more concentrated media, have enzymes with increased specific activity, or grow at higher temperatures to help prevent contamination. Recombinant DNA technology may lead to the development of completely new products or modification of important existing ones. In the past, some potentially useful bioprocesses have

- It is often said that biochemical catalysis is many times more effective than conventional chemical catalysis. ‘1’his contention is based on the very high specific activities observed for individual enzymes in vitro. Such rates are seldom encountered under large-scale conditions. In general, bioprocesses are extremely slow in comparison with conventional chemical processes.

not been economical. Now, however, a combination of improved engineering design and procedures and rDNA technology may yield bioprocesses that are more efficient than they have been in the past and therefore more competitive.

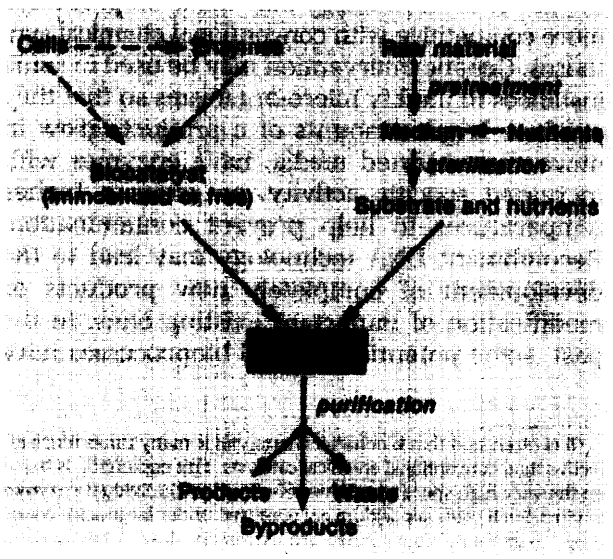
*Bioprocess essentials**

The steps in bioprocessing are presented schematically in figure 9. The substrate and nutrients are prepared in a sterile medium and are put into the process system with some form of biocatalyst–free or immobilized cells or enzymes. Under controlled conditions, the substrate is converted to the product and, when the desired degree of conversion has been achieved, byproducts and wastes are separated.

Water is the dominant component of the medium for virtually all current bioprocesses. Even when micro-organisms are grown on solid materials, an unusual processing mode, the substrate must be dampened in order to permit microbial growth and enzyme action. Products must usually be purified from dilute, aqueous solutions.

*The bioprocesses discussed here exclude uncontrolled environmental applications.

Figure 9.—Steps in Bioprocessing



SOURCE: Office of Technology Assessment.

Bioprocesses require a closely controlled environment, and this necessity markedly influences their design. Biocatalyst generally exhibit great sensitivity to changes in temperature, pH, and even concentrations of certain nutrients or metal ions. The success of a bioprocess depends on the extent to which these factors are controlled in the medium where interaction between biocatalyst and substrate takes place.

SUPPLY OF NUTRIENTS

In addition to establishing a suitable environment, the medium must provide for the nutritional needs of living cells. A primary requirement is a source of carbon, In addition to supplying the energy needed for metabolism and protein synthesis, carbon sources contribute structural elements required for the formation of complex compounds. Often, the carbon source may itself be the substrate for the catalyzed reaction, as in the fermentation of sugar to ethanol. Sugars, starches, and triglycerides, and, to a lesser extent, petroleum fractions, serve as carbon sources.

Other important nutrients required by living cells are nitrogen, phosphorus, and sometimes oxygen. Nitrogen and phosphorus are incorporated into structural and functional molecules of a cell and may also become pafi of product molecules. Most of the microorganisms currently used by industry are highly aerobic and require an adequate supply of oxygen, but others are strictly anaerobic and must be protected from oxygen. A number of other nutrients, such as vitamins and metal ions, though required only in very small amounts, are nevertheless essential. Some of these nutrients, especially metals, may appear in the product.

In order to make the substrate and nutrients accessible to the biocatalyst, the medium must be thoroughly mixed. Most bacteria and some yeasts used in bioprocesses commonly grow as individual cells or as aggregates of a few cells suspended in the medium, whereas fungi and actinomycetes grow in long strands. As they grow, all these types of cells increase the viscosity of the fluid in which they are growing in a batch process, making the fluid more difficult to mix, and thus more difficult for nutrients to reach them.

Since most of the micro~rganisms currently used by industry perform their conversions aerobically, they demand a constant supply of oxygen. Oxygen's low volubility in water represents a significant stumbling block to efficient bioprocessing. Since oxygen is depleted during conversion, the medium must be constantly aerated; the more viscous the medium, however, the more difficult it becomes to supply oxygen. Approaches to maintaining an adequate oxygen supply include:

- increasing reactor pressure to increase oxygen volubility,
- the use of oxygen-rich gas for aeration, and
- changes in process design and operation.

PURE CULTURES AND STERILIZATION

Most of the products of bioprocesses are formed through the action of a single biocatalyst, either a microorganism or an enzyme. * If foreign organisms contaminate the process system, they may disrupt its operation in a variety of ways. They can directly inhibit or interfere with the biocatalyst, whether it is a single enzyme or a complete cell, and they may even destroy the biocatalyst completely. Alternatively, contaminating organisms may leave the catalyst unaffected, but modify or destroy the product. Foreign organisms can also generate undesirable substances that are difficult to separate from the primary product. In the manufacture of pharmaceutical products, the risk of toxic impurities is of particular concern.

