Biotechnology has been around for a long time, as this example of winemaking in ancient Egypt indicates. (Illustration reprinted from *Chemical Technology*, edited by F. A. Henglein. Pergamon Press, 1969.)

Biotechnology for Fun and Profit

by JAMES E. BAILEY

Biotechnology will be defined here as the application of chemical processes that use either biological catalysts or biological raw materials and that occur in vats or tanks of man's design. We can illustrate each component of this definition with the example of home winemaking, in which the biological raw material is sugar and the biological catalyst is yeast. As the fermentation occurs, alcohol is formed as the major product, along with carbon dioxide and more yeast cells.

This way of making alcohol dates to ancient times. The Babylonians, for example, were brewing beer as early as 7000 B.C. The original manufacture of wine and beer was done on a small scale, but it did not prove too difficult to make fermented alcohol in large casks and vats and probably our ancestors were thankful for that.

The situation was much different in another very old process of biotechnology — vinegar manufacture. Here early practitioners were not successful in translating the small-scale process (carried out in shallow wooden tubs) into big tanks in order to make large batches of vinegar. Why not? The basic difference is in the chemical reactions involved. In vinegar manufacture, microorganisms grow on the surface of a liquid where they convert alcohol into vinegar. In the process, they require oxygen, and they generate heat. That reaction causes no problems in shallow vats because the oxygen comes from the air over the liquid, and the cells grow as a film on the liquid surface. Any heat that is released is easily dissipated into the atmosphere. Productivity is not increased in a deeper tank because everything occurs on the surface.

An ingenious invention was developed in the early 19th century, however, in order to "scale-up" the process. A large chamber was filled with wood shavings, on the surfaces of which the microorganisms could grow and convert ethanol (alcohol) into vinegar. In this case, the reaction occurs throughout the system by creating on each wood shaving exactly the conditions that are necessary for the microorganisms to carry out the reaction. The heat generated from the reaction in the pile of shavings causes air to flow by natural convection through the bed of shavings,

From *Industrial Microbiology*, 1968, L. E. Casida Jr. John Wiley & Sons.

Early vinegar manufacturing processes used shallow vats; the chemical reactions involved in vinegar production required large open surfaces for oxygen supply and release of heat.
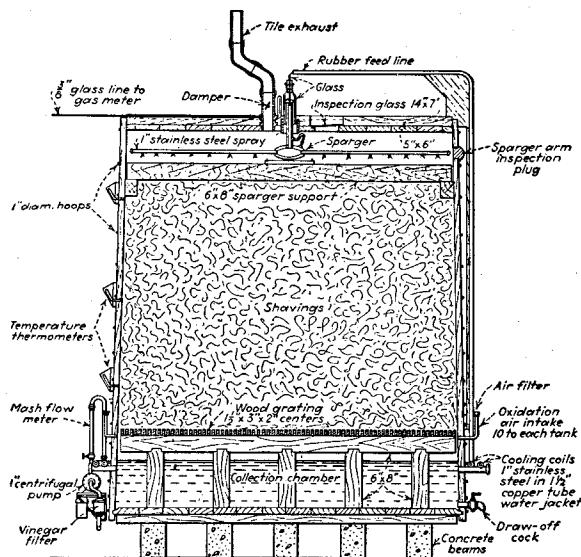
The 19th-century solution to large-scale vinegar manufacture made use of wood shavings to provide surfaces for the microorganisms to convert alcohol to vinegar. Heat generated by the reaction causes air to flow through the chamber by natural convection, simultaneously cooling it and supplying oxygen.

cooling it and supplying fresh air to the bugs.

Something else about this process illustrates an important engineering concept. In the early vinegar process and the making of wine, things are done in batches, but that isn't a very efficient way to manufacture products in quantity. Whenever a vat sits empty and idle, capital investment is being wasted. In addition, someone must be paid to fill the vats, and, when the batch operation is over, further labor is necessary to empty the vats, clean them, and start the process again. It is much cheaper to try to keep the desired chemical conversion processes operating continuously. In the 19th-century vinegar-making procedure, alcohol is sprayed continuously through nozzles on the top and trickles through the wood shavings to produce a continuous flow of vinegar at the bottom.

