Part I
Biotechnology

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Biotechnology—an introduction

Biotechnology involves the use in industry of living organisms or their components (such as enzymes). It includes the introduction of genetically engineered micro-organisms into a variety of industrial processes.

The pharmaceutical, chemical, and food processing industries, in that order, are most likely to take advantage of advances in molecular genetics. Others that might also be affected, although not as immediately, are the mining, crude oil recovery, and pollution control industries.

Because nearly all the products of biotechnology are manufactured by micro-organisms, fermentation is an indispensable element of biotechnology's support system. The pharmaceutical industry, the earliest beneficiary of the new knowledge, is already producing pharmaceuticals derived from genetically engineered micro-organisms. The chemical industry will take longer to make use of biotechnology, but the ultimate impact may be enormous. The food processing industry will probably be affected last.

This report examines many of the pharmaceutical industry's products in detail, as well as some of the secondary impacts that the technologies might have. Because the chemical and food industries will feel the major impact of biotechnology later, specific impacts are less certain and particular products are less identifiable. The mining, oil recovery, and pollution control industries are also candidates for the use of genetic technologies. However, because of technical, scientific, legal, and economic uncertainties, the success of applications in these industries is more speculative.

The generalizations made with respect to each of the industries should be viewed as just that—generalizations. Because a wide array of products can be made biologically, and because different factors influence each instance of production, isolated examples of success may appear throughout the industries at approximately the same time. In almost every case, specific predictions can only be made on a product-by-product basis; for while it may be true that biotechnology's overall impact will be profound, identifying many of the products most likely to be affected remains speculative.

Fermentation

There are several ways that DNA can be cut, spliced, or otherwise altered. But ***engineered*** DNA by itself is a static molecule. To be anything more than the end of a laboratory exercise, the molecule must be integrated into a system of production; to have an impact on society at large, it must become a component of an industrial or otherwise useful process.

The process that is central to the economic success of biotechnology has been around for centuries. It is fermentation, essentially the process used to make wine and beer. It can also produce organic chemical compounds using micro-organisms or their enzymes.

Over the years, the scope and efficiency of the fermentation process has been gradually improved and refined. Two processes now exist, both of which will benefit from genetic engi-

neering. In fermentation technology, living organisms serve as miniature factories, converting raw materials into end products. In enzyme technology, biological catalysts extracted from those living organisms are used to make the products.

Fermentation industries

The food processing, chemical, and pharmaceutical industries are the three major users of fermentation today. The food industry was the first to exploit micro-organisms to produce alcoholic beverages and fermented foods. Mid-16th century records describe highly sophisticated methods of fermentation technology. Heat processing techniques, for example, anticipated pasteurization by several centuries.

[n the early 20th century, the chemical industry began to use the technology to produce organic solvents like ethanol, and enzymes like amylase, used at the time to treat textiles. The chemical industry's interest in fermentation arose as the field of biochemistry took shape around the turn of the century. But it was not until World War I that wartime needs for the organic solvent acetone—to produce the cordite used in explosives–substantially increased research into the potential of fermentation. Thirty years later after World War 11, the pharmaceutical industry followed the chemical industry's lead, applying fermentation to the production of vitamins and new antibiotics.

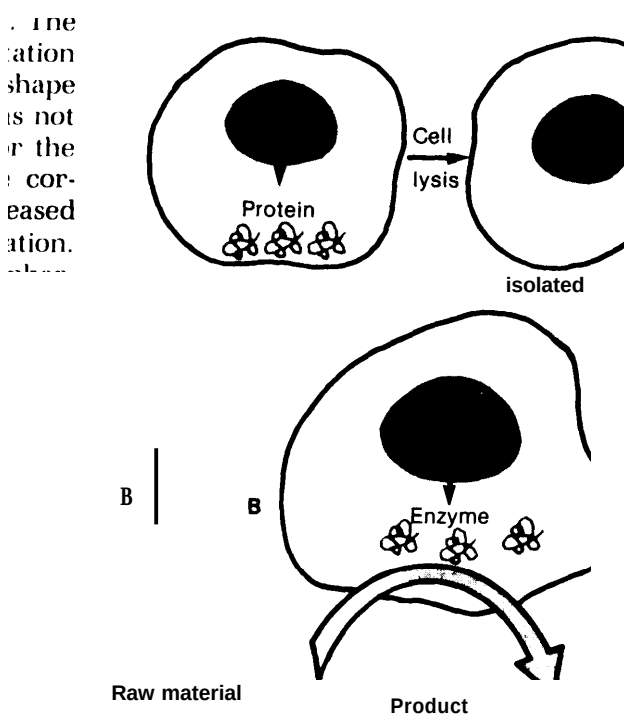
Today, approximately ***200*** companies in the United States and over 500 worldwide use fermentation technologies to produce a wide variety of products. Most use them as part of production processes, usually in food processing. But others manufacture either proteins, which can be considered primary products, or a host of secondary products, which these proteins help produce. For genes can make enzymes, which are proteins; and the enzymes can help make alcohol, methane, antibiotics, and many other substances.