To avoid or minimize contamination, most current bioprocess technologies employ pure culture techniques. The medium and its container are sterilized, and a pure culture consisting of a population of a particular species is introduced. In order to avoid subsequent contamination, all materials entering the system, including the large amounts of air required for aerobic processes, are sterilized. The apparatus must be designed and operated so that opportunities for invasion by unwanted organisms are minimized.

*A significant exception to this generalization is the broad group of biological waste treatment processes. These processes use mixed and varied populations of microorganisms developed naturally and adapted to the waste stream being treated.

Processing modes

Bioprocesses may, in principle, use any of the operating modes employed by conventional chemical technology. These modes range from batch processing to continuous steady-state processing.

In batch processing, the reaction vessel is filled with the medium containing the substrate and nutrients, the medium is sterilized, the biocatalyst is added, and conversion takes place over a period ranging from a few hours to several days. During this period, nutrients, substrates, agents for pH control, and air are supplied to, and product gases are removed from, the reaction vessel. When conversion is complete, the reaction vessel is emptied, and the purification process begins. Turnover time between batches can account for a significant portion of total processing time.

In continuous steady-state processing, which lies at the other end of the operational spectrum from batch processing, raw materials are supplied to, and spent medium and product are withdrawn from, the reaction vessel continuously and at volumetrically equal rates. Potential advantages offered by continuous processing over batch processing include significantly higher productivity, greater ease of product recovery due to the lack of contaminating biocatalyst, and lower cost due to reuse of biocatalyst.

The simplest approach to the implementation of a continuous processing system is to modify a batch reactor so that fresh substrate and nutrients can continually be added while a product stream is removed. This simple arrangement has one serious drawback: the biocatalyst leaves the reactor continuously with the outlet stream and must be separated from the product. Several techniques, all of which involve fixing the biocatalyst in some reame,) have been developed to avoid the biocatalyst's escape with the reaction mixture and allow its repeated use. The development of techniques for the immobilization of biocatalyst has greatly expanded the possibilities for continuous bioprocesses. Although still not widely employed for large-scale bioprocesses, the biocatalyst immobilization techniques now available offer a diversity of new opportunities for more effective bioprocessing (see Box ***A.-Continuous Bioprocessing).***

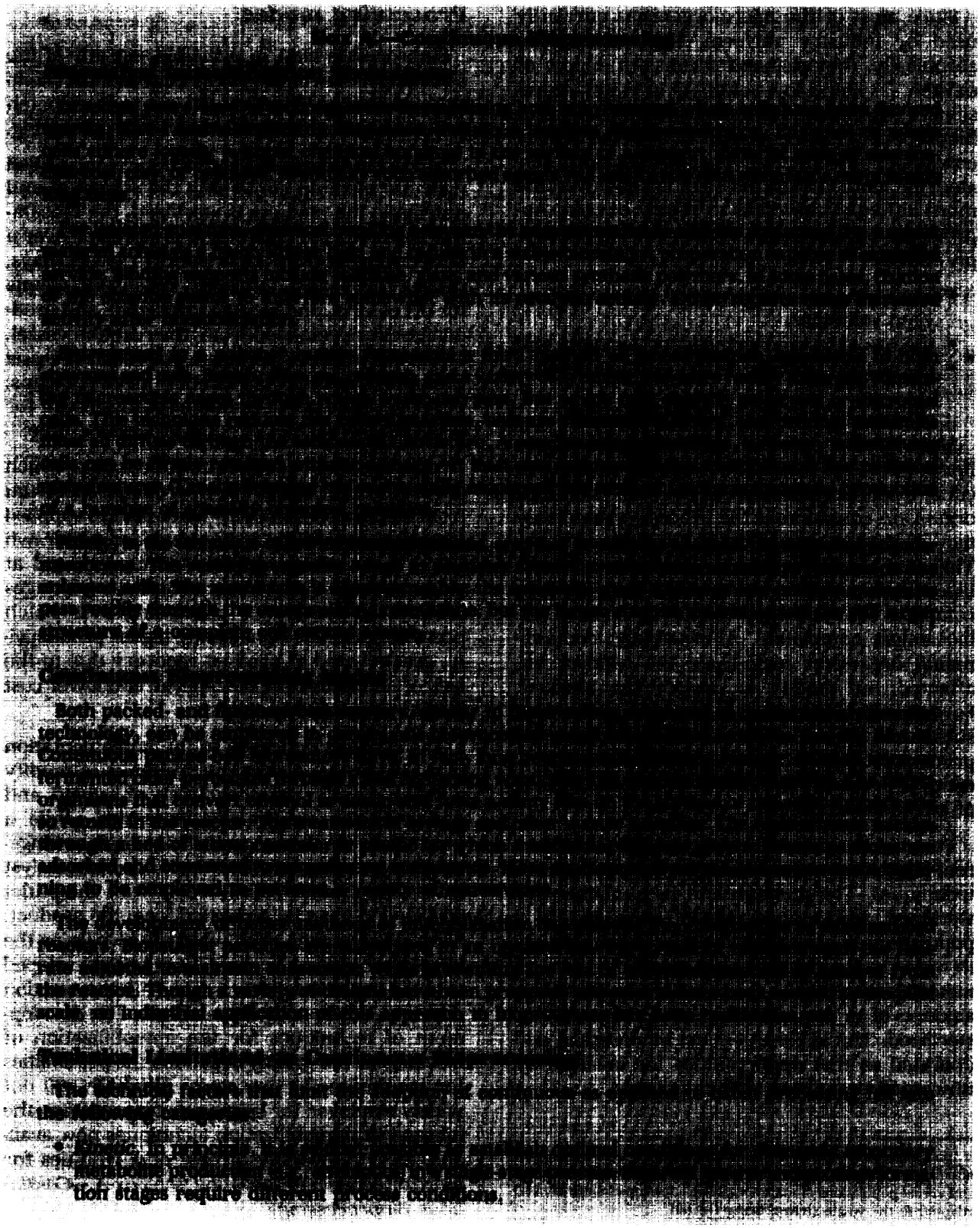
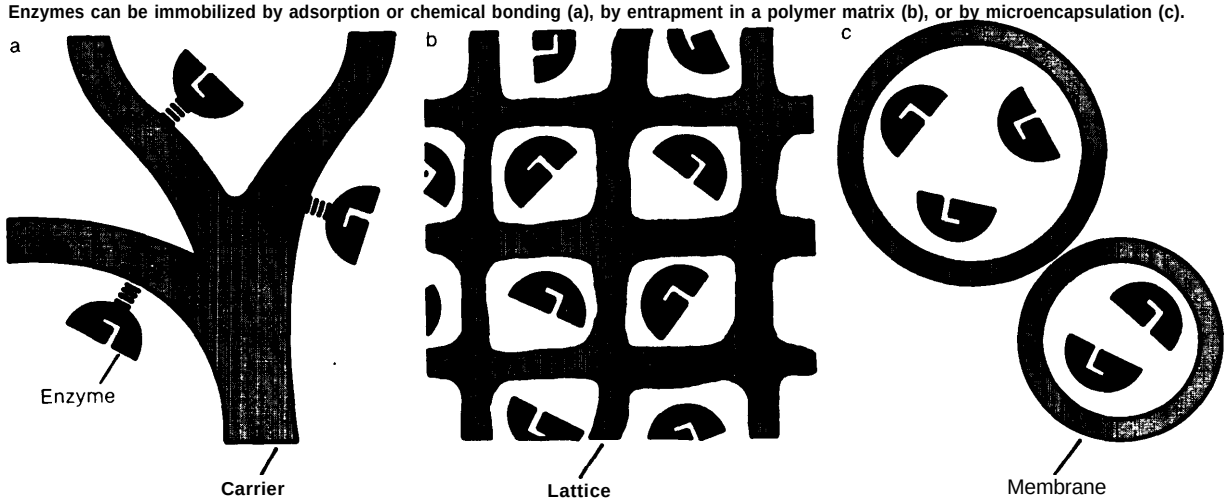