Conducting these early processes was really an art rather than engineering. In fact, the participation of microorganisms in catalyzing these chemical changes wasn't realized until Pasteur's work in the mid-19th century. It was Alexander Fleming's discovery of penicillin in 1928 that eventually led to the development of a large-scale industrial process. This story has historical interest and also provides an opportunity to discuss several engineering ideas.

Although penicillin was discovered in the late 1920s, it wasn't until the fire bombing of London in early World War II that it was recognized — on a crisis basis — that there was a need to find better treatments for burn victims. At that point intensive efforts were launched to find a way to make large amounts of penicillin. The vessel for the original discovery of penicillin was a petri dish, and it's clear that such a ''reactor'' is of virtually no value in making practically useful quantities of penicillin. By the early

1940s, the largest available reactor produced about twice as much penicillin in a single batch — in a bottle or flask. There were two ways of growing the mold on this scale: on solid bran or under water.

The mold grew readily on bran and produced penicillin, but that process couldn't be scaled up because in a large mass of bran the amount of heat released by the oxidation reaction kills the mold. If the mold is grown under water, controlling temperature is much easier, but there are problems in supplying oxygen to the mold. This consideration was already mentioned for the vinegar process, but there's an important difference between growing penicillin mold and growing the organism that makes vinegar. When vinegar is produced, if any stray contaminating microorganisms happen to find their way into the process, they won't grow well in the alcohol and acid environment. Thus contamination is not a serious problem for vinegar manufacture, and that process can be operated in the open air. On the other hand, the liquid solution that is used to grow penicillin is a very good nutrient for many natural organisms, and consequently they must be prevented from entering the penicillin culture. In the early 1940s, cotton stoppers were used to prevent contamination of flask cultures, but no one knew how to provide a large amount of air to a large volume of growing microorganisms while at the same time keeping the air sterile.

At first, in the absence of any engineering knowledge as to how to make penicillin in bigger batches, a brute force approach was adopted — the *Penicillium* mold was grown in many small bottles. To treat one person with penicillin then required the output of about 40 bottles. But penicillin was so urgently needed that in the space of about a year all of the engineering problems were solved, and the transition was made to growing the mold in agitated vessels holding about 10,000 gallons. By 1944 it was possible to make in one of these tanks enough penicillin to treat 2,500 people, and similar vessel designs remain in many respects the workhorses of today's pharmaceutical industry.

At first it took the contents of 40 of these bottles to treat one person with penicillin.
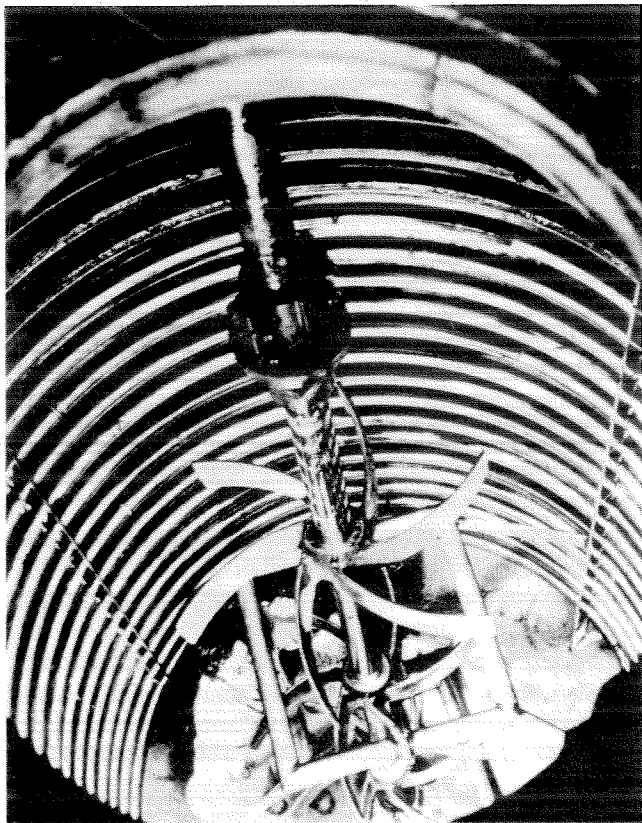
By 1944 the engineering problems had been solved, and enough penicillin to treat 2,500 people could be made in one of these 10,000-gallon tanks.

Biochemical engineering was really born with the development of this large-scale penicillin process. Since then, the attention of biochemical engineers has gone beyond physical problems such as aeration and mixing to problems involving the chemical reactions that occur in biological systems and the biological catalysts involved.

