CHAPTER 42

Industrial Microbiology and Biotechnology

Biodegradation often can be facilitated by changing environmental conditions. Polychlorinated biphenyls (PCBs) are widespread industrial contaminants that accumulate in anaerobic river muds. Although reductive dechlorination occurs under these anaerobic conditions, oxygen is required to complete the degradation process. In this experiment, muds are being aerated to allow the final biodegradation steps to occur.

Outline

Concepts

1. Microorganisms are used in industrial microbiology and biotechnology to create a wide variety of products and to assist in maintaining and improving the environment.
2. Most work in industrial microbiology has been carried out using microorganisms isolated from nature or modified through mutations. In modern biotechnology, microorganisms with specific genetic characteristics can be constructed to meet desired objectives.
3. Most microorganisms have not been grown or described. A major challenge in biotechnology is to be able to grow and characterize these observed but uncultured microorganisms in what is called "bioprospecting."
4. Forced evolution and adaptive mutations now are part of modern biotechnology. These can be carried out in processes termed "natural genetic engineering."
5. The development of growth media and specific conditions for the growth of microorganisms is a large part of industrial microbiology and biotechnology. Microorganisms can be grown in controlled environments with specific limitations to maximize the synthesis of desired products.
6. Microbial growth in soils, waters, and other environments, where complex microbial communities already are present, cannot be completely controlled, and it is not possible to precisely define limiting factors or physical conditions.
7. Microbial growth in controlled environments is expensive; it is used to synthesize high-value microbial metabolites and other products for use in animal and human health. In comparison, microbial growth in natural environments usually does not involve the creation of specific microbial products; microorganisms are used to carry out lower-value environmental and agriculture-related processes.
8. In controlled growth systems, different products are synthesized during growth and after growth is completed. Most antibiotics are produced after the completion of active growth.
9. Antibiotics and other microbial products continue to contribute to animal and human welfare. Newer products include anticancer drugs. Combinatorial biology is making it possible to produce hybrid antibiotics with unique properties.
10. The products of industrial microbiology impact all aspects of our lives. These often are bulk chemicals that are used as food supplements and acidifying agents. Other products are used as biosurfactants and emulsifiers in a wide variety of applications.
11. Degradation is critical for understanding microbial contributions to natural environments. The chemical structure of substrates and microbial community characteristics play important roles in determining the fate of chemicals. Anaerobic degradation processes are important for the initial modification of many compounds, especially those with chlorine and other halogenated functions. Degradation can produce simpler or modified compounds that may not be less toxic than the original compound.
12. Biosensors are undergoing rapid development, which is limited only by the advances that are occurring in molecular biology and other areas of science. It is now possible, especially with streptavidin-biotin-linked systems, to have essentially real-time detection of important pathogens.
13. Gene arrays, based on recombinant DNA technology, allow gene expression to be monitored. These systems are being used in the analysis of complex microbial systems.
14. Bacteria, fungi, and viruses are increasingly employed as biopesticides, thus reducing dependence on chemical pesticides.
15. Application of microorganisms and their technology has both positive and negative aspects. Possible broader impacts of applications of industrial microbiology and biotechnology should be considered in the application of this rapidly expanding area.

The microbe will have the last word.

—Louis Pasteur

Industrial microbiology and biotechnology both involve the use of microorganisms to achieve specific goals, whether creating new products with monetary value or improving the environment. Industrial microbiology, as it has traditionally developed, focuses on products such as pharmaceutical and medical compounds (antibiotics, hormones, transformed steroids), solvents, organic acids, chemical feedstocks, amino acids, and enzymes that have direct economic value. The microorganisms employed by industry have been isolated from nature, and in many cases, were modified using classic mutation-selection procedures.

The era of biotechnology has developed rapidly in the last several decades, and is characterized by the modification of microorganisms through the use of molecular biology, including the use of recombinant DNA technology (*see chapter 14*). It is now possible to manipulate genetic information and design products such as proteins, or to modify microbial gene expression. In addition, genetic information can be transferred between markedly different groups of organisms, such as between bacteria and plants.

Selection and use of microorganisms in industrial microbiology and biotechnology are challenging tasks that require a solid understanding of microorganism growth and manipulation, as well as microbial interactions with other organisms. The use of microorganisms in industrial microbiology and biotechnology follows a logical sequence. It is necessary first to identify or create a microorganism that carries out the desired process in the most efficient manner. This microorganism then is used, either in a controlled environment such as a fermenter or in complex systems such as in soils or waters to achieve specific goals.