Figure BXA-1 .—Techniques for Immobilizing Enzymes and Whole Cells

Enzymes can be immobilized by adsorption or chemical bonding (a), by entrapment in a polymer matrix (b), or by microencapsulation (c).



SOURCE Adapted from E Gaden, "Production Methods in Industrial Microbiology)" *Scientific American,* September 1981, p. 182,

Table BXA"l.—Characteristics of Immobilization Methods for Enzymes and Cells

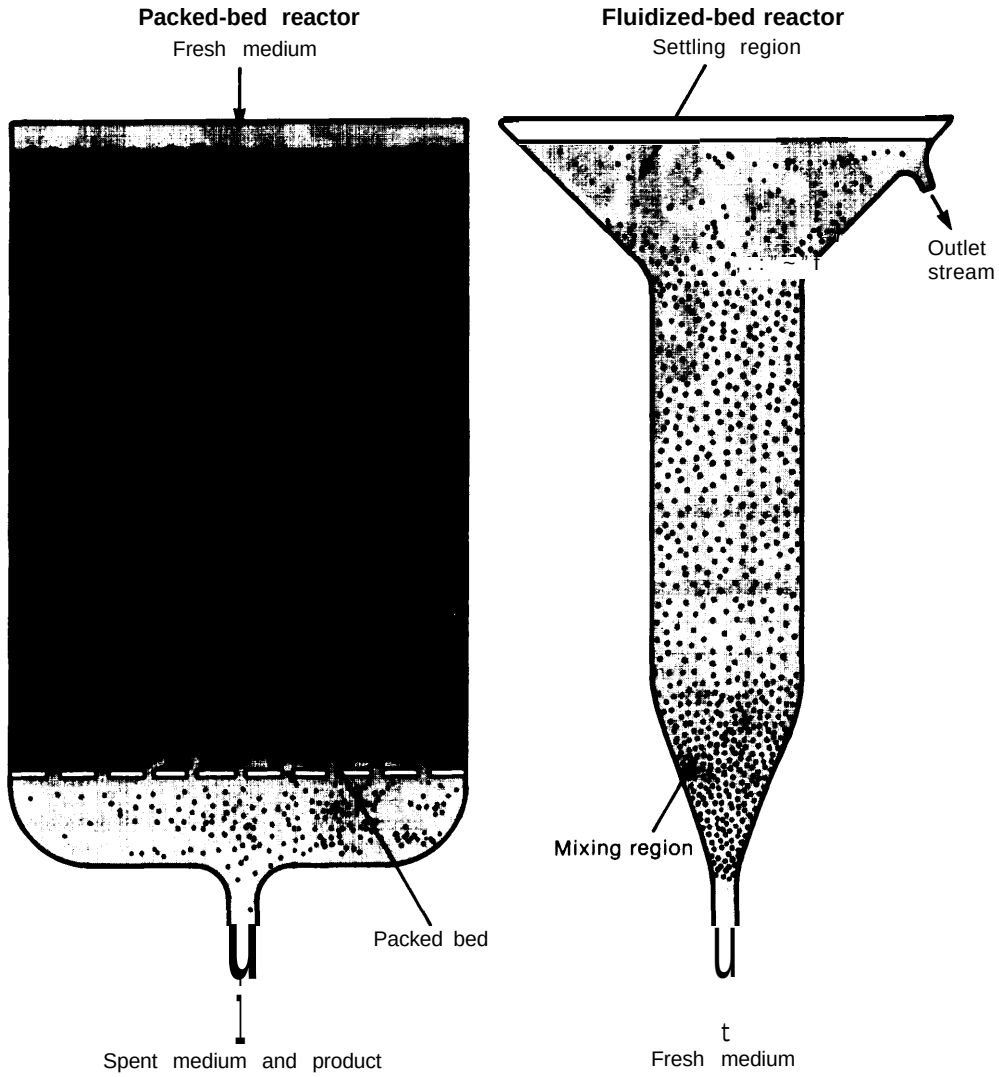
Characteristic	Immobilization method		
	Physical adsorption	Chemical bonding	Entrapment; encapsulation
Preparation	Easy	Difficult	Moderate
Activity	Low	High	High
Specificity	Unchanged	Changeable	Unchanged
Binding force (retention)	Weak	Strong	Strong
Regeneration	Possible	Impossible	Impossible
cost	Low	High	Low