The elementary unit of catalysis in biological systems is the enzyme. Enzymes are protein molecules that are synthesized by living cells, but they don't grow or reproduce by themselves. There are many different kinds of enzymes, and they are necessary for life processes.

In biochemical engineering, enzymes are now applied in many ways which relate to the specificity of their catalytic action. If we want to decompose starch molecules to form simple sugars, for instance, a catalyst is needed that works on many different sizes of starch molecules — the enzyme glucoamylase has this property. If we want to remove stains from fabric, it's desirable that the enzyme not care whether the stain is chicken or beef, and several protein-splitting, or protease, enzymes will serve this purpose.

On the other hand, most enzymes are extremely selective, and this is one reason why there is now great impetus in industry to expand application of enzyme catalysts. One example of the use of an extremely selective enzyme is analysis of blood sugar. In the presence of the very com-plex mixture of different sugars, proteins, and fats in a blood sample, this enzyme will carefully and fastidiously select only the glucose, causing it to form, for one thing, hydrogen peroxide. The amount of hydrogen peroxide liberated can be determined, thus providing an accurate measurement of the glucose concentration in the blood. Other kinds of selective enzymes can be used to cut DNA at specific points.

It should be obvious, since enzymes are necessary for all life processes, that they work well at about one atmosphere pressure and at, say, 20 degrees C. Very active catalysis at these temperatures and pressures is of great interest in the chemical industry because most processes there are done at high temperatures and pressures, which require complicated equipment and great amounts of energy. Since energy is becoming increasingly scarce and precious, major efforts are now in progress to discover enzymes that can conduct on a more energy-efficient basis some of the reactions important in the chemical industry.

Until about 1960, all of the applications using enzymes were started by mixing together enzymes and reacting molecules such as starch. At the end of the reaction, the product — here sugar solution — was harvested and used, perhaps by pumping it into a fermentation tank to make alcohol. A major problem with this approach is loss of the enzymes with the product, an often uneconomical situation also sometimes causing undesirable contamination of the end product. We now know that the enzymes can be attached to solids or trapped in a cage of solid material or fiber.

This development actually had its origin in bionics, which means "the study of systems that either mimic or carry out functions similar to those found in biological and natural systems." Since many enzymes are attached to surfaces in the cell interior rather than floating around loose inside, biochemists wanted to study these enzymes in an attached state and therefore invented enzyme immobilization in the early 1960s. Study of the preparation of enzyme catalysts and the influence that binding to a solid surface has on them is one area of my research.

One recent discovery in my laboratory pertains to the glucose oxidation reaction mentioned earlier. The hydrogen peroxide produced quickly deactivates the enzyme involved, limiting the useful lifetime of the enzyme. By chemical coupling of the enzyme to activated carbon, which itself is an effective catalyst for hydrogen peroxide decomposition, a multifunctional solid surface-enzyme catalyst is obtained in which the hydrogen peroxide enzyme poison is converted to harmless compounds as it is produced. This strategy should prove useful in enzymatic processes for manufacturing fructose and gluconic acid, important in the food and pharmaceutical industries.

As an example of an immobilized enzyme reactor, consider a tube filled with solid particles in which enzymes are trapped. These catalyst particles are held in place by screens on either end of the tube. With the enzymes im-

NATURAL
PENICILLIN

IMMOBILIZED ENZYME

REACT

SEMISYNTHETIC
PENICILLIN
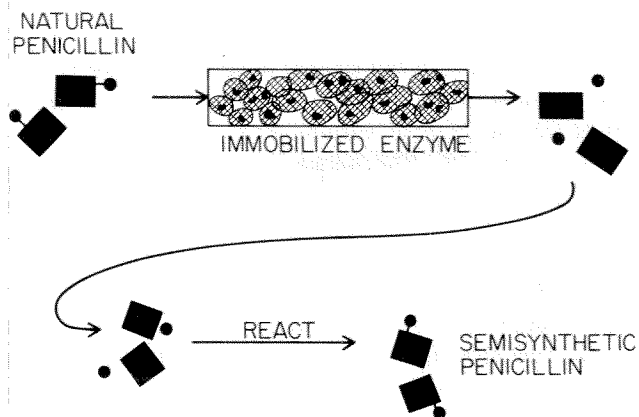
Immobilized enzymes — trapped inside a tube so a continuous process can use them over and over — can selectively remove side groups from the core of natural penicillin. Substituted artificial side groups create a semisynthetic penicillin with more desirable properties.

