**Industrial Microbiology and Biotechnology**

*Never underestimate the power of the microbe.*
—Microbiologist Jackson W. Foster of the University of Texas

Humans probably discovered alcoholic beverages by accident. It is conceivable that sunlight warmed some sort of fallen grape or other fruit and accelerated the fermentation of its juices by yeasts. Humans must have sampled this “spoiled” fruit with curiosity, and if the taste of the aromatic concoction was not especially pleasing, the euphoric feeling that followed probably brought them back for more. By trial and error, humans discovered the important factors in fermentation and soon learned to control the process. In doing so, they became the first industrial microbiologists.

How fermentation occurs was worked out by Louis Pasteur when he studied the souring of French wines. With this understanding, the process of industrial fermentation, using the metabolic products of microorganisms for commercial purposes was developed. By the 1930s, several types of fermentations were being carried out to produce ethyl alcohol and butyl alcohol. For the British war effort, Chaim Weizmann's development of a microbial fermentation process for acetone using *Clostridium acetobutylicum* was critical toward the manufacture of gunpowder.

However, industrial microbiology is not restricted to alcohol fermentations. It includes bread baking and cheese production, as well as many of the foods resulting from fermentation. Industrial fermentation enhances the metabolic reactions already present in (or genetically engineered into) microbes. **Biocatalysis** is the term used to describe the metabolic reactions carried out at this industrial scale.

To produce a significantly useful amount of product from microorganisms, tremendously large numbers of microorganisms are needed. This means scaling up microbial metabolic processes to a level significant enough to produce...
the valuable commercial product (Figure 27.1A). On this large scale, the term fermentation has come to have a slightly different definition. Industrial fermentation procedures are not necessarily anaerobic processes; rather, they are large-scale microbial methods that may be aerobic or anaerobic.

Such biocatalytic processes are carried out in large containers or tanks called bioreactors or fermentors (Figure 27.1B). Technicians or scientists must continually monitor the growth conditions in these containers to ensure that microbes continue to either grow or reach a state in which maximal product is produced. Temperature, pH, oxygen, and nutrients—all of the typical physical and chemical conditions that apply to growing microbes in a culture tube—apply here, just on a grander scale.

The exploitation of microbes for use in industrial processes is closely linked to biotechnology. Thus, many of the products of industrial microbiology contribute to public health, interrupt the spread of disease, or improve the quality of life. Microbial cells or their products are used, for example, to produce enzymes, insecticides, food additives, protein hormones, antibiotics, monoclonal antibodies, interferon, and the myriad products of genetic engineering that are at the heart of the biotechnology industry today.

As we see in this chapter, industrial microbiology is an extremely diversified field, in which inexpensive raw materials are converted to valuable commodities through the metabolism of microorganisms.

Chapter Challenge

Many of the foods we eat are the result of microbial metabolism, especially fermentation. However, there are many other products and medicines that we use every day that are the result of microbial activity. Make a short list right now of those products and medicines that you can think of that you have used. As we progress through this chapter, check off the products on your list that are described and add to your list as we discover other products that you forgot about or did not know had a microbial origin.
Certain properties make microorganisms well suited for industrial processes. Microorganisms not only possess a broad variety of enzymes to make an array of chemical conversions possible, they also have a relatively high metabolic activity that allows conversions to take place rapidly. In addition, they have a large surface area for the quick absorption of nutrients and release of end products. Moreover, they usually multiply at a high rate, as evidenced by the 20-minute generation time for Escherichia coli under ideal conditions.

In the industrial process, microorganisms act like chemical factories. To be effective, they should liberate a large amount of a single product that can be extracted and purified efficiently. The organisms should be easy to maintain and cultivate, and should have genetic stability with infrequent mutations. Their value is enhanced if they can grow on an inexpensive, readily available medium that is a byproduct of other industrial processes. For example, a large amount of whey is produced in cheese manufacturing, and microorganisms that convert whey components to lactic acid add to the overall profit of the cheese industry. Figure 27.2 displays some possible conversions from a single metabolic substance.

**Microorganisms Produce Many Useful Organic Compounds**

Microorganisms are used in industry to produce a variety of organic compounds, including acids, growth stimulants, and enzymes. In some cases, the production results from a microorganism manufacturing many thousands of times the amount of product necessary for its own metabolism.

Microorganisms may produce one of two types of metabolites that are important to industrial microbiology. **Primary metabolites** are directly involved in the normal growth, development, and reproduction of the microbe. Pyruvate and the end products of the fermentation pathways shown in Figure 27.2 are examples of primary metabolites. **Secondary metabolites** form the bulk of products of industrial interest. These metabolites...
are not directly involved in growth and reproduction, but usually have important ecological functions and often are species specific. Secondary metabolites usually are produced near or at the end of the microbe’s growth phase; that is, in the stationary phase.

One of the first secondary metabolites to be made in bulk by industrial fermentation was citric acid. Manufacturers use this organic compound in soft drinks, candies, inks, engraving materials, and a variety of pharmaceuticals, such as anticoagulants and effervescent tablets (e.g., Alka-Seltzer®). The organism most widely used for citric acid production is the fungus *Aspergillus niger*. Microbiologists inoculate the mold into a medium of cornmeal, molasses, salts, and inorganic nitrogen in huge aerobic fermentors. The absence of a citric acid cycle enzyme in the fungus prevents the metabolism of citric acid (citrate) into the next component of the cycle, and the citric acid accumulates in the medium. \[\text{FIGURE 27.3}\] outlines this chemistry.

Another important microbial product is lactic acid, a compound employed to preserve foods, finish fabrics, prepare hides for leather, and dissolve lacquers. Lactic acid is commonly produced by bacterial activity on the whey portion of milk. *Lactobacillus bulgaricus* is widely used in the fermentation because it produces only lactic acid from lactose. Gluconic acid (gluconate), another valuable organic acid, is useful in medicine as a carrier for calcium, because gluconic acid is easily metabolized in the body, leaving a store of calcium for distri-

\[\text{Gluconic acid (gluconate)} \text{ is useful in medicine as a carrier for calcium, because gluconic acid is easily metabolized in the body, leaving a store of calcium for distribution.}\]

\[\text{FIGURE 27.3} \text{ The Chemistry of Citrate Production. Aspergillus niger is grown in a mixture of nutrients, where it digests glucose into pyruvate. The pyruvate then is converted to acetyl-CoA, which condenses with oxaloacetate in the citric acid cycle to yield citric acid or citrate. However, the chemistry goes no further, because the next enzyme in the cycle is absent. Citric acid therefore accumulates and is isolated for use in various products, as shown. }\]

\[\text{Because the rest of the citric acid cycle does not occur in this strain of Aspergillus, how does the organism make ATP?}\]
KEY CONCEPT 27.1 Microorganisms Are Used to Produce Many Industrial Products

...This acid is produced from carbohydrates by A. niger and by species of the bacterial genus *Glucobacter* cultivated in fermentors. Calcium gluconate also is added to the feed of laying hens to provide calcium to strengthen the eggshells.

When the amount of amino acid produced by a microorganism exceeds what the microbe needs, the remainder is excreted into the environment. Such is the case with glutamic acid (glutamate) produced by certain species of *Micrococcus*, *Arthrobacter*, and *Brevibacterium*. Glutamic acid is a valuable food supplement for humans and animals, and its sodium salt, monosodium glutamate, is used in food preparations.

In the industrial production of lysine, another amino acid, two organisms are involved. *E. coli* is first cultivated in a medium of glycerol, corn steep liquor, and other ingredients, resulting in the accumulation of diaminopimelic acid (DAP). Several days later, *Enterobacter aerogenes* is added to the fermentor. This organism produces an enzyme that removes the carboxyl group from DAP to produce the lysine used in breads, breakfast cereals, and other foods.

Two important vitamins, riboflavin (vitamin B2) and cyanocobalamin (vitamin B12), also are products of microbial growth. Riboflavin is a product of *Asbya gossypii*, a mold able to produce 20,000 times the amount of riboflavin it needs for metabolism. Cyanocobalamin is produced by selected species of *Pseudomonas*, *Propionibacterium*, and *Streptomyces* grown in a cobalt-supplemented medium. The vitamin prevents pernicious anemia in humans and is used in bread, flour, cereal products, and animal feeds.

**Microorganisms Also Produce Important Enzymes and Other Products**

The production of microbial enzymes for commercial exploitation has been an important industry since the emergence of industrial microbiology. Currently, over two dozen types of microbial enzymes are in use, and several others are in the research or developmental stage. Industrial enzymes have reached an annual market of $1.6 billion.

Among the important microbial enzymes used commercially are amylase, pectinase, and several proteases (TABLE 27.1). Amylase is produced by the mold *Aspergillus oryzae*. It is used as a spot remover in laundry presoaks, as an adhesive, and in baking, where it digests starch to glucose. Pectinase, a product of a *Clostridium* species, is employed to ret flax for linen. In this process, manufacturers mix the flax plant with pectinase to decompose the pectin “cement” holding cellulose fibers together. The cellulose fibers then are spun into linen. **MicroFocus 27.1** describes a more traditional process for retting. Pectinase also is used to clarify fruit juices.

**TABLE 27.1 Some Enzymes and Their Microbial Sources Used in Commercial Markets**

<table>
<thead>
<tr>
<th>Commercial Market</th>
<th>Enzyme</th>
<th>Source</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>Proteases</td>
<td>Bacteria and molds</td>
<td>Hydrolysis of whey proteins; coagulant in cheese production</td>
</tr>
<tr>
<td></td>
<td>Lactase</td>
<td>Mold and yeasts</td>
<td>Hydrolysis of lactose to produce lactose-free milk</td>
</tr>
<tr>
<td>Brewing</td>
<td>Cellulases</td>
<td>Molds</td>
<td>Liquefaction and clarification processes</td>
</tr>
<tr>
<td></td>
<td>Amylases and proteases</td>
<td>Bacteria and molds</td>
<td></td>
</tr>
<tr>
<td>Wine and juices</td>
<td>Pectinases</td>
<td>Bacteria and molds</td>
<td>Increase juice yield and clarification</td>
</tr>
<tr>
<td>Meat</td>
<td>Proteases</td>
<td>Bacteria and molds</td>
<td>Meat tenderizing</td>
</tr>
<tr>
<td>Confectionary</td>
<td>Invertase</td>
<td>Yeasts</td>
<td>Liquefaction of sucrose</td>
</tr>
<tr>
<td>Textiles</td>
<td>Pectinase</td>
<td>Bacteria and molds</td>
<td>Flax retting</td>
</tr>
<tr>
<td>Pulp and paper</td>
<td>Xylanases, hemicellulases, lipases</td>
<td>Molds and yeasts</td>
<td>Cleaner and more efficient pulp and paper processing</td>
</tr>
<tr>
<td>Detergents</td>
<td>Proteases, amylases, lipases, cellulases</td>
<td>Bacteria and molds</td>
<td>Better cleansing of laundry and dishes</td>
</tr>
</tbody>
</table>
Proteases are a group of protein-digesting enzymes produced by Bacillus subtilis, A. oryzae, and other microorganisms. Certain proteases are used for bating hides in leather manufacturing, a process in which organic tissue is removed from the skin to yield a finer texture and grain. Other proteases find value as liquid glues, laundry pre-soaks, meat tenderizers, drain openers, and spot removers.

One of the most appreciated but lesser known uses of a microbial enzyme is in making soft-centered chocolates. Invertase, an enzyme from yeast, is mixed with flavoring agents and solid sucrose, and then covered with chocolate. The enzyme converts some of the sucrose to liquid glucose and fructose, forming the soft center of the chocolate.

In medical microbiology, doctors use another microbial enzyme, streptokinase, to break down blood clots formed during a heart attack. Still another enzyme, hyaluronidase, is used to facilitate the absorption of fluids injected under the skin. The microbial roles for these enzymes as virulence factors are discussed in another chapter.

Although most natural food flavoring ingredients are produced by traditional processes using plants, new biotechnology methods have made it possible to produce novel flavoring ingredients by converting relatively cheap starting materials into higher-value flavor and aroma additives. The latter are used in foods, beverages, cosmetics, and other consumer items; examples include fruit, peach, and coconut flavoring agents called lactones. Although lactones can be generated from long-chain fatty acids derived from sweet potatoes, the fatty acids occur in too limited a quantity and are expensive to modify. To circumvent this problem, microbiologists use species of Mucor and other fungi to convert medium-chain fatty acids to compounds other microorganisms can easily transform to lactones. Another example is the methylketones, which confer strong cheese-associated flavors in dairy products. The flavoring agents are derived industrially from Penicillium roqueforti incubated in lipase-treated milk fats.

In addition to the major products we have surveyed, microorganisms provide a number of specialized materials. Typical of the miscellaneous microbial products is alginate, a sticky substance used as a thickener in ice cream, soups, and other foods. Other products of microbial origin are some perfumes. Musk oil, for example, is prepared from ustilagic acid, a product of the mold Ustilago zeae, which, ironically, causes smut disease (see Chapter 11). Moreover, there are numerous pharmaceutical products derived from the ergot poisons of the mold Claviceps purpurea. These derivatives are prescribed to induce labor, treat menstrual disorders, and control migraine headaches.

**Concept and Reasoning Check 1**

a. Assess the importance of microbial secondary metabolites to industry and commercial markets.

b. Identify some of the enzymes for industry that are produced by microorganisms.
The fermentation of beer, wine, and other alcoholic beverages is one of the most venerable and universal of human domestic activities. The origin of beer fermentation, for example, has been traced as far back as 4000 B.C., when legend tells us that Osiris, the god of agriculture, taught Egyptians the art of brewing once they had learned how to farm the land (Figure 27.4). Wine production apparently has an equally long history because archaeologists have discovered evidence of grape cultivation in the Nile Valley during the same period. Furthermore, scientists have found evidence of wine in jars excavated from an Iranian site 7,000 years old. Thus, it is conceivable that the Egyptians, Sumerians, Assyrians, and other Near East peoples were among the earliest consumers, if not connoisseurs, of alcoholic beverages.

**Beer Is Produced by the Fermentation of Malted Barley**

As early as 3400 B.C., a tax was placed on beer in the ancient Egyptian city of Memphis on the Nile River. The Greeks later brought the art of brewing to Western Europe, and the Romans refined it. Indeed, the main drink of Caesar’s legions was beer. During the Middle Ages, monasteries were the centers of brewing, and by the 1200s, breweries and taverns were commonplace in Great Britain.

The word beer is derived from the Anglo-Saxon baere, meaning “barley.” Thus, beer is traditionally a product of yeast fermentations of barley grains. However, yeasts cannot digest barley starch, and therefore, it must be predigested for them to work. The malt is ground with water to achieve further digestion of the starch. This process, called mashing, often includes corn as a starch supplement. Brewers then remove the liquid portion, or wort, and boil it to inactivate the enzymes. Dried petals of the vine Humulus lupulus, commonly called hops, are added to the wort, giving it flavor, color, and stability. Hops also prevent contamination of the wort, because the leaves contain at least two antimicrobial substances. At this point, the fluid is filtered, and yeast is added in large quantities.

The yeast usually employed in beer fermentation is one of two species of Saccharomyces developed for centuries by brewers. One species, S. cerevisiae, gives a uniform dark cloudiness to beer and is carried to the top of the fermentation vat by foaming carbon dioxide. This yeast, therefore, is called a “top yeast.” It is used primarily in English-type brews such as ale and stout. The second species, S. carlsbergensis, ferments the malt more slowly and produces a lighter, clearer beer, having less alcohol. This yeast sediments and is therefore, called a “bottom yeast.” Its product is pilsner or lager beer. Almost three-quarters of the world’s beer is lager beer.

A normal fermentation requires approximately 7 days in a fermentation tank. The young beer
Beer, called hqt by the ancient Egyptians, was a very important drink. They often used beer in religious ceremonies and, because water could be a source of illness, they served beer at mealtimes to both adults and children. In fact, archaeologists have discovered that workers at the great pyramids had five types of beer that they drank three times a day. It was the staple drink of the poor (wages often were paid in beer), a drink of the pharaohs, and a drink offered to the gods.

Because of the prevalence of beer in Egyptian life, many Egyptologists have studied beer residue from ancient Egyptian vessels. The traditional view held that their beer was made by crumbling lightly baked well-leavened bread into water. After straining through a vat, the water was allowed to ferment because of the yeast from the bread. It then was flavored with date juice or honey.

In 1996, these traditional views were challenged. Delwen Samuel, an archaeobotanist at the University of Cambridge, examined just what cereals and grains the Egyptians used to brew beer by looking at 2,000-year-old beer residues using a scanning electron microscope. Her findings suggested that the ancient Egyptians used barley to make malt and a type of wheat, called emmer, instead of hops. She says they heated the mixture and then added yeast and uncooked malt to the cooked malt. After adding the second batch of malt, the mixture was allowed to ferment. In her analysis, Samuel says she found no traces of flavorings.

Samuel and her colleagues tried brewing the beer using the recipe derived by the analysis. They brewed it at a modern brewery and found the beer to be fruity and sweet because it lacked the bitterness of hops. The beer was reported to have an alcoholic content of between 5% and 6%. Samuel gave her recipe to Scottish and Newcastle Breweries in London that then bottled a limited edition, 1,000-bottle batch of “King Tut Ale.” It was sold at Harrods department store for $100 per bottle, the proceeds going toward further research into Egyptian beer making.

In 2002, a major Japanese brewery claimed to have recreated a 4,000-year-old Egyptian beer by following a recipe from ancient hieroglyphs in Egyptian tomb paintings. Kirin Brewery Company Ltd., Japan’s second-largest beer maker, said the 30 liters of brewed beer was dark brown, contained no froth, had a strong sour taste, and an alcohol content of about 10%. The company does not plan to sell the beer commercially—it was developed for research purposes only!

then is transferred to vats for secondary aging, or lagering, which may take an additional 6 months. If the beer is intended for canning or bottling, it is pasteurized at 60°C for 55 minutes to kill the yeasts, or filtered through a membrane filter. Some yeast is allowed to seed new wort, and the remainder may be dried for animal feed or pressed to tablets for human consumption. The alcoholic content of beer is approximately 4%.

**Wine Is Produced by the Fermentation of Fruit or Plant Extracts**

During the Middle Ages and the centuries thereafter, wine was called *aqua vitae*, the “water of life.” The title was appropriate because wine was one of the few safe things to drink. Indeed, until the late 1800s, safe drinking water was virtually nonexistent in the Western world (note that the Bible makes no references to water for drinking purposes). Wine, by contrast, was generally free of pathogens due to its acidity and alcohol content, and it provided a few minerals and vitamins to the diet, while serving as a pain reliever. Of course, it also bred a society somewhat inebriated much of the time.

Essentially, all wines are derived from the natural conversion of grape or other fruit sugars to ethyl alcohol by fermentation with *Saccharomyces*. Wild yeasts, which occur naturally on the grapes, can produce undesirable qualities in a wine, so they are killed by adding sulfur dioxide (“sulfites”) during the production process.

Wine may be made from fruit, fruit juice, or plant extracts such as dandelions. Among the grapes, the species *Vitis vinifera* is recognized as the highest-quality fruit. The winemaking process begins with crushing to produce the juice, or **must** (Figure 27.5). For red wine, black grapes are used, including skins and sometimes, the stems. White wine, by contrast, is made from black or white
Figure 27.4 A Generalized Process for Producing Beer. Barley grains are held in malting tanks while the seeds germinate to yield fermentable sugars. The digested grain, or malt, is then mashed in a mashing tank and the fluid portion, the wort, is removed. Hops are added to the wort in the next step, followed by yeast growth and alcohol production during fermentation. The young beer is aged in primary and secondary aging tanks. When it is ready for consumption, it is transferred to kegs, bottles, or cans. Why must beer be pasteurized or filtered before packaging?
grapes without their skins or stems. Cultured yeasts (S. ellipsoideus) are added to begin the alcoholic fermentation process. Anaerobic conditions soon are established as carbon dioxide evolves and takes up all the air space within the vats or steel tanks.

Alcohol production requires only a few days, but the aging process in wooden casks may go on for weeks or months. During this time wine develops its unique flavor, aroma, and bouquet. These result from the array of alcohols, acids, aldehydes, and other organic compounds produced by the yeast during aging. In fact, wine contains thousands of compounds, a few of which have been identified (MicroFocus 27.3). Soil and climate conditions (the terroir, as it is called in French) also contribute to the wine because they determine what organic compounds are present in the grape. The type of yeast and nature of wood derivatives from the fermentation casks are other determining factors. Thus, there are “vintage years” and poor years. For mass production, wine is pasteurized to increase its shelf life, filtered, and bottled.

The broad variety of available wines results from modifications of the basic fermentation process. In dry wines, for example, most or all of the sugar is metabolized, while in sweet wines, fermentation is stopped while there is residual sugar. Sparkling wines, including champagne, sparkle because of a second fermentation taking place inside the bottle. For a sweet sauterne, vintners enhance the sugar content of grapes by a controlled infection with the mold Botrytis cinerea. The mold literally sucks water out of the grapes, thereby increasing the sugar concentration.

The strongest natural wines measure about 15% alcohol because yeasts cannot tolerate alcohol much above this level. Most table wines average about 10% to 12% alcohol, with fortified wines reaching 22% alcohol. In fortified wines, brandy or other spirits are added to produce such wines as port, sherry, and Madeira.
In 1992, scientists from the Bordeaux region of France did a population and epidemiological study and noted that while French and other Mediterranean peoples ate large amounts of fatty foods, they suffered a relatively low incidence of coronary artery disease. A 1996 report by the television news magazine program 60 Minutes also pointed out that fatty meats, creams, butters, and sauces had little apparent effect on French hearts. This so-called “French Paradox” was due, in part, to the apparent medicinal properties of wine, especially drinking red wine. So, is red wine good for your health?

In 1996, researchers identified phenol-based compounds in red wine that inhibited the oxidation of low-density lipoproteins (LDLs) in the blood, and by doing so, prevented the buildup of cholesterol and blood platelets in the arteries. The scientists pointed out that red wine contains more phenolics than white wine, and far more than beer. The phenolics also were present in other foods (e.g., raisins and onions), but not in the quantity found in the skins of grapes used for red wine, which concentrate even more after fermentation has taken place.

A compound known as resveratrol, which belongs to the family of phenols, naturally exists in grapes and red wine. This compound was shown to extend the lifespan of yeast cells by up to 80% and might help explain why moderate consumption of red wine has been linked to a lower incidence of heart disease in humans and cancer in experimental mice.

Red wine and the resveratrol it contains also has an inhibitory effect on the foodborne pathogens Escherichia coli, Salmonella enterica, and Listeria monocytogenes. Helicobacter pylori, which is the main cause of stomach ulcers, is especially susceptible.

However, red wine is not for everyone. Indeed, alcohol should be avoided by pregnant women, people taking medications, those under the legal drinking age, and anyone with a family history of alcoholism. For these and for anyone else wishing to stay away from alcohol, alcohol-free red wines are appearing in the marketplace. They offer the opportunity to take advantage of a natural health ingredient while enjoying a glass of nature’s bounty. Perhaps Louis Pasteur was correct when he said, “Wine is the most healthful and hygienic of beverages.”

**Distilled Spirits Contain More Alcohol than Beer or Wine**

Distilled spirits are alcoholic beverages containing ethyl alcohol resulting from distillation of a fermented substance, such as fruits or grains. Gin, vodka, tequila, and whiskey are a few types of spirits. Such spirits contain considerably more alcohol than beer or wine. Most of the world measures the alcohol content by its percentage of the total liquid; that is, by volume. However, in the United States, distilled beverages are designated with a “proof number,” which is twice the percentage of the alcohol content. For example, a 90 proof product contains 45% alcohol.

The production of distilled spirits begins like a wine fermentation. A raw product is fermented by Saccharomyces species, then aged, and finally matured in casks. At this point, the process diverges as manufacturers concentrate the alcohol by a distillation apparatus using heat and vacuum pressure. Next, they mature the product in wooden casks to introduce unique flavors from various chemicals, such as aldehydes and volatile acids. Finally, the alcohol is standardized by diluting it with water before bottling.

Four basic types of distilled spirits are produced: brandy, whiskey, rum, and neutral spirits. Brandy is made from fruit or fruit juice, while rum is produced from molasses. Whiskey is a product of various malted cereal grains, such as scotch from barley, rye from rye grain, and bourbon from corn. The final type, neutral spirits, includes vodka, made from potato starch and left unflavored; gin, flavored with the oils of juniper berries; and tequila, containing the fermented juice of the blue agave.

**Concept and Reasoning Checks 2**

a. How does a lager differ from an ale, microbiologically speaking?
b. Explain why the alcohol produced in wine represents a primary metabolite.
c. Summarize how distilled spirit production produces a higher alcohol content than beer or wine.
Chapter Challenge

You may or may not be a wine or beer drinker, or partake of distilled spirits. Yet all are the product of microbial action and alcohol fermentation. So, if you wish you can add these to your list if you haven’t already.

QUESTION B: Try to wrap your head around the reality that, partaking in alcoholic beverages or not, these fermentation products (and distilled products) of microbial origin have a large influence on human behavior, both financially and societally. List some of the impacts they have daily.

Answers can be found on the Student Companion Website in Appendix D.

KEY CONCEPT 27.3 Microorganisms Also Produce Many Other Valuable Commercial Products

In addition to the products we have discussed, microorganisms are the sources of antibiotics and a number of valuable insecticides. Moreover, they are the producers of enzymes that break down natural and synthetic wastes in bioremediation, and they are the biological factories for the genetic engineering technology revolutionizing the biotechnology industry today. In the final section of this text, we study the methods for antibiotic and insecticide production and bioremediation, while discussing some details of the genetic engineering process.

Many Antibiotics Are the Result of Industrial Production

Penicillin was the first antibiotic to be produced on an industrial scale. In 1941, Robert H. Coghill of the Fermentation Division of the United States Department of Agriculture (USDA) suggested adapting the deep-tank method used to produce vitamins to produce penicillin. In the ensuing months, he offered several modifications to stimulate the growth of Penicillium notatum and increase the penicillin yield. For example, corn steep liquor in the culture medium increased the output 20 times, and the substitution of lactose for glucose made penicillin production still more efficient. Moreover, the search for a higher-yielding producer of the drug led researchers to Penicillium chrysogenum, a mold isolated from a rotten cantaloupe purchased in a Peoria, Illinois, supermarket. Treatment with ultraviolet light resulted in a mutant with still higher penicillin yields. By 1943, the United States was producing enough penicillin for the Allied forces, and by 1945, sufficient amounts were available for the civilian population.

To the present time, over 8,000 antibiotic substances have been described and approximately 100 such drugs are available to the medical practitioner. Although most antibiotics are produced by species of Streptomyces (Figure 27.6), several are also produced of Penicillium or Bacillus species. The worldwide annual production of antibiotics exceeds 500 metric tons, half of which is prescribed for human use.

Antibiotic production may involve fermentation processes producing natural antibiotics or semisynthetic drugs, which represent a modification to a natural antibiotic. This is especially true for the penicillins (see text on antimicrobial drugs). The fermentation process is carried out in large aerated, stainless steel fermentors. A typical fermentor may hold 110,000 liters of medium. Older methods employed enormous mats of fungi or actinomycetes on the surface of the tank. Newer technology, however, employs small fragments of submerged fungal hyphae or bacterial cells, rotated and agitated in the medium with a constant stream of oxygen. After several weeks of growth, the stationary phase microorganisms produce the antibiotic. At the appropriate time, the antibiotic is filtered out, extracted, and purified from the growth medium. The remaining brown mash of microorganisms may be dried and sold.
KEY CONCEPT 27.3 Microorganisms Also Produce Many Other Valuable Commercial Products

as an animal feed additive. Another alternative is to process it for use as human food.

Some Microbial Products Can Be Used to Control Insects

To be useful as an insecticide, a toxin from a microorganism should be relatively specific for an insect pest and should act rapidly. It should be stable in the environment and easily dispersed as well as inexpensive to produce. It helps if its odor is pleasant.

In the early part of this century, a scientist named G. S. Berliner discovered that sporulating cells of a Bacillus species were inhibitory to moth larvae. Berliner named the organism Bacillus thuringiensis after the German state Thuringia where he lived. The Bacillus remained in relative obscurity until recently, when scientists learned that older cells of B. thuringiensis produce toxic crystals during the process of sporulation (Figure 27.6A). The toxic substance, an alkaline protein, is deposited on leaves and ingested by caterpillars (the larval forms of butterflies, moths, and related insects). In the caterpillar gut, the insecticide lyases the cells of the gut wall. As gut liquid diffuses between the cells, the larvae experience paralysis, and bacterial invasion soon follows (Figure 27.6B).

Bacillus thuringiensis toxin (Bt toxin) appears to be harmless to plants and other animals. It is produced by harvesting bacterial cells at the onset of sporulation and drying them into a commercially available dusting powder. The product is useful on butterfly and moth caterpillars. Other strains of the bacterial species produce toxins to beetle and fly larvae, and to mosquitoes. Today, Bt toxin is widely used; more than 32 million hectares (larger than the size of Italy) of land worldwide has been treated with the pesticide.

However, bacterial cells sprayed onto plants can soon wash off, so the protective effect of the insecticide can be limited. DNA technologists realized that long-term protection can be provided by inserting the genes for Bt toxin directly into plants. To date, Bt cotton and Bt corn plants have been developed in the United States. In both cases, the isolated Bt gene has been spliced into plant genes by genetic engineers. The bacterial toxin gene is inserted as the carrier (or vector).

Genetic engineering also is being used to develop a single Bt toxin to control many different pest species. Genetic engineers can engineer a gene encoding the toxic factor along with the factor able to infect different plant pests. Such transformed bacterial cells can be grown in large fermentors to produce the Bt toxin.

Another biotechnological approach to gene-related plant resistance has been used in corn infested with corn borers. Researchers isolated the B. thuringiensis gene for toxin production and spliced it into a bacterial species living harmlessly with corn plants. The researchers then forced a colony of gene-altered bacterial cells into corn seeds. When the seeds were sown, the Bt toxin-producing bacterial cells flourished along with the plant, and when an insect ate the plant, it consumed the toxin. This approach is advantageous
because only the insect attacking the plant is subject to the toxin.

Another useful species is Bacillus sphaericus, which kills at least two species of mosquitoes that ingest its poison. To increase the bacterial cell's efficiency, researchers inserted two of its genes into the bacterium Asticcacaulis excentris and achieved insecticidal activity against the mosquitoes transmitting malaria, filariasis, and St. Louis encephalitis. Using the gene carrier A. excentris is advantageous because it is easier to grow in large quantity; it tolerates sunlight better than B. sphaericus; and it floats in water, where mosquitoes feed (the heavier Bacillus species sinks because of its spores).

Many bacteriologists are also investigating the insecticidal abilities of the gram-negative rod Photorhabdus luminescens. Its toxin, known as Pht, attacks the gut lining of larvae (as Bt does), and is contained in large cytoplasmic crystals; however, its spectrum of activity is wider than for Bt and includes numerous types of caterpillars as well as cockroaches. The Pht genes have been isolated, and efforts are underway to introduce them to plant cells. Normally, P. luminescens lives in the intestines of soilborne nematodes. The latter invade insect tissues in the soil, and the bacteria-derived toxin kills the insect.

Viruses also show promise as pest-control devices, partly because they are more selective in their activity than bacterial species. Once released in the field, the viruses spread naturally. It also is possible to harvest infected insects, grind them up, and use them to disseminate the virus to new locations. Among the insects successfully controlled with viruses are the cotton bollworm, cabbage looper, and alfalfa caterpillar.

Researchers also can develop insecticides by using a toxin from the venom of a scorpion. The toxin paralyzes the larvae of moths and other lepidopteran insects. It is attached to a baculovirus, a virus with a high affinity for lepidopteran tissues. Then, the virus is sprayed on lettuce and cotton plants infested with moth larvae. At the conclusion of the field trial, the plot was sprayed with 1% bleach to destroy any remaining viruses.

Viral genes also have been used to protect grapevines. French biotechnologists have incorporated genes from the grape fanleaf virus (GFLV) into champagne grapevines. This virus is transmitted by a nematode and is endemic in the soils of many French regions. It causes malformation of
Commercially Developed Fungal Organisms Also Are Being Environmental Protection Agency (EPA).

Even a fungus is being employed in the pesticide wars. California researchers have used Lagenidium giganteum to protect against crop-damaging mosquitoes in soybeans and rice, and in mosquito-infested nonagricultural settings such as wetlands. The fungus forces its spores into mosquito larvae, which then die in a day or two. Marketed as Laginex®, the fungal preparation has been approved for certain uses by the U.S. Environmental Protection Agency (EPA).

**Fungal Organisms Also Are Being Commercially Developed**

From the previous sections, it should be obvious that yeasts are involved in many fermentation processes, including alcoholic beverages and baking. Today, yeast cells or their products are of great commercial value.

Yeast cells can be grown in fermentors that are kept aerated. To grow at a maximal rate, plenty of ATP must be made, so aerobic respiration is essential. To keep the cells growing, the energy and carbon source is molasses, which is introduced into the fermentor in small amounts to prevent the yeast cells from switching to fermentation and producing alcohol.

After the growth period, the yeast cells are collected and processed for the commercial market as either dry yeast or as compressed yeast cakes for baking. The product may also be added to commercial wheat or corn flour. Also, because yeast cells are rich in protein and B vitamins, the yeast cells can be dried and marketed in health food stores as a nutritional supplement.

Besides the unicellular fungal yeasts, filamentous fungi also have nutritional value. The most commercial form for human consumption is mushrooms (Figure 27.8A). In recent years, more and more species of mushrooms are being grown commercially, and now include crimini, shitake, oyster, and portabella. Mushrooms are not grown in fermentors, but rather in special buildings called "mushroom farms" where the temperature and humidity can be closely controlled (Figure 27.8A). High humidity and cool temperatures are necessary to trigger mushroom formation.

The beds in which the mushrooms will be grown consist of soil mixed with rich organic matter. The soil is inoculated with a spawn, which is a pure culture of the mushroom mycelium. In the bed, the spawn spreads throughout the soil for several weeks. Casing soil, a nonnutritious soil layer providing the mycelium with more moisture for mushroom formation, is then added. The appearance of mushrooms on the bed is called a flush. Once the flush appears, the mushrooms must be collected while they remain fresh. Commercial farms then package and keep the mushrooms cool for delivery to the market.

**Figure 27.8 Commercial Mushroom Farming.** (A) Many types of mushrooms are grown commercially today. © Jones and Bartlett Publishers. Photographed by Kimberly Potvin. (B) A mushroom farm often consists of a rich bed of soil in which the fungal mycelium grows and produces mushrooms in flushes, such as these Agaricus bisporus. © Mashkov Yuri; Itar-Tass/Landov. Why must the beds be kept in moist and often damp places?
Bioremediation Helps Clean Up Pollution Naturally

Recruiting bacterial species and other microorganisms to break down synthetic waste is an immensely appealing idea. It signals a willingness to work with nature and adapt to its sophisticated sanitation systems, rather than trying to reinvent them. Putting microorganisms to work in this manner is at the crux of bioremediation, which is the use of microorganisms to degrade or neutralize contaminants in the soil or water.

The concept of bioremediation is not new. In the 1800s, for example, night soil men would, for a small fee, travel from house to house collecting sewage and excrement. After making their rounds, they would scatter their collections on fields, to be broken down by naturally occurring bacterial species in the soil. Although modern waste-disposal systems have replaced the night soil men, a new concern is the plethora of environmental pollutants contaminating the land. Bioremediation seeks to exploit microorganisms to degrade these pollutants.

The advantages of bioremediation were displayed following a major oil spill from the tanker Exxon Valdez in 1987 along the Alaska coastline (Figure 27.9). Studies have shown that where oil has spilled, the composition of the natural bacterial microbiota rapidly changes to where the oil-degrading species become dominant. Certainly this was the case for the 2010 Deepwater Horizon oil spill in the Gulf of Mexico. Investigating the Microbial World 27 examines these oil-degrading bacterial communities. In the case of the Exxon Valdez spill, technologists only needed to encourage bacterial growth. Thus, technologists “fertilized” the oil-soaked water with nitrogen sources (e.g., urea), phosphorous compounds, and other mineral nutrients to modify the environment and stimulate the growth of naturally occurring microorganisms. Areas treated this way were cleared of oil significantly faster than nonremediated shorelines. Indeed, the oil degraded five times faster when microorganisms were put to work.

Bioremediation also can be applied to help eliminate polychlorinated biphenyls (PCBs) from the environment. PCBs were used widely in industrial and electrical machinery before their threat to environmental quality was realized. These inert compounds contain numerous chlorine atoms and chlorine-containing groups, and researchers have identified anaerobic bacterial species able to remove the atoms and their daughter groups, and thus to reduce the compounds to smaller molecules. Aerobic bacterial species now take over and reduce the molecular size still further. Field demonstrations in New York’s Hudson River have shown the value of the combination anaerobic–aerobic degradation; where once there was an accumulation of PCBs, now only carbon dioxide, water, and hydrogen chloride remain.

Many years ago, trichloroethylene (TCE) was a much-used cleaning agent and solvent. At the time, scientists did not realize TCE would diffuse through the soil and contaminate underground wells and water reservoirs (aquifers). To combat this problem, scientists have exploited bacterial
Oil-Eating Microbes

The explosion of the Deepwater Horizon (DH) drilling platform in the Gulf of Mexico in 2010 produced the world’s largest accidental release of oil (hydrocarbons) into a body of water. Almost 5 million barrels of light crude were released. By June, 2010, oil had reached the shoreline and beaches in the Gulf where it had some severe ecological consequences.

- **OBSERVATIONS:** Biodegradation of hydrocarbons in the marine environment is primarily mediated by indigenous microbial communities. More than 200 bacterial, algal, and fungal genera making up over 500 species have been identified that can degrade hydrocarbons.

- **QUESTION:** In the DH oil spill, what bacterial species are the predominant oil-degrading organisms in contaminated beach ecosystems and how does the bacterial community respond to influx and prolonged oil contamination?

- **HYPOTHESIS:** The indigenous bacterial communities will increase in numbers in oil-contaminated sands. If so, then by identifying those species in clean and oiled sands, there should be an increase in species population sizes in oiled beach sands.

- **EXPERIMENTAL DESIGN:** Beach sand samples (cores) and trench-excavated sand samples were collected from Pensacola Beach that had been exposed to oil. Control sands came from St. George Island prior to the oil spill.

- **EXPERIMENT 1:** Chemical analysis was used to compare hydrocarbon compositions between oil-contaminated sands and source oil (DH wellhead).

- **EXPERIMENT 2:** Most probable number (MPN) counts and ribosomal RNA (rRNA) gene abundances were used to estimate bacterial populations in experiment 1 samples and compared to clean sand samples.

- **EXPERIMENT 3:** Ribosomal RNA (rRNA) gene sequencing was used to identify the major hydrocarbon-degrading bacterial taxa in the oiled sands.

**RESULTS:**

- **EXPERIMENT 1:** All beach sand samples contained detectable oil hydrocarbons. However, the sand samples had a 1 to 2 orders of magnitude reduction in the ratio of light molecular weight hydrocarbons (chains of 6 to 16 carbons) to heavy molecular weight hydrocarbons (chains of 16 to 35 carbons) compared to source oil.

- **EXPERIMENT 2:** See table and figure.

### TABLE: Enumeration of Oil-Degrading Bacteria—by MPN and rRNA Gene Abundance

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>MPN Counts (per ml)</th>
<th>rRNA Gene Abundance (copies/g sand)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oiled sands</td>
<td>$2.4 \times 10^{10}$ cells</td>
<td>$0.44 \times 10^{7}$ to $14.2 \times 10^{7}$</td>
</tr>
<tr>
<td>“Clean” sands</td>
<td>$2.4 \times 10^{6}$ to $9.3 \times 10^{6}$</td>
<td>$0.02 \times 10^{7}$ to $1.57 \times 10^{7}$</td>
</tr>
</tbody>
</table>

species that grow on methane to degrade the TCE. During their metabolism, the bacterial cells produce a methane-digesting enzyme that breaks down TCE. Technologists pump methane and other nutrients into the TCE-contaminated water, and as the bacterial cells grow, they digest the TCE as well as the methane. Once again, the deliberate enhancement of microbial growth yields an environmental cleanup.

Deinococcus radiodurans is a bacterial species able to withstand 3,000 times more radiation than humans. The bacterial cells were found in a tin of irradiation-sterilized ground beef. Researchers are hoping to use this organism in the daunting task of cleaning up thousands of toxic-waste sites where radioactive materials such as plutonium and uranium are present. Genetic engineering methods have produced a D. radiodurans strain that degrades...
ion by degrading flame-retardants, phenols, and mercury to a less toxic form.

During the 1940s through the 1960s, a major component of weaponry was the explosive compound 2,4,6-trinitrotoluene (TNT). Like other synthetic wastes, this compound has contaminated the soil from residues deposited around weapons plants. Scientists have discovered they can reduce the level of contamination by encouraging bacterial growth with molasses. In a pilot study, researchers mixed water with TNT-laced soil and added molasses at regular intervals. In a matter of weeks, the TNT concentration plummeted. 

Plants also can conscript bacterial species for the task of environmental cleanup. Following the Gulf War of 1991, oily devastation remained in much of the Arabian Desert. Within 4 years, however, plant life returned, aided in large measure by Arthrobacter species. When researchers dug into oil-soaked desert, they found healthy plant roots surrounded by reservoirs of these oil-degrading gram-negative rods.

For many years, the “haul-and-bury” technique was the prevailing method for disposing of synthetic waste. As the public becomes increasingly intolerant of such an approach, the importance of bioremediation will become more apparent. When researchers dug into oil-soaked desert, they found healthy plant roots surrounded by reservoirs of these oil-degrading gram-negative rods.

Microinquiry 27 explores a few more examples with microorganisms.

Industrial Genetic Engineering Continues to Make Advances

In 1973, Herbert Boyer, of the University of California at San Francisco, and Stanley Cohen, of Stanford University, performed the first practical experiment in genetic engineering. Working with E. coli, they removed the genes for kanamycin resistance and spliced them into a plasmid already carrying genes for tetracycline resistance. The plasmids then were mixed with E. coli cells not resistant to either antibiotic. Finally, the cells were streaked on plates of culture medium containing both kanamycin and tetracycline. The bacterial cells that grew had taken up the plasmids and now were resistant to both antibiotics. The cells had been transformed; they could now do something they could not do before. Feats like these launched the modern era of biotechnology.

Genetic engineering based in plasmid technology has been hailed as the beginning of modern industrial microbiology. Plasmids are small, circular, double-stranded DNA molecules that exist apart from the bacterial chromosome (Figure 27.10). A single plasmid may contain between 2 and 250 genes. With plasmid technology, a gene of interest is spliced into the plasmid DNA and then the plasmid serves as the vector to carry the foreign gene into the targeted cell. The mechanics of this genetic engineering process are explored in another chapter. Some contemporary products already obtained by plasmid technology include interferon, insulin, and human growth hormone.

When microbiologists first developed plasmid technology, the likely choice for the prototype “bacterial factory” was E. coli. Nonpathogenic strains had long been used as test organisms in the laboratory, and the genetics of E. coli were well understood. In the 1980s, however, attention shifted to Bacillus subtilis and yeasts as host organisms. B. subtilis is a gram-positive rod that normally secretes the proteins it makes, while E. coli retains them. Also, B. subtilis is not regarded as a human pathogen, in contrast to some strains of E. coli, nor does it contain endotoxins in its cell wall. Yeast also is a key “factory” due to its traditional role in the fermentation processes and as an organism without disease potential. In fact, Saccharomyces species have been reengineered to produce a synthetic vaccine for hepatitis B and to obtain rennin, the enzyme used to make cheese.

The implications of genetic engineering are almost limitless. Biotechnologists are trying to introduce the antibiotic-producing genes of Streptomyces species directly into more rapidly growing organisms. They also are trying to amplify the number of plasmids in antibiotic producers to obtain a higher yield of product.

In the 1960s, a “Green Revolution” took place in which genetically modified high-yielding supergrains were exported to poor countries throughout the world to encourage self-sufficiency. However, the program stalled when stocks of essential petroleum fertilizers declined due to high oil prices. Agriculturalists now hope for a second Green Revolution sparked by genetic engineering (Figure 27.11). They foresee the day when the...
Working with Microorganisms

As we approach the end of this text, you now should have a deep appreciation for the diverse roles microorganisms can have. Certainly, their roles go beyond causing human disease. In this MicroInquiry, let’s examine some additional ways microbes are being put to beneficial use. Answers can be found on the Student Companion Website in Appendix D.

Oil Exploration Through Microbial Chemistry

For decades, we have been hearing about the impending oil shortage. Indeed, it is becoming harder for oil companies to find deposits of light (“sweet”) crude oil, which is the form usually refined. This means about 60% of the world’s reserves are a very thick and sticky form called heavy (“sour”) crude oil. Much of it remains below ground because it is very difficult to recover and costly to refine into the liquid gasoline you put in your automobile.

27.1a. Outline a microbial way to recover this sour crude oil more reasonably.

Another problem with heavy crude oil is that it contains large amounts of potential contaminants, including sulfur compounds and heavy metals, which could be spread into the atmosphere through refining. The release of these waste products would reverse the trend to cut sulfur emissions in the air. Add to this the high cost of refining such sulfur-rich crude, and you have a dilemma.

27.1b. How can this heavy crude be more economically and safely extracted and refined?

Prospecting with Microbes

The technology of metal extraction has been around for a long time. More than 2,000 years ago, the Romans used iron, copper, lead, gold, silver, and alloys such as bronze and brass for tools, weapons, coins, and jewelry. Unknown to them, microbes had solubilized these metals, which the Roman metallurgists had collected from mines in Spain and Wales.

The twentieth century saw an explosion in the production of such high-grade ores, which, like crude oil, are becoming depleted. With this expansion of metal production, besides disfiguring the land, comes the environmental pollution from the smelting process that produces sulfur dioxide in the atmosphere and toxic chemicals in the water and soil.

27.2 Outline a biotechnological scheme to provide for a continued supply of metals.

Superfund and Bioremediation

Historically, and still in use today, are such chemical and physical methods as excavation and incineration, which degrade many of the toxic materials found at Superfund sites. However, the Environmental Protection Agency (EPA) has tested the feasibility of bioremediation at several of these sites.

Creosote Contamination

Between 1973 and 1985, a lumber company in southern Missouri operated a wood treating facility that preserved railroad ties with a creosote/diesel fuel mixture. These operations contaminated the soil at the site with polynuclear aromatic hydrocarbons (PAHs), which were major components of the creosote/diesel mixture. After the facility was shut down and designated as a Superfund site in 1987, the EPA oversaw construction and operation of a land treatment unit to remove the PAH-contaminated soils at the site. EPA studies indicated indigenous microorganisms could digest aromatic hydrocarbons existing in the soil.

27.3 Outline a process whereby excavated contaminated soil could be decontaminated.

Petrochemical Wastes

In Texas, an industrial waste disposal facility contained an estimated 70 million gallons of petrochemical wastes that were disposed of onsite between 1966 and 1971. Contaminants in the lagoon sediments included PAHs, chlorinated organics, and metals.

27.4 Outline a process whereby lagoon sediments could be decontaminated.

Superfund:

Common name for an environmental law giving the government broad authority to respond directly to releases or threatened releases of hazardous substances endangering public health or the environment.

Transgenic:

Referring to an organism containing a stable gene from another organism.

genes for nitrogen fixation can be extracted from bacterial organisms like Rhizobium and inserted into grain plants such as wheat, rye, and barley. The most optimistic planners look to the future and foresee fertilizers becoming obsolete, plants using microbial toxins to drive off insects, and crops living for weeks without water.

Another dream of biotechnologists is using replacement organisms to interrupt disease cycles in nature. For example, a transgenic snail that resists invasion of Schistosoma species could conceivably interrupt the life cycle of the parasite causing schistosomiasis. A slightly different strategy is being employed by DNA technologists who are attempting to produce transgenic cotton bollworms by inserting a gene activating a toxin-producing gene in offspring of the bollworm. Work continues to progress on the engineering of a transgenic mosquito unable to harbor and transmit malarial parasites. Released in large numbers, the mosquitoes could dilute or overwhelm native mosquito populations and break the chain of disease transmission.
Other research projects in genetic engineering have equally important goals. Research in genetic engineering holds the promise of new strategies for cancer prevention, new diagnostic procedures for microbial diseases and genetic abnormalities, new methods to correct genetic disorders, new hormones, antibiotics, and vaccines, and a generally improved quality of life. The discoveries and insights made possible by genetic engineering have been described as breathtaking. In the future, we can expect startling developments in medicine, agriculture, and the pharmaceutical and chemical industries. Indeed, it is an exciting time to be a microbiologist.

**CONCEPT AND REASONING CHECKS 3**

a. Why has it been necessary for industry to develop semi-synthetic antibiotics, such as many of the penicillins?

b. How are bacterial toxins being produced industrially to kill insect larvae?

c. Summarize the steps involved in mushroom farming.

d. Describe some of the ways bacterial species can be used in bioremediation.

e. Justify the need for industrial microbiology to the advances in biotechnology.
27.1 Microorganisms Are Used to Produce Many Industrial Products
1. Among the organic compounds (secondary metabolites) synthesized on an industrial scale are organic acids (such as citric, lactic, and gluconic acids), along with various amino acids and vitamins. (Fig. 27.2)
2. Microbes also produce a variety of enzymes such as amylase, pectinase, and protease. Microorganisms produce other key products and save much expense and time for the chemist. (Fig. 27.3; Table 27.1)

27.2 Alcoholic Beverages Are Products of Fermentation
3. Yeasts of the genus Saccharomyces ferment barley grains to produce beer under anaerobic conditions. The process requires an aging step to develop the full flavor of the product. (Fig. 27.4)
4. Yeasts of the genus Saccharomyces ferment grape juice to wine under anaerobic conditions. This process also requires an aging step to develop the full flavor of the product. (Fig. 27.5)
5. To produce spirits, alcohol produced from fermentation is distilled off to produce brandy, rum, whiskey, or neutral spirits.

27.3 Microorganisms Also Produce Many Other Valuable Commercial Products
6. The industrial production of penicillins involves fermentation processes carried out in large fermentors.
7. The spores of Bacillus thuringiensis are used in the live form as insecticides for plants and are successfully employed against the caterpillars of various insect pests. In addition, scientists are investigating the use of bacterial insecticides, such as the toxin produced by Photorhabdus. Scorpion toxins attached to insect viruses are being developed as potent insecticides.
8. Several fungi have uses as food or food additives. Besides their use in the alcoholic beverage industries, yeasts have commercial value in a dried or cake form in the baking industries. Nutritional yeasts can be a source of protein and B vitamins in the human diet. Mushroom farms have developed the technology to grow many commercial types of wild mushrooms.
9. Bioremediation is still another innovative use for industrial microorganisms. In this process, naturally occurring microorganisms are encouraged to grow in a polluted environment and break down pollutants. Bioremediation has been used successfully to degrade the oil in oil spills and to help eliminate polychlorinated biphenyls and trichloroethylene from the environment. Researchers are hoping to use the process in the daunting task of cleaning toxic waste sites that contain radioactive materials.
10. The future of industrial microbiology will center on genetic engineering and plasmid technology. By inserting foreign genes into vector organisms, such as bacterial species and yeast, biotechnologists can produce rare proteins on an industrial scale and provide treatments for such diseases as diabetes, hemophilia, and cancer. Genetic engineers predict plants will be developed with inborn resistance to disease, animals will be engineered to produce human proteins, and novel treatments will be designed to cure or prevent specific illnesses. Medicine, agriculture, and industry eagerly anticipate the fruits of modern biotechnology that are made possible with microorganisms.

CHAPTER SELF-TEST
For STEPS A–D, answers to even-numbered questions and problems can be found in Appendix C on the Student Companion Website at http://microbiology.jbpub.com/10e. In addition, the site features eLearning, an online review area that provides quizzes and other tools to help you study for your class. You can also follow useful links for in-depth information, read more MicroFocus stories, or just find out the latest microbiology news.

STEP A: REVIEW OF FACTS AND TERMS

Multiple Choice
Read each question carefully, then select the one answer that best fits the question or statement.
1. Which one of the following is NOT true of a primary metabolite?
   A. It is directly involved with normal growth.
   B. It is normally produced during the stationary growth phase.
   C. Alcohol is a primary metabolite.
   D. It is a product essential to the survival of the microbe.
2. Industrially produced streptokinase is a/an ____ used to ____.
   A. enzyme; dissolve blood clots
   B. vitamin; prevent pernicious anemia
   C. enzyme; facilitate the absorption of fluids
   D. enzyme; digest glucose
3. The brewing of a lager requires
   A. a bottom yeast
   B. Saccharomyces cerevisiae
   C. a top yeast
   D. wild yeasts
4. In wine making, wild yeasts often ____.
   A. are killed with sulfur dioxide
   B. produce undesirable wine qualities
   C. are found on the grapes
   D. All the above (A–C) are correct.
5. A distilled spirit that is 60 proof contains ____.
   A. 15% alcohol
   B. 30% water
   C. 30% alcohol
   D. 60% alcohol
6. Most antibiotics are the products of ____ species.
   A. Streptomyces
   B. Penicillium
   C. Streptococcus
   D. Bacillus

7. The toxin produced by Bacillus thuringiensis is ____.
   A. a protein
   B. an acid
   C. a sugar
   D. a cell wall component

8. A spawn is a ____.
   A. complex set of nutrients
   B. cluster of mushrooms
   C. bed in which mushrooms grow
   D. mushroom mycelium

### Matching

Many different microorganisms find value in industrial microbiology. To test your knowledge of these organisms, match the microorganism on the right to the characteristic on the left by placing the correct letter in the space. A letter may be used once, more than once, or not at all.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>11. Used to ferment grapes.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12. Used in riboflavin production.</td>
</tr>
<tr>
<td></td>
<td>13. Produces many antibiotics.</td>
</tr>
<tr>
<td></td>
<td>14. Enhances grape sugar content.</td>
</tr>
<tr>
<td></td>
<td>15. Used in lysine production.</td>
</tr>
<tr>
<td></td>
<td>16. Biological insecticide.</td>
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<tr>
<td></td>
<td>17. Source of musk oil.</td>
</tr>
<tr>
<td></td>
<td>18. Used for citric acid production.</td>
</tr>
<tr>
<td></td>
<td>19. Fungal source of amylase.</td>
</tr>
<tr>
<td></td>
<td>20. Produces ergot poisons.</td>
</tr>
<tr>
<td></td>
<td>21. Used in top fermentation of beer.</td>
</tr>
<tr>
<td></td>
<td>22. Yeast for making pilsner beer.</td>
</tr>
<tr>
<td></td>
<td>23. Able to degrade oil.</td>
</tr>
<tr>
<td></td>
<td>24. Used for genetic engineering.</td>
</tr>
<tr>
<td></td>
<td>25. Degrades ionic mercury compounds.</td>
</tr>
</tbody>
</table>

### Microorganism

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>A. Acetobacter acetii</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. Ashbya gossypii</td>
</tr>
<tr>
<td></td>
<td>C. Aspergillus niger</td>
</tr>
<tr>
<td></td>
<td>D. Aspergillus oryzae</td>
</tr>
<tr>
<td></td>
<td>E. Bacillus thuringiensis</td>
</tr>
<tr>
<td></td>
<td>F. Bacillus subtilis</td>
</tr>
<tr>
<td></td>
<td>G. Botrytis cinerea</td>
</tr>
<tr>
<td></td>
<td>H. Claviceps purpurea</td>
</tr>
<tr>
<td></td>
<td>I. Deinococcus radiodurans</td>
</tr>
<tr>
<td></td>
<td>J. Enterobacter aerogenes</td>
</tr>
<tr>
<td></td>
<td>K. Pseudomonas species</td>
</tr>
<tr>
<td></td>
<td>L. Rhizopus nigricans</td>
</tr>
<tr>
<td></td>
<td>M. Saccharomyces carlsbergensis</td>
</tr>
<tr>
<td></td>
<td>N. Saccharomyces cerevisiae</td>
</tr>
<tr>
<td></td>
<td>O. Saccharomyces ellipsoideus</td>
</tr>
<tr>
<td></td>
<td>P. Streptomyces species</td>
</tr>
<tr>
<td></td>
<td>Q. Ustilago zaeae</td>
</tr>
</tbody>
</table>

### Step B: Concept Review

   (Key Concept 1)
27. Summarize the types of enzymes produced by the industrial fermentation of microorganisms for commercial and medical needs.  
   (Key Concept 1)
28. Construct a concept map for beer production.  
   (Key Concept 2)
29. Describe the steps in the production, fermentation, and aging of wine.  
   (Key Concept 2)
30. Compare and contrast the production of distilled spirits from wine making.  
   (Key Concept 2)
31. Explain why antibiotics produced through industrial fermentation represent secondary metabolites.  
   (Key Concept 3)
32. Evaluate the role of microorganisms to the industrial production of insecticides.  
   (Key Concept 3)
33. Identify the usefulness of fungi to food microbiology and as a food supplement.  
   (Key Concept 3)
34. Explain how bioremediation can be accelerated using microorganisms.  
   (Key Concept 3)
35. Describe how plasmid technology is advancing biotechnology.  
   (Key Concept 3)
**CHAPTER 27  Industrial Microbiology and Biotechnology**

### STEP C: APPLICATIONS AND PROBLEMS

36. Many beer companies have developed strains of yeasts that break down more of the carbohydrate in barley malt than traditional yeasts. What do you think their product is called?

37. The discovery of the extremely high resistance of *Deinococcus radiodurans* to radioactivity has prompted hopes that this organism can be used in bioremediation. Suppose you were to go on a hunt for novel organisms that could be used for other environmental cleanups. Where might you look?

38. A product called Dipel dust contains *Bacillus thuringiensis*. It is used in the Northeast for destruction of the gypsy moth caterpillar. What dangers might result from extensive application of this product?

39. A friend who also has taken a class in microbiology wonders if it would be possible to engineer certain bacterial species to produce antibiotics and then give these species to diseased people to serve as antibiotic producers within the body. How would you respond to your friend’s idea?

### STEP D: QUESTIONS FOR THOUGHT AND DISCUSSION

40. The poet John Donne once wrote: “No man is an island, entirely of itself.” This maxim applies not only to humans, but to all living things in the natural world. What are some roles the microorganisms play in the interrelationships among living things?

41. When the Mayflower set sail for the New World, its intended destination was Virginia. Instead, it landed at Plymouth, Massachusetts, because, as one diarist put it, “We could not now take time for further search or consideration, our victuals being much spent, especially our beer.” What do these last few words tell you about the Pilgrims?

42. In several places in this text, we have noted how apparently harmless organisms have been discovered later to be dangerous in humans. Yeasts have been consumed in breads and alcoholic beverages for centuries, and still no pathogenic signs have been observed. Can you postulate why?

43. Certain bacterial species produce many thousands of times their required amount of specific vitamins. Some biologists suggest that this makes little sense because the excess is wasted. Can you suggest a reason for this apparent overproduction in nature?

44. How many times in the last 24 hours have you had the opportunity to use or consume the industrial product of a microorganism?