Proteins, the primary products, function as:

- enzymes such as asparaginase which are used in the treatment of leukemia;
- structural components, such as collagen, used in skin transplants following burn trauma;
- certain hormones, such as insulin and human growth hormone;
- substances in the immune system, such as antibodies and interferon; and
- specialized functional components, such as hemoglobin.

Fermentation technologies are so useful for producing proteins partly because these are the direct products of genes. But proteins (as enzymes) can also be used in thousands of additional conversions to produce practically any organic chemical and many inorganic ones as well: (See figure 16.)

Figure 16.-Diagram of Products Available From Cells



In (A) DNA directs the formation of a protein, such as insulin, which is itself the desired product. In (B), DNA directs the formation of an enzyme which, in turn, converts some raw material, such as sugar, to a product, such as ethanol.

SOURCE: Office of Technology Assessment.

- vc carbohydrates, such as fructose sweeteners;
- u lipids, such as vitamins A, E, and K;
- s, alcohols, such as ethanol;
- r{ other organic compounds, such as acetone; and
-)i(inorganic chemicals, such as ammonia, for use in fertilizers.

Fermentation is not the only way to manufacture or isolate these products. Some are traditionally produced by other methods. If a change from one process to another is to occur, both economic and societal pressures will help determine whether an innovative approach will be used to produce a particular product. Alan Bull has identified four stimuli for change and innovation: [1]

1. abundance of a potentially useful raw material;
2. scarcity of an established product;
3. discovery of a new product; and
4. environmental concerns.

And conditions existing today have added a fifth stimulus:

5. scarcity of a currently used raw material.

Each of these factors has tended to accelerate the application of fermentation.

1. ***Abundance of a potentially useful raw material.***—The use of a raw material can be the driving force in developing a process. When straight chain hydrocarbons (n-alkanes) were produced on a large scale as petroleum refinery byproducts, fermentation processes were developed to convert them to single-cell proteins for use in animal feed.
2. ***Scarcity*** of ***an established product.—The*** new-found potential for producing human hormones through fermentation technology is a major impetus to the industry today. Similarly, many organic compounds once obtained by other processes—like citric acid, which was extracted directly from citrus fruits—are now made by fermentation. As a result of more efficient technology, products from vitamin B_{12} to steroids have come into wider use.
3. ***Discovery*** of ***a new product.—The*** **discovery that antibiotics were produced by microorganisms sparked searches for an entirely new group of products. Several thousand antibiotics have been discovered to date, of which over a hundred have proved to be clinically useful.**
4. ***Environmental concerns.*** —The problems of sewage treatment and the need for new sources of energy have triggered a search for methods to convert sewage and municipal wastes to methane, the principal component of natural gas. Because micro-organisms play a major role in the natural cycling of organic compounds, fermentation has been one method used for the conversion.
5. ***Scarcity*** of ***a currently used raw materia/.***—Because the Earth's supplies of fossil fuels are rapidly dwindling, there is intense interest in finding methods for converting other raw materials to fuel. Fermentation offers a major approach to such conversions.

Fermentation technologies can be effective in each of these situations because of their outstanding versatility and relative simplicity. The **processes of fermentation are basically identical, no matter what organism is selected, what medium used, or what product formed. The same apparatus, with minor modifications, can** be used to produce a drug, an agricultural product, a chemical, or an animal feed supplement.