SOURCE Gaden, personal communication, 1983

- *Biological.* Biocatalwvst stability may be difficult to maintain for long periods of continuous operation. The phenomenon of "culture degeneration," reported in many instances, deserves careful study. The results of such studies will surely be case-specific and may simply reflect inadequacies in the lmoivledge of nutrient requirements necessary to sustain long-term productivity. As the use of rDNA organisms grows, this matter will require close attention because of concerns over the stability of these types of organisms.

(Operationaf. The primary technical factors acting to limit continuous bioprocessing in the past have been difficulties in maintaining sterile conditions and in handling biocatalytic suspensions, especially those of filamentous fungi or large cell clumps. The perplexing contamination problem has focused improvement efforts on the deficiencies of equipment (mainly pumps) for moving liquids and slurries and on valves and transfer lines. Many specific difficulties have already been overcome in connection with batch operations, and improved equipment design and more rigorous operating procedures may result in successful continuous processes.

Figure BXA.2.– Packed-Bed and Fluidized-Bed Reactors



SOURCE: Adapted from E. Gaden, "Productlon Methods in Industrial Microbiology," *Sckwrtlfk Arnerlcan,* September 19S1, p. 19S.

Batch operation currently dominates specialty chemical and pharmaceutical bioprocesses and is likely to continue to do so in the near future, In addition to technical limitations on continuous processing (see box A), other considerations have led manufacturers to choose the batch mode. Batch processing is often used, for example, because it offers the operational flexibility needed when a large number of products are manufactured, each at fairly low production levels; each process unit, more or less standard in design, can easily and rapidly be switched from one product line to another. Furthermore, a switch from batch to continuous processing is expensive, and, if a company has unused batch equipment, it may find that a switch to continuous processing is not economically warranted in the near term.

Increased use of genetically manipulated biocatalyst could affect the design and operation of bioconversion units. Harvey Blanch points out (33):

> . . . one of the difficulties which arises from the insertion of foreign DNA into the organism is re -

version. This can be minimized by placing the cell in an environment in which cellular replication is minimized, while cellular activity, such as the production of enzymes and products, is maintained at high levels.

Achieving the dual objective of minimal growth and maximum conversion activity requires restrictive nutrient supplies and high cell densities. Immobilized biocatalyst could be used to achieve these objectives.

Bioprocesses, unlike petroleum refining or petrochemical operations which completely convert raw materials to products or consume them as process fuels, regularly produce large amounts of waste, mainly cell matter and residual nutrients. Bioprocesses also require large volumes of clean water, discharge equivalent amounts of dilute, high biological oxygen demand wastes, and produce products in low concentrations. One solution to problems associated with bioprocessing might be the use of cleaner, more defined media, which produce fewer byproducts. Another solution might be the use of more concentrated media. The latter option is normally considered in bioprocess development, but the microorganisms now in use are limited in their tolerance for high nutrient concentrations. Genetic manipulation may provide micro-organisms that are less sensitive to increased product concentration.

Raw materials

Current bioprocess technology uses an extremely limited range of raw materials. Just a few agricultural commodities—starch, molasses, and vegetable oil—are employed as raw materials in many of the existing industrial bioprocesses, Industry chooses these feedstocks for several reasons. There are established markets for these materials and, for primary products like starch, reasonably defined quality standards and assay procedures. Several competing suppliers guarantee uniform quality and fairly stable prices. Bioprocess applications constitute only a relatively small fraction of the market for agricultural commodities. The need for raw materials for bioprocesses, however, could become a major factor in commodity grain markets if bioprocesses find a place in large-scale fuel or chemical production.