mobilized in this way, we can use the enzymes and carry out the desired reaction continuously as the reaction mixture flows through the tube. One application of this technology is found in the manufacture of semisynthetic penicillin. The natural penicillin molecule can be viewed as having two parts: a core and a side group. An enzyme is known that will selectively remove the side group without disturbing the core. If we pass natural penicillin through a reactor containing this immobilized enzyme, we can obtain the core, which, after separation from the cleaved natural side group, can be reacted with a new and artificial side chain to produce a semisynthetic penicillin.

These semisynthetic penicillins have some interesting properties relative to natural penicillin. Some are absorbed more easily through the intestine than many natural penicillins, which means they can be taken orally rather than by injection. Several semisynthetic penicillins tend to cause fewer allergic reactions than do their natural counterparts, and, by varying the synthetic side group, the drug becomes lethal to certain bacteria which are resistant to natural penicillins.

A much more complicated subject is the catalysis of desirable reactions in the living cell. The living cell is truly an amazing chemical reactor, and we have only begun to learn how to use it to make useful and valuable products. In this discussion, cells may be viewed as self-contained chemical factories with many assembly lines which loop and branch in many directions. Each machine in one of these assembly lines is a particular enzyme, so the cell synthesizes a different enzyme for each of many different chemical reactions.

To use the chemical factory for our purposes, its major control systems must be considered. First of all, the cell's DNA contains all of the blueprints to make the enzyme machines and other important cell components. Based on the environment in which the cell finds itself, appropriate segments of the DNA instructions are turned on or off. In a solution that is rich in the amino acid histidine, for example, a bacterial cell will not waste energetic and

chemical resources to make the enzymes needed for histidine synthesis. If that assembly line isn't needed, it isn't produced.

Besides acting on the DNA to determine which enzymes are made, the environment also influences the enzymes to determine the rates at which the various assembly lines work. A major objective in biotechnology is to learn how to manipulate the factory for particular purposes. There are two basic kinds of such manipulation: One is regulation of the environment, and the other is changing the blueprint, changing the DNA.

A recent strategy for maximizing production of food yeast illustrates an engineering contribution. Yeast is sensitive to the amount of sugar in its environment. At high sugar concentrations the yeast makes alcohol and carbon dioxide. However, if producing yeast cells is the objective, sugar shouldn't be wasted to form alcohol. This undesired result can be avoided by maintaining a sufficiently low sugar content in the yeast growth medium. Since no one has yet found a way to measure the sugar level or to keep track of how rapidly the yeast are growing inside large fermentation tanks, an indirect method for estimating these important process parameters has been devised. The fermenter exhaust gas carbon dioxide content is measured, and based upon that data, a computer controls the rate of sugar addition to the growing yeast culture. A high CO_2 level corresponds to too much sugar in the growth medium, so addition of sugar is stopped. When the CO_2 level falls, more sugar can be pumped in. My colleague Greg Stephanopoulos is extending this strategy, using the latest sensor technology and modern control and estimation theory, to improve process controllers for microbial reactors.

In considering the motivation for changing the DNA of an industrial microorganism, it is important to recognize that the control system of the cell has been designed over time by evolution to fulfill its own natural objective — the survival and growth of the organism. But often maximizing cell growth is not man's main purpose. In the case of making penicillin, our objective is not mold production but penicillin synthesis. To improve a microorganism's productivity, it's desirable to find ways to manipulate the cellular chemical factories to make them produce more of the desired compound. This has been done quite successfully in the case of penicillin by classical methods of industrial genetics. The original penicillin culture in 1941 produced about 4 units per tank. After a worldwide search to find the best penicillin producer in nature and then after some mutation programs, yields increased to around 1,000 units. Since then, by mutation and selection processes, the productivity has been improved to more than 10,000 units per tank.

These improvements were done by a very slow, arduous, and imprecise technique. The mold was exposed to radiation, such as ultraviolet light, or to chemicals that caused changes in the DNA blueprints of the mold. Which

16

part of the DNA would be changed in this fashion and what effect the change would have on penicillin productivity was unknown. Most of the mutated organisms produced by such treatment died. However, a small fraction of cells produced by this shotgun program was able to grow and to synthesize more penicillin. It's by this strategy that many genetic improvements in commercial microorganisms have been obtained — a sequence of carefully selected good luck.

Recently there has been a dramatic improvement in man's ability to manipulate DNA. Often called genetic engineering, recombinant DNA, or gene-splicing technology, these methods can instruct a bacterial cell to synthesize new products that aren't ordinarily found in microorganisms. For example, we can now put into microorganisms DNA molecules that will cause them to produce human proteins. This has tremendous potential. It means that drugs such as insulin and interferon can be produced in bacteria very rapidly, cheaply, and in relatively large quantities.

Interferons are proteins that have potent effects against many viruses. Until quite recently, the world's major source of interferons was the Finnish Red Cross, which extracted the protein from white blood cells. The total amount of interferons produced was minute — about 2/1000 of an ounce per year. Last year it was announced that the interferon blueprint had been moved into bacteria using recombinant DNA technology. Using the published yields that have already been achieved, one 10,000-liter vessel can produce six times the current world's production of interferon (assuming the same productivity on that scale as can now be obtained in the lab). Making that batch would take several days at most. It is therefore anticipated that the current interferon price of about $50 per million units will be reduced to pennies or even fractions of a cent thanks to gene-splicing technology.

Genetic engineering has many more potential applications. One which has been recently explored is a process designed to make animal feed. In this case the goal is to produce microbial cells, but to do this the original cell has to use some of its internal energy to assimilate nitrogen from the environment. Genetic engineering adds a missing enzyme and makes it possible to incorporate nitrogen without using any of the cell's energy. Presumably this energy can be redirected, used in other places, and the end result should be more cells for the amount of raw material used. At this point, the modified organism produces only about 4 percent more cell material as a result of the genetic modification, but the cell's chemical factory is very complicated and we have only begun to try to improve it with the methods of recombinant DNA. If we consider the cell as a factory with a collection of assembly lines, we have now managed to upgrade the loading dock. Improving the factory itself is something for the future.

Gasohol, which is of great public interest now, is a liquid motor fuel that is a mixture of 90 percent gasoline and 10 percent ethanol. In order to qualify for some current tax incentives, the ethanol must be made by a biological process using natural materials. Gasohol production has been granted special tax treatment because, according to its proponents, the use of domestic agricultural and waste materials to make fuel reduces the need for imported oil. Gasohol gives mileage comparable to that of gasoline, a slight increase in octane, and a little bit higher emissions.

The alcohol portion of gasohol is currently produced by treating corn physically to make small particles, which are subjected to decomposing enzymes to break down the starch to form sugars. The sugars are then fed to yeast — essentially the same process as in brewing and home winemaking — producing a solution which contains about 10 percent ethanol in water. The water then must be separated from the ethanol. The solid residue from this process — the yeast and the debris from corn — is good animal feed.

However, using corn to make alcohol is controversial, partly because of the net energy benefit. There are widely divergent opinions on this; according to a recent report by the Alcohol Fuels Policy Review, fermentation ethanol production yields only about 5 percent more energy than must be used in the ethanol manufacturing process using corn as a raw material. This is a major concern of gasohol opponents. Net useful energy yields can be enhanced by using low-grade fuels for the distillation separation of alcohol from water (the most energy-demanding part of the process), or by using other separation methods. Vigorous research on these problems is now in progress.

Another debate surrounding gasohol is the propriety of using corn that could be used for food to make a motor fuel. Without using any of the corn that currently goes into food, sufficient grain is available in the United States to produce 5 billion gallons a year of alcohol. That may sound like a tremendous amount, but it is a small fraction of the estimated 1990 gasoline consumption of over 100 billion gallons per year. Consequently, it is important to identify new raw materials for this process — perhaps cellulose. There are vast amounts of cellulose in agricultural and urban wastes and in plant matter. If we could find an economical way to break this cellulose down into sugar, it would yield significantly more alcohol and other useful products.

My research group is working to support and expand microbial reactor technology through several research projects which are investigating protein and DNA synthesis in individual microbial cells, immobilized cell physiology, and scale-up problems and opportunities for reactors using recombinant microorganisms. The major aim of this research is to improve the fundamental bases for microbial reactor design and operation by synthesizing the key biological and engineering factors into a quantitative process description. The research problems are fascinating, and the scope of application of their solutions is rapidly expanding. It is an exciting time for all involved with biotechnology. □