Fermentation using whole living cells

Originally, fermentation used some of the most primitive forms of plant life as cell factories. Bacteria were used to make yogurt and antibiotics, yeasts to ferment wine, and the filamentous fungi or molds to produce organic acids. More recently, fermentation technology has begun to use cells derived from higher plants and animals under growth conditions known as cell or tissue culture. In all cases, large quantities of cells with uniform character-

C. Ellwood and J. Ratledge, *Mic* **Rat ledge,** *nology: plant Future Prospects,* **Prospects,** in o **Symposium** fo **the Society** obiology at Universit c Cambrid , pril 1979 ngland: Cambridge University Press, 1979), pp. 4-8 pp. urg

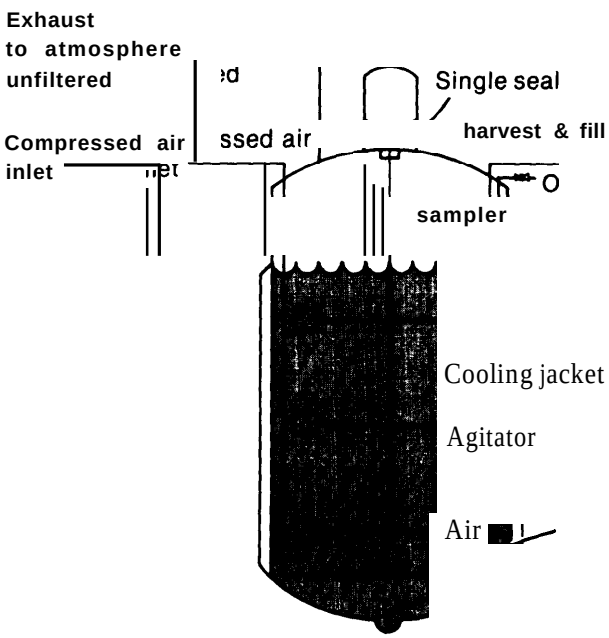
istics are grown under defined, controlled conditions.

In its simplest form, fermentation consists of mixing a micro-organism with a liquid broth and allowing the components to react. More sophisticated large-scale processes require control of the entire environment so that fermentation proceeds efficiently and, more importantly, so that it can be repeated exactly, with the same amounts of raw materials, broth, and micro-organisms producing the same amount of product. Strict control is maintained of such variables as pH (acidity/alkalinity), temperature, and oxygen supply. (See figure l?.) The newest models are regulated by sensors that are monitored by computers. The capacity of industrial-sized fermenters can reach ***50,000*** **gal or** more. The one-shot system of fermentation is called batch fermentation—i.e., fermentation in which a single batch of material is processed from start to finish.

In continuous fermentation, an improvement on the batch process, fermentation goes on without interruption, with a constant input of raw materials and other nutrients and an attendant output of fermented material. The most recent approaches use micro-organisms that have been immobilized in a supporting structure. (See figure 18.) As the solution containing the raw material passes over the cells, the micro-organisms process the material and release the products into the solution flowing out of the fermenter.

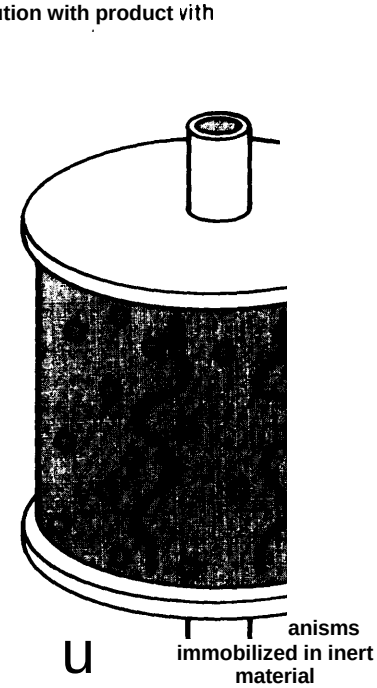
In general, products obtained by fermentation also can be produced by chemical synthesis, and to a lesser extent can be isolated by extraction from whole organs or organisms. A fermentation process is usually most competitive when the chemical process requires several

Figure 17.- Features of a Standard Fermenter



SOURCE: Eli Lilly & Co.

Figure 18.-immobilized Cell System

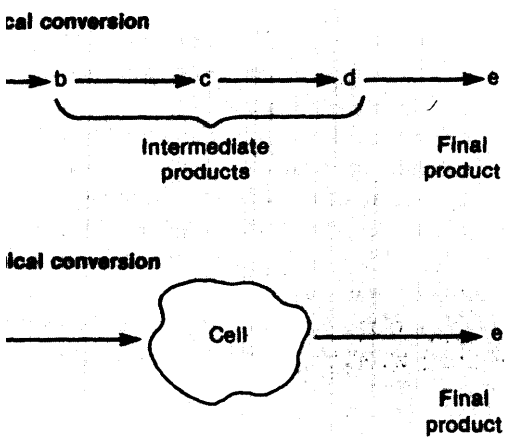


Typically, a solution of raw materials is pumped through a bed of immobilized micro-organisms which convert the materials to the desired product.