Less important raw materials are some byproducts of agricultural and food processing, such as "corn steep liquor" and "distillers solubles. " Petroleum hydrocarbons are little used because of their high cost. The potential for relatively pure cellulose (e.g., delignified wood) remains unrealized. * For various carbohydrate wastes—agricultural, food, industrial, or municipal—in spite of frequent claims of their availability and low cost, no economical bioprocess applications have yet been found.

Biocatalyst

The substances that actually cause chemical change in bioprocesses are the enzymes produced by a living cell. For simple enzymatic conversions, isolated enzymes can be used as biocatalyst. When biological transformation of the substrate involves several sequential and interrelated chemical reactions, each catalyzed by a separate enzyme, however, whole cells (most commonly, but not exclusively, micro-organisms-bacteria, yeast, or fungi) are used as biocatalyst. Bioproc - esses used for the synthesis of complex molecular structures (e.g., antibiotics or proteins such as insulin), for example, require entire systems of enzymes, Such systems do not yet function in concert outside a living cell. Indeed, when the desired product is the cell itself (e.g., baker's yeast or single-cell protein), all the enzymes comprising the cell's growth machinery are components of the catalytic system.

An inspection of the immense spectrum of organisms whose biochemical capabilities have been reasonably well defined reveals that bioprocesses employ only a small, select group of biocatalyst. If one eliminates those organisms considered "natural populations" in food fermentation or biological waste treatment, the range of biocatalyst employed in bioprocesses is even more limited. Some animal cells and tissues are employed for vaccine production and related activities, but the catalytic capabilities of plant cells, except for some algae, have not yet been employed commercially. It is possible **that biotech**nology will provide a means whereby important catalytic activities from poorly understood

● **See Chapter 9: Commodity Chemicals and Energy** Production,

organisms can be transferred to cells whose large-scale growth is well understood.

Wider availability of thermotolerant biocatalyst is important for all industries using bioprocesses. Recent research on the development of thermotolerant biocatalytic agents has advanced the potential efficiency of bioprocesses. The advantages of thermotolerance include:

- reduced susceptibility to contamination;
- easier removal of metabolic heat;
- more complete and rapid conversions when volatile inhibitors are present (but oxygen volubility is reduced); and
- easier recovery of volatile products (e.g., ethanol).

Biocatalyst that can withstand high pressure may also be useful industrially. For instance, higher pressures will increase the volubility of oxygen.

Finally, research investigating the relationship between the structure and the function of enzymes is proceeding. Ultimately, the aim is to be able to design, with the help of computers, an enzyme to perform any specific catalytic activity under given conditions. Although this procedure will not be done routinely for many years, it will soon be possible, using rDNA technology, to modify the structure of an enzyme to improve its function in a given condition, such as at a particular pH or temperature. Thus, biotechnology could greatly affect the efficiency of bioprocesses.

Bioprocess monitoring and associated instrumentation

Despite the need for close control of process variables during a bioprocess operation, the techniques available for making measurements on-line are extremely limited. Existing equipment can readily monitor only temperature, pH, dissolved oxygen concentration, and evolution of gases. Although many other sensors have been developed to measure other variables (e.g., glucose levels), all are sensitive to steam sterilization. Thus, their usefulness in monitoring most bioprocesses is limited. Many critical variables are able to be monitored only by withdrawing samples from the reaction vessel and analyzing them off-line, and, even then, it is difficult to determine key characteristics accurately. When measuring cell mass (an indicator of growth), for example, most process operators simply note such crude indicators as packed cell volume, turbidity, or, at best, dry weight.

It is possible to measure the compositions and flow rates of gaseous streams entering and leaving the reactor and to use the values obtained from such measurements to help estimate key process conditions indirectly. Such procedures have been greatly facilitated by the use of computers. The real potential of computer control, however, will not be realized until a greater range of reliable on-line sensors becomes available. *

A number of European, Japanese, and American groups have developed improved sensors for bioprocess control, but, so far, most devices require removal of samples for off-line analysis because the sensors cannot withstand sterilization. Continuous sampling combined with various types of rapid instrumental analyzers offers a reasonable compromise, but, with this approach, there is a time lag between the actual sample time and the time at which the assay information becomes available.

Sophisticated instrumentation will have increasing use in bioprocess monitoring. High performance liquid chromatography, for example, is used to identify particular compounds in a mix of compounds and is one of the fastest growing instrumentation fields. Flow cytometry has potential use in measuring process variables such as cell size (an indicator for adjusting nutrient flows) and cell viability. Other instrumentation will surely be used as bioprocess monitoring becomes more widely investigated.

Computer-coupled bioprocesses can greatly improve monitoring and controlling the growth conditions during a bioprocess run. Computers can be used to analyze the data from sensors and other monitoring instrumentation and respond to these data by adjusting process variables, such as nutrient flow. Additionally, computer interfaces can be used:

- to schedule efficiently the use of equipment;
- . to alarm operators when necessary;

*For a discussion of biosensors, see ***Chapter*** *10: Bioeleetronics.*

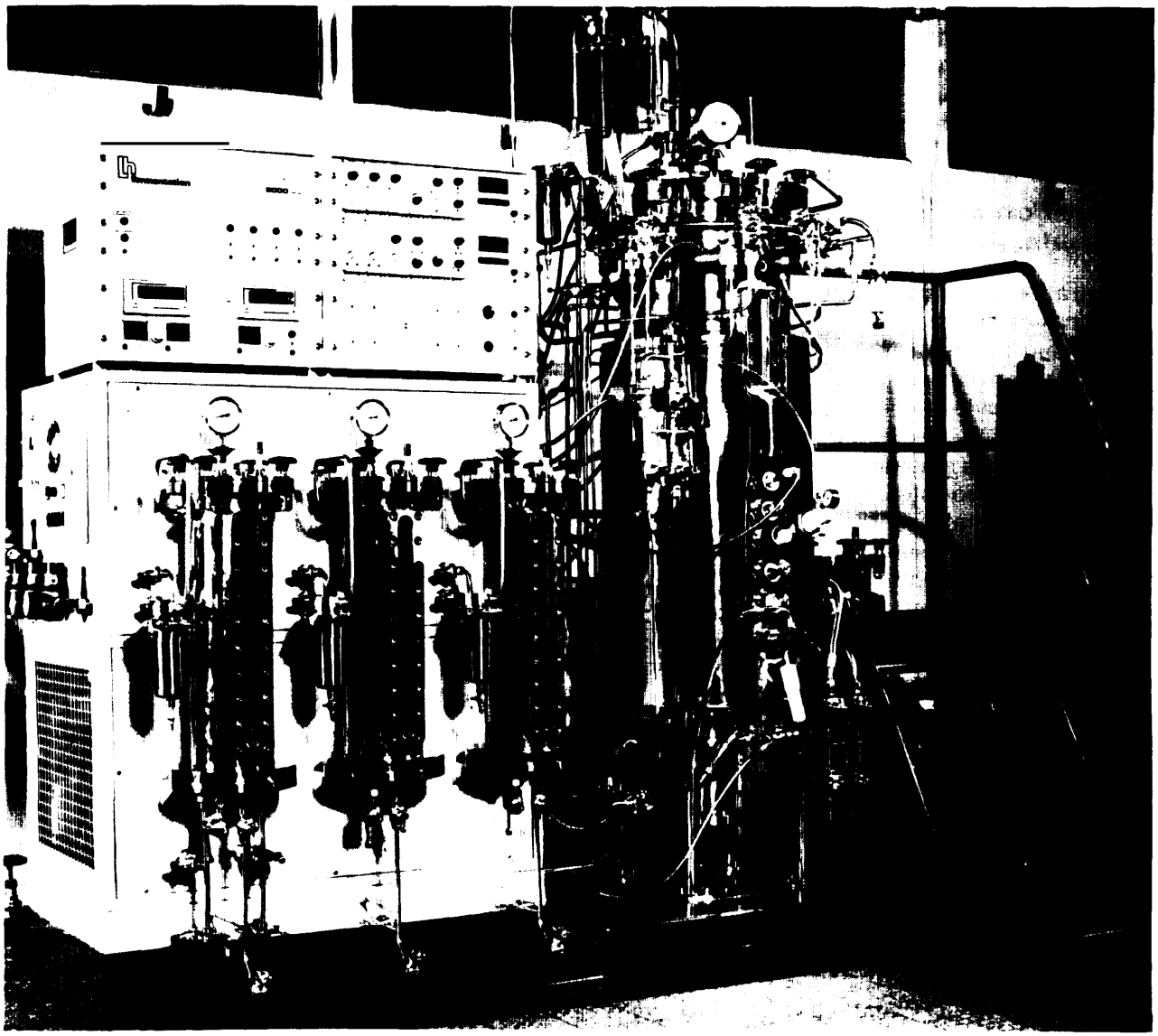


Photo credif: Porfon/LH /nternationa/

100-liter pilot plant bioreactor with computer controls

- to log, store, and analyze data; and
- to inventory raw material depletion and product synthesis.

These functions optimize the methodology and organization of bioprocessing within a plant. Com - panies are only now starting to use computer-controlled bioprocesses because of cost, lack of good sensors, and interfacing problems. Yet advances in this field are sure to occur soon because of increased interest in bioprocesses by electronics experts, as evidenced by the recent joint venture between Genentech and Hewlett-Packard. *

The automation of bioprocessing will be of crit - ical importance in the future as companies com - pete for shares in biotechnology product markets.

'See Chapter 4: F'irrns Commercializing Biotechnolo@.

As automation reduces the labor intensity of laboratory tasks, the pace of competition will quicken, and countries with sophisticated software to direct the automation will possess an advantage in the commercialization of biotechnology.

Separation and purification of products

Separation and purification techniques used in bioprocesses are the aspect of bioprocess engineering most in need of attention, especially for the production of novel products such as proteins. Research is needed to find highly selective recovery techniques that leave as little residual product as possible in the medium and thus lessen the labor intensity associated with downstream processing. An example of the effort expended in downstream processing is provided by the new plant Eli Lilly built to produce human insulin (Humulin"). The plant employs 220 people, 90 percent of whom are involved in recovery processes.

Some of the possibilities for improving recovery techniques now under consideration include the following:

- ***Ultrafiltration.*** Membranes and other filtration systems, such as porous metals, offer many advantages, and considerable experience in other areas of chemical technology is already available. Some U.S. companies, such as Millipore, Amicon, and Nucleopore are making advances in this area.
- ***Continuous chromatography and high performance liquid chromatography.*** If these approaches, already available on the laboratory scale, could be scaled-up, it would be possible, in principle, to collect a crude product from the medium and then, by selective elution, recover product, reusable nutrients, and inhibitory substances separately, one American manufacturer (Waters, a Millipore subsidiary) claims to have developed a pilot-scale chromatographic unit.
- ***Electrophoresis.*** Electrophoretic methods, especially continuous flow, can separate proteins, peptides, and nucleic acids on the basis of their electrical charge. The advantage of this separation method over some others is that it can run continuously and can effectively separate molecules in large sample volumes. The potential of continuous-flow electrophoresis for producing commercial quantities of high purity substances such as pharmaceuticals was demonstrated on a recent space shuttle mission. The electrophoresis experiment, cosponsored by McDonnell Douglas and the National Aeronautics and Space Administration, demonstrated that under weightless conditions an electrophoresis system, identical to one tested on Earth, separated about 700 times more material in a given period of time and also achieved four times the purity while processing 250 times more material.
- ***Monoclinal antibodies.*** Immobilized MAbs are being used as purification agents for protein products (see "Monoclinal Antibody Technology" section above). This technique best suits large molecular weight and high-value-added products such as proteins,

Genetic modifications of micromganisms used in bioprocessing could also aid in recovery processes. Two changes in particular would greatly improve the yield and ease recovery of proteins. First, microorganisms could be developed that have minimal intracellular proteindegrading enzymes. The presence of these enzymes will decrease the yield of protein product. Second, a protein is much more easily purified if it is secreted from the cell into the surrounding medium. The genetic incorporation of protein secretion mechanisms will lower production costs dramatically.

Although purification and separation protocols have been developed for existing bioprocesses, new bioprocesses will present new challenges. For example, rDNA technology has led to a new set of bioprocesses that synthesize protein products, and substantial work is needed to improve recovery strategies for large-scale protein purifications. In addition, one of the factors that restricts the use of bioprocesses for producing commodity chemicals is the expense of recovering these low-value-added chemicals from dilute aqueous solutions.

Culture of higher eukaryotic cells

The organisms used most extensively in large-scale bioprocesses are prokaryotes (e.g., bacteria) or simple eukaryotes (e.g., yeast). These are hardy organisms which grow to high cell densities and consequently give high product yields.

Certain products can be obtained in some situations only from the large-scale cultivation of higher eukaryotic cells. As noted in table 2, for instance, many proteins that are potentially useful (e.g., in medicine) have not been isolated in large enough quantities to study adequately. If eukaryotic cell culture made these proteins available in larger quantities, their amino acid sequence could be determined, their genes cloned, and even more of the proteins could be produced. Furthermore, some proteins probably need "post-translational modifications" (changes in protein structure after the protein is made from mRNA) that only higher eukaryotes can perform. These modified proteins may onty be made in eukaryotic cells, Also, in many cases, the production of secondary metabolizes in plant cells is a function of several enzymatic functions, most of which are not known. Therefore, the growth of plant cells in culture might be the easiest way to produce useful plant compounds. Finally, many individuals think that the growth of hybridomas would be easier and more economic in culture if the culture technology were better developed (see "Monoclinal Antibody Technology" section above). As biotechnology becomes more integrated into the industrial structure, the development of more efficient and economic bioprocess technologies for higher eukaryotic cells will increase in importance.

Photo credit: Sci&Jce Photo Library and Porion/LH International

Laboratory tissue culture production

The technologies developed for the growth of microorganisms have limited applicability to the growth of higher eukaryotic cells because of dif - ferences between microbial and mammalian cells (see table 3). Mammalian cells are larger, more fragile, and more complex than microbial cells. *

* **Most cell** culture research has been **done with mammalian cells, so the work reported here focuses on those cells. Problems with plant cell culture are similar to those of mammalian cell culture.**

Table 2.—Situations Potentially Requiring Large-Scale Eukaryotic Cell Culture

Cell culture system	Reason for large-scale eukaryotic cell culture
Cells producing useful proteins	Not large enough quantity to determine amino acid sequence; therefore cannot use rDNA technology
Cells producing modified proteins	Modification systems present only in higher cells
Plant cells producing useful secondary metabolizes	Enzymatic pathways for metabolic production not well understood; therefore cannot use rDNA technology
Hybridomas	Mouse acites system has limited capacity and applicability

SOURCE: Office of Technology Assessment.

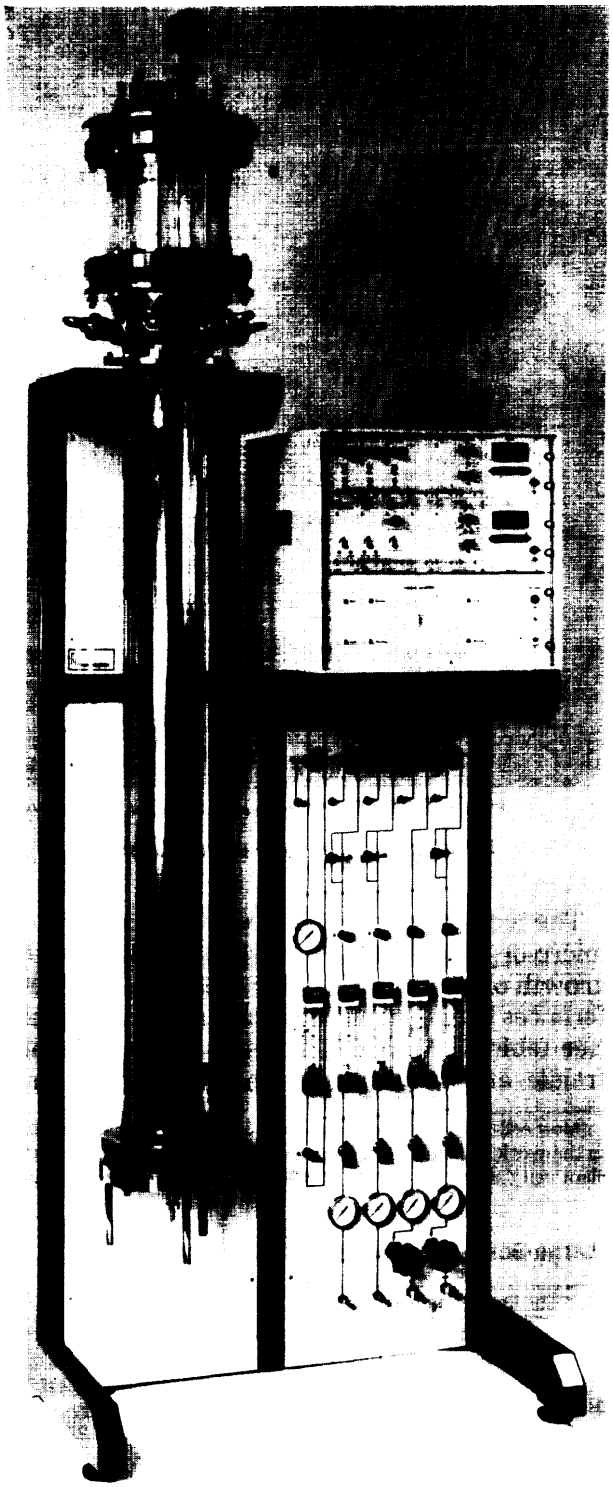


Photo cmdlt: *Porton/LH International*

Bioreactor specially designed for the growth of plant or animal cells

Furthermore, mammalian cells have very complex nutritional requirements, which have not been completely defined. They require serum from blood for growth, and the essential composition of serum is not well characterized. In contrast to microbial cells, mammalian cells are not normally exposed to the environment, but are constantly surrounded by a circulatory system that supplies nutrients and removes wastes. When these cells are grown in culture, the medium is initially clean and nutritionally balanced; as the cells take up the nutrients and excrete waste products, however, the medium becomes much less like the cells' normal environment. This problem, along with the problem of fragility, requires modified reactor design (39).

Some mammalian cells grow in suspension like microbial cells, but most higher cells must attach to a solid surface. A major problem with large-scale cell growth of mammalian cells has been the availability of large, accessible surfaces for cell growth. The attachment of cells to microcarriers, or very small beads, has begun to solve many of the problems associated with large-scale mammalian cell culture. The beads provide a large amount of surface area and can be placed in a column where either a continuous-flow or fluidized-bed bioreactor can be used for cell growth and product formation (see box A). Either of these bioreactors is gentler than a stirred tank reactor. Additionally, because of the continuous nature of these bioreactors, fresh nutrients are added and wastes are removed continuously.

The instrumentation requirements for mammalian cell growth are different than those for microbial growth. The lower rates of metabolism and lower density of mammalian cells require more sensitive sensor systems than for microbial cell growth. Additionally, because the nutritional requirements are so much more complex, different strategies are needed to monitor and control cell growth. These problems are just beginning to be addressed (4o).

Priorities for future research

Priorities for future generic research in bioprocess engineering that would be applicable

Table 3.—Comparison of Microbial and Mammalian Cells

Characteristic	Microbial cells	Mammalian cells
Size (diameter)	1-10 microns	10-100 microns
Metabolic regulation	Internal	Internal and hormonal
Nutritional spectrum	Wide range of substrates	Very fastidious nature
Doubling time	Typically 0.5-2.0 hours	Typically 12-60 hours
Environment	Wide range of tolerance	Narrow range of tolerance
Other characteristics		Limited life span of normal cells Lack of protective cell wall

SOURCE: Office of Technology Assessment, adapted from R. J. Fleischaker, Jr., "An Experimental Study in the Use of instrumentation To Analvze Metabolism and Product Fermentation in Cell Culture," thesis, Massachusetts Institute of Technology, Cambridge, Mass., June 19S2.

to all industries using biotechnology include research in the following areas:

- continued work on the practical use of and design of bioreactors for immobilized cell and enzyme systems;
- development of a wider range of sterilizable sensors for process monitoring and control;
- improved product recovery techniques, especially for proteins;
- general reactor design and practical approaches to better oxygen transfer;
- inhibition of intracellular protein-degrading enzymes;
- improving the genetic stability of rDNA organisms;
- protein secretion mechanisms;
- improved methods for heat dissipation during bioprocessing; and
- biochemical and physiological mechanisms for temperature and pressure tolerance.

The large-scale culture of eukaryotic cells is beginning to receive some research attention. Because of the complex nutritional requirements of eukaryotic cells, the cost of the medium is high. If industry is going to adopt eukaryotic cell culture technology, the development of economic artificial media is important. Also important is the development of new bioreactor design and instrumentation for the control of cell growth.

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