42.1 Choosing Microorganisms for Industrial Microbiology and Biotechnology

The first task for an industrial microbiologist is to find a suitable microorganism for use in the desired process. A wide variety of alternative approaches are available, ranging from isolating microorganisms from the environment to using sophisticated molecular techniques to modify an existing microorganism.

Finding Microorganisms in Nature

Until relatively recently, the major sources of microbial cultures for use in industrial microbiology were natural materials such as soil samples, waters, and spoiled bread and fruit. Cultures from all areas of the world were examined in an attempt to identify strains with desirable characteristics. Interest in "hunting" for new microorganisms continues unabated.

Because only a minor portion of the microbial species in most environments has been isolated or cultured (**table 42.1**), there is a continuing effort throughout the world to find new microorganisms, even using environments that have been examined for decades. In spite of these long-term efforts, few microorganisms have been cultured and studied; most microbes

Box 42.1

The Potential of Archaea from High-Temperature Environments for Use in Biotechnology

There is great interest in the characteristics of archaeans isolated from the outflow mixing regions above deep hydrothermal vents that release water at 250 to 350°C. This is because these hardy organisms can grow at temperatures as high as 113°C. The problems in growing these microorganisms are formidable. For example, to grow some of them, it will be necessary to use special culturing chambers and other specialized equipment to maintain water in the liquid state at these high temperatures.

Such microorganisms, termed hyperthermophiles, with optimum growth temperatures of 80°C or above (*see p. 126*), confront unique challenges in nutrient acquisition, metabolism, nucleic acid replication, and growth. Many of these are anaerobes that depend on elemental sulfur as an oxidant and reduce it to sulfide. Enzyme stability is critical. Some DNA polymerases are inherently stable at 140°C, whereas many other enzymes are stabilized in vivo with unique thermoprotectants. When these enzymes are separated from their protectant, they lose their unique thermostability.

These enzymes may have important applications in methane production, metal leaching and recovery, and for use in immobilized enzyme systems. In addition, the possibility of selective stereochemical modification of compounds normally not in solution at lower temperatures may provide new routes for directed chemical syntheses. This is an exciting and expanding area of the modern biological sciences to which environmental microbiologists can make significant contributions.

Table 42.1 Estimated Total and Known Species from Different Microbial Groups

Group	Estimated Total Species	Known Species[a]	Percent Known
Viruses	130,000[b]	5,000	[4][c]
Archaea	?[d]	<500	?
Bacteria	40,000[b]	4,800	[12]
Fungi	1,500,000	69,000	5
Algae	60,000	40,000	67

[a]Mid-1990 values and should be increased 10–50%.

[b]These values are substantially underestimated, perhaps by 1–2 orders of magnitude.

[c][ ] indicates that these values are probably gross overestimates.

[d]Small subunit (SSU) rRNA data indicate much higher in situ diversity than given by culture-based studies.

Adapted from: D. A. Cowan. 2000. Microbial genomes—the untapped resource. *Tibtech* 18:14–16. Table 1, p. 15.

Table 42.2 Estimates of the Percent "Cultured" Microorganisms in Various Environments

Environment	Estimated Percent Cultured
Seawater	0.001–0.100
Fresh water	0.25
Mesotrophic lake	0.1–1.0
Unpolluted estuarine waters	0.1–3.0
Activated sludge	1–15
Sediments	0.25
Soil	0.3

Source: D. A. Cowan. 2000. Microbial genomes—the untapped resource. *Tibtech* 18:14–16. Table 2, p. 15.

that can be observed in nature have not been cultured or identified, although molecular techniques are making it possible to obtain information on these uncultured microorganisms (**table 42.2**). With increased interest in microbial diversity and microbial ecology, and especially in microorganisms from extreme environments (**Box 42.1**), microbiologists are rapidly expanding the pool of known microorganisms with characteristics desirable for use in industrial microbiology and biotechnology. They are also identifying microorganisms involved in mutualistic and protocooperative relationships with other microorganisms and with higher plants and animals. There is continuing interest in bioprospecting in all areas of the world, and major companies have been organized to continue to explore microbial diversity and identify microorganisms with new capabilities. Uncultured microorganisms and microbial diversity (section 6.5)

Genetic Manipulation of Microorganisms

Genetic manipulations are used to produce microorganisms with new and desirable characteristics. The classical methods of microbial genetics (*see chapter 13*) play a vital role in the development of cultures for industrial microbiology.

Mutation

Once a promising culture is found, a variety of techniques can be used for culture improvement, including chemical mutagens and ultraviolet light (*see chapter 11*). As an example, the first cultures of *Penicillium notatum,* which could be grown only under static conditions, yielded low concentrations of penicillin. In 1943 a strain of *Penicillium chrysogenum* was isolated—

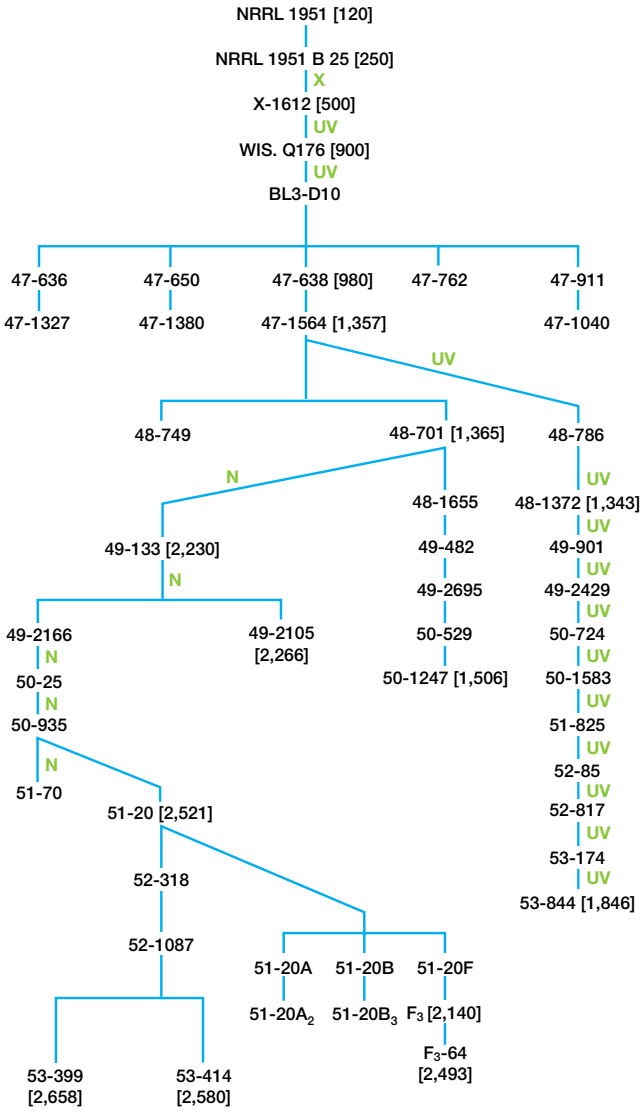


Figure 42.1 Mutation Makes It Possible to Increase Fermentation Yields. A "genealogy" of the mutation processes used to increase penicillin yields with *Penicillium chrysogenum* using X-ray treatment (X), UV treatment (UV), and mustard gas (N). By use of these mutational processes, the yield was increased from 120 International Units (IU) to 2,580 IU, a 20-fold increase. Unmarked transfers were used for mutant growth and isolation. Yields in international units/ml in brackets.

strain NRRL 1951—which was further improved through mutation (**figure 42.1**). Today most penicillin is produced with *Penicillium chrysogenum,* grown in aerobic stirred fermenters, which gives 55-fold higher penicillin yields than the original static cultures.

Protoplast Fusion

Protoplast fusion is now widely used with yeasts and molds. Most of these microorganisms are asexual or of a single mating type, which decreases the chance of random mutations that could lead to strain degeneration. To carry out genetic studies with these microorganisms, protoplasts are prepared by growing the cells in an isotonic solution while treating them with enzymes, including cellulase and beta-galacturonidase. The protoplasts are then regenerated using osmotic stabilizers such as sucrose. If fusion occurs to form hybrids, desired recombinants are identified by means of selective plating techniques. After regeneration of the cell wall, the new protoplasm fusion product can be used in further studies.

A major advantage of the protoplast fusion technique is that protoplasts of different microbial species can be fused, even if they are not closely linked taxonomically. For example, protoplasts of *Penicillium roquefortii* have been fused with those of *P. chrysogenum.* Even yeast protoplasts and erythrocytes can be fused.

Insertion of Short DNA Sequences

Short lengths of chemically synthesized DNA sequences can be inserted into recipient microorganisms by the process of **site-directed mutagenesis.** This can create small genetic alterations leading to a change of one or several amino acids in the target protein. Such minor amino acid changes have been found to lead, in many cases, to unexpected changes in protein characteristics, and have resulted in new products such as more environmentally resistant enzymes and enzymes that can catalyze desired reactions. These approaches are part of the field of **protein engineering.**

Site-directed mutagenesis (p. 323)

Enzymes and bioactive peptides with markedly different characteristics (stability, kinetics, activities) can be created. The molecular basis for the functioning of these modified products also can be better understood. One of the most interesting areas is the design of enzyme-active sites to promote the modification of "unnatural substrates." This approach may lead to improved transformation of recalcitrant materials, or even the degradation of materials that have previously not been amenable to biological processing.

1. How are industrial microbiology and biotechnology similar and different?
2. Based on recent estimates, what portion of the microorganisms have been cultured from soils and from aquatic and marine environments?
3. What is protoplast fusion and what types of microorganisms are used in this process?
4. Describe site-directed mutagenesis and how it is used in biotechnology.
5. What is protein engineering?

Table 42.3 Combinatorial Biology in Biotechnology: The Expression of Genes in Other Organisms to Improve Processes and Products

Property or Product Transferred	Microorganism Used	Combinatorial Process
Ethanol production	*Escherichia coli*	Integration of pyruvate decarboxylase and alcohol dehydrogenase II from *Zymomonas mobilis.*
1,3-Propanediol production	*E. coli*	Introduction of genes from the *Klebsiella pneumoniae* dha region into *E. coli* made possible anaerobic 1,3-propanediol production.
Cephalosporin precursor synthesis	*Penicillium chrysogenum*	Production 7-ADC and 7-ADCA[a] precursors by incorporation of the expandase gene of *Cephalosoporin acremonium* into *Penicillium* by transformation.
Lactic acid production	*Saccharomyces cerevisiae*	A muscle bovine lactate dehydrogenase gene (LDH-A) expressed in *S. cerevisiae.*
Xylitol production	*S. cerevisiae*	95% xylitol conversion from xylose was obtained by transforming the XYLI gene of *Pichia stipitis* encoding a xylose reductase into *S. cerevisiae,* making this organism an efficient organism for the production of xylitol, which serves as a sweetener in the food industry.
Creatininase[b]	*E. coli*	Expression of the creatininase gene from *Pseudomonas putida* R565. Gene inserted with a pUC18 vector.
Pediocin[c]	*S. cerevisiae*	Expression of bacteriocin from *Pediococcus acidilactici* in *S. cerevisiae* to inhibit wine contaminants.
Acetone and butanol production	*Clostridium acetobutylicum*	Introduction of a shuttle vector into *C. acetobutylicum* by an improved electrotransformation protocol, which resulted in acetone and butanol formation.

[a]7-ACA = 7-aminocephalosporanic acid; 7-ADCA = 7-aminodecacetoxycephalosporonic acid.

[b]T.-Y. Tang; C.-J. Wen; and W.-H. Liu. 2000. Expression of the creatininase gene from *Pseudomonas putida* RS65 in *Escherichia coli. J. Ind. Microbiol. Biotechnol.* 24:2–6.

[c]H. Schoeman; M. A. Vivier; M. DuToit; L. M. Y. Dicks; and I. S. Pretorius. 1999. The development of bactericidal yeast strains by expressing the *Pediococcus acidilactici* pediocin gene (pedA) in *Saccharomyces cerevisiae. Yeast* 15:647–656.

Adapted from S. Ostergaard; L. Olsson; and J. Nielson. 2000. Metabolic engineering of *Saccharomyces cerevisiae.* Microbiol. Mol. Biol. Rev. 64(1):34–50.

Transfer of Genetic Information between Different Organisms

New alternatives have arisen through the transfer of nucleic acids between different organisms, which is part of the rapidly developing field of **combinatorial biology** (**table 42.3**). This involves the transfer of genes for the synthesis of a specific product from one organism into another, giving the recipient varied capabilities such as an increased capacity to carry out hydrocarbon degradation. An important early example of this approach was the creation of the "superbug," patented by A. M. Chakarabarty in 1974, which had an increased capability of hydrocarbon degradation. The genes for antibiotic production can be transferred to a microorganism that produces another antibiotic, or even to a non-antibiotic-producing microorganism. For example, the genes for synthesis of bialophos (an antibiotic herbicide) were transferred from *Streptomyces hygroscopicus* to *S. lividans.* Other examples are the expression in *E. coli,* of the enzyme creatininase from *Pseudomonas putida* and the production of pediocin, a bacteriocin, in a yeast used in wine fermentation for the purpose of controlling bacterial contaminants. Bacteriocins (pp. 297, 712)

DNA expression in different organisms can improve production efficiency and minimize the purification steps required before the product is ready for use. For example, recombinant baculoviruses (*see p. 415*) can be replicated in insect larvae to achieve rapid large-scale production of a desired virus or protein. Transgenic plants (*discussed on pp. 335–36*) may be used to manufacture large quantities of a variety of metabolic products. A most imaginative way of incorporating new DNA into a plant is to simply shoot it in using DNA-coated microprojectiles and a gene gun (*see section 14.6*).

A wide range of genetic information also can be inserted into microorganisms using vectors and recombinant DNA techniques. Vectors (*see section 14.5*) include artificial chromosomes such as those for yeasts (YACs), bacteria (BACs), P1 bacteriophage-derived chromosomes (PACs), and mammalian artificial chromosomes (MACs). YACs are especially valuable because large DNA sequences (over 100 kb) can be maintained in the YAC as a separate chromosome in yeast cells. A good example of vector use is provided by the virus that causes foot-and-mouth disease of cattle and other livestock. Genetic information for a foot-and-mouth disease virus antigen can be incorporated into *E. coli,* followed by the expression of this genetic information and synthesis of the gene product for use in vaccine production (**figure 42.2**).

Genetic information transfer allows the production of specific proteins and peptides without contamination by similar products that might be synthesized in the original organism. This approach can decrease the time and cost of recovering and purifying a product. Another major advantage of peptide production with modern biotechnology is that only biologically active stereoisomers are produced. This specificity is required to avoid the possible harmful side effects of inactive stereoisomers, as occurred in the thalidomide disaster.

In summary, modern gene-cloning techniques provide a considerable range of possibilities for manipulation of microorganisms and the use of plants and animals (or their cells) as factories for the expression of recombinant DNA. Newer molecular techniques continue to be discovered and applied to transfer genetic information and to construct microorganisms with new capabilities.

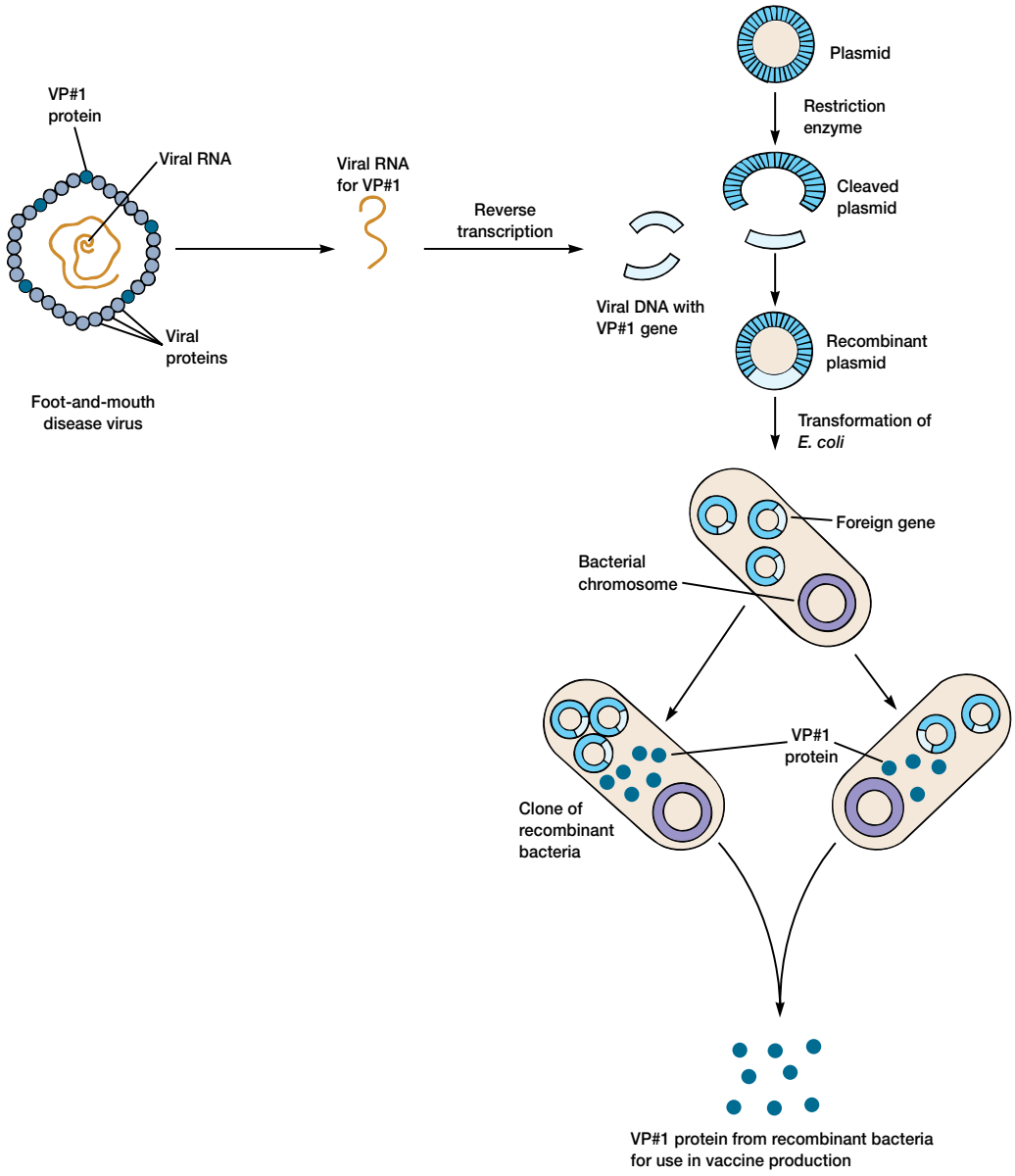


Figure 42.2 **Recombinant Vaccine Production.** Genes coding for desired products can be expressed in different organisms. By the use of recombinant DNA techniques, a foot-and-mouth disease vaccine is produced through cloning the vaccine genes into *Escherichia coli*.

Table 42.4 Examples of Recombinant DNA Systems Used to Modify Gene Expression

Product	Microorganism	Change
Actinorhodin	*Streptomyces coelicolor*	Modification of gene transcription
Cellulase	*Clostridium* genes in *Bacillus*	Amplification of secretion through chromosomal DNA amplification
Recombinant protein albumin	*Saccharomyces cerevisiae*	Fusion to a high-production protein
Heterologous protein	*Saccharomyces cerevisiae*	Use of the inducible strong hybrid promoter UAS_{gal}/CYCl
Enhanced growth rate[a]	*Aspergillus nidulans*	Overproduction of glyceraldehyde-3-phosphate dehydrogenase
Amino acids[b]	*Corynebacterium*	Isolation of biosynthetic genes that lead to enhanced enzyme activities or removal of feedback regulation

[a,b]S. Ostergaard; L. Olsson; and J. Nielson. 2000. Metabolic engineering of *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 64(1):34–50. Table 1, p. 35

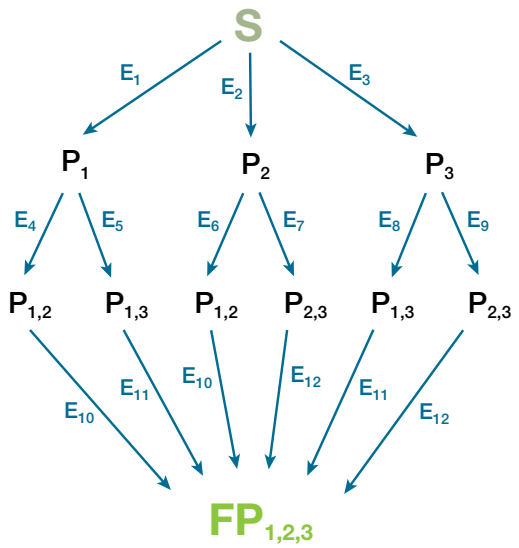


Figure 42.3 Pathway Architecture, a Critical Factor in Metabolic Engineering. Alternative steps for addition of three functional groups to a basic chemical skeleton may have different efficiencies