SOURCE: Office of Technology Assessment.

individual steps to complete the conversion. In a chemical synthesis, the raw material (shown in figure 19 as ***a)*** might have to be transformed to an intermediate b, which, in turn, might have to be converted to intermediates c and d before final conversion to the product e–each step necessitating the recovery of its products before the next conversion. In fermentation technology, all steps take place within those miniature chemical factories, the micro-organisms; the microbial chemist merely adds the raw material ***a*** and recovers the product e.

A wide variety of carbohydrate raw materials can be used in fermentation. These can be pure substances (sucrose or table sugar, glucose, or fructose) or complex mixtures still in their original form (cornstalks, potato mash, sugarcane, sugar beets, orcellulose). They can be of recent biological origin (biomass) or derived from fossil fuels (methane or oil). The availability of raw materials varies from country to country and even from region to region within a country; the economics of the production process varies accordingly.



Under the Chemical n of raw n of raw to fina to product e, intermediates d c, and e must be synthesized. Each intermediate must be recovered and purified before it can be used in the next step of the conversion.

A cell can perform the same conversion of *a* to e, but with the advantage that the chemist does not have to deal with the intermediates: the raw material *a* simply added and the final product e, recovered.

ce of Tec Office of Technology Assessment.

The cost of the raw material can contribute significantly to the cost of production. Usually, the most useful micro-organisms are those that consume readily available inexpensive raw materials. For large volume, low-priced products (such as commodity chemicals), the relationship between the cost of the raw material and the cost of the end product is significant. For low volume, high-priced products (such as certain pharmaceuticals), the relationship is negligible.

The process of enzyme technology

Although live yeast had been used for several thousand years in the production of fermented foods and beverages, it was not until 1878 that the active agents of the fermentation process were given the name "enzymes" (from the Greek, meaning "in yeast"). The inanimate nature of enzymes was demonstrated less than two decades later when it was shown that extracts from yeast cells could effect the conversion of glucose to ethanol. Finally, their actual chemical nature was established in 1926 with the purification and crystallization of the enzyme urease.

Fermentation carried out by live cells provided the conceptual basis for designing fermentation processes based on isolated enzymes. A single enzyme situated within a living cell is needed to convert a raw material into a product. A lactose-fermenting organism, e.g., can be used to convert the sugar lactose, which is found in milk, to glucose (and galactose). But if the actual enzyme responsible for the conversion is identified, it can be extracted from the cell and used in place of a living cell. The purified enzyme carries out the same conversion as the cell, breaking down the raw material in the absence of any viable micro-organism. An enzyme that acts inside a cell to convert a raw material to a product can also do this outside of the cell.

Both batch and continuous methods are used in enzyme technology. However, in the batch method, the enzymes cannot be recovered eco-

nomically, and new enzymes must be added for each production cycle. Furthermore, the enzymes are difficult to separate from the end product and constitute a potential contaminant. Because enzymes used in the continuous method are reusable and tend not to be found in the product, the continuous method is the method of choice for most processes. Depending on the desired conversion, the immobilized micro-organisms of figure 18 could be replaced by an appropriate immobilized enzyme.

Although more than 2,000 enzymes have been discovered, fewer than 50 are currently of industrial importance. Nevertheless, two major features of enzymes make them so desirable: their specificity and their ability to operate under relatively mild conditions of temperature and pressure. (The most frequently used. enzymes are listed in table 2.)

Comparative advantages of fermentations using whole cells and isolated enzymes

At present, it is still uncertain whether the use of whole cells or isolated enzymes will be more useful in the long run. There are advantages and disadvantages to each. The role of genetic engineering in the future of the industry, however, will be partly determined by which method is chosen. With isolated enzymes, genetic manipulation can readily increase the supply of enzymes, while with whole organisms, a wide variety of manipulations is possible in constructing more productive strains.

Table 2.—Enzyme Products

Source/name	Commercially available before: 1900	1950	1980	Current production tons/yr
Animal				
Rennet	X			2
Trypsin		x		15
Pepsin		x		5
Plant				
Malt amylase	X			10,000
Papain		x		100
Microbial				
Koji	X			
Fungal protease	X			10
Bacillus protease		x		500
Amyloglucosidase			x	300
Fungal amylase	X			10
Bacterial amylase		x		300
Pectinase		x		10
Glucose isomerase			x	50
Microbial rennet			x	10

SOURCE: Office of Technology Assessment.

The relationship of genetics to fermentation

Applied genetics is intimately tied to fermentation technology, since finding a suitable species of micro-organism is usually the first step in developing a fermentation technique. Until recently, geneticists have had to search for an organism that already produced the needed product. However, through genetic manipulation a ***totally new capability*** can be engineered; micro-organisms can be made to produce substances beyond their natural capacities. The most striking successes have been in the pharmaceutical industry, where human genes have been transferred to bacteria to produce insulin, growth hormone, interferon, thymosin a-1, and somatostatin, (See ch. 4.)

In general, once a species is found, conventional methods have been used to induce mutations that can produce even more of the desired compound. The geneticist searches from among hundreds of mutants for the one micro-organism that produces most efficiently. Most of the many methods at the microbiologist's disposal involve trial-and-error. Newer genetic tech. nologies, such as the use of recombinant DNA (rDNA), allow approaches in which useful genet ic traits can be inserted directly into the micro organism.

The current industrial approach to fermenta tion technologies therefore considers two prob lems: First, whether a biological process car produce a particular product; and second, wha micro-organism has the greatest potential for production and how the desired characteristic; can be engineered for it. Finding the desired micro-organism and improving its capability i so fundamental to the fermentation industry that geneticists have become important mem bers of fermentation research teams.

Genetic engineering can increase an organism's productive capability (a change that can make a process economically competitive); but it can also be used to construct strains with characteristics other than higher productivity. Properties such as objectionable color, odor, or slime can be removed. The formation of spores that could lead to airborne spread of the micro-organism can be suppressed. The formation of harmful byproducts can be eliminated or reduced. Other properties, such as resistance to bacterial viruses and increased genetic stability, can be given to micro-organisms that lack them.

Applying recent genetic engineering techniques to the production of industrially valuable enzymes may also prove useful in the future. For example, a strain of micro-organism that carries the genes for a desired enzyme may be pathogenic. If the genes that express (produce) the enzyme can be transferred to an innocuous micro-organism, the enzyme can be produced safely.

CURRENT TECHNICAL LIMITS ON GENETIC ENGINEERING

Despite the many genetic manipulations that are theoretically possible, there are several notable technical limitations:

- Genetic maps—the identification of the location of desired genes on various chromosomes have not been constructed for most industrially useful micro-organisms.
- Genetic systems for industrially useful micro-organisms, such as the availability of useful vectors, are at an early stage of development.
- Physiological pathways—the sequence of enzymatic steps leading from a raw material to the desired product, are not known for many chemicals. Much basic research will be necessary to identify all the steps. The ***number*** of genes necessary for the conversion is a major limitation. Currently, rDNA is most useful when only a ***single*** easily identifiable gene is needed. It is more difficult to use when several genes must be transferred. Finally, the problems are formidable, if not impossible, when the genes have not yet been identified. This is the case with many traits of agronomic importance, such as plant height.
  Even if the genes are identified and successfully transferred, methods must be developed to ***recognize*** the bacteria that received them. Therefore, the need to develop appropriate selection methods has impeded the application of molecular genetics.

As a consequence of these limitations, genetic engineering will be applied to the development of capabilities that require the transfer of only one or a few identified genes.

Fermentation and industry

Genetic engineering is not in itself an industry, but a technology used at the laboratory level. It allows the researcher to alter the hereditary apparatus of a living cell so that the cell can produce more or different chemicals, or perform completely new functions. The altered cell, or more appropriately the population of altered identical cells is, in turn, used in industrial production. It is within this framework that the impacts of applied genetics in the various industries is examined.

Regardless of the industry, the same three criteria must be met before genetic technologies can become commercially feasible. These criteria represent major constraints that industry must overcome before genetic engineering can play a part in bringing a product to market. They include the need for:

1. a useful biochemical product;
2. a useful biological fermentation approach to commercial production; and
3. a useful genetic approach to increase the efficiency of production.

The three criteria interrelate and can be met in any order; the demonstration of usefulness can begin with any of the three. Insulin, e.g., was first found to have value in therapy; fermentation was then shown to be useful in its production; and, now genetic engineering promises to make the fermentation process economically competitive. In contrast, the value of thymosin **a-1,** has not yet been proved, although

the usefulness of genetic engineering and fermentation in its production have been demonstrated.

As these examples indicate, the limits on a product's commercial potential vary with the product. In some cases, the usefulness of the product has already been shown, and the usefulness of genetic technologies must be proved. In others, the genetic technologies make production at the industrial level possible, but their market has not yet been established. In still others, the feasibility of fermentation is the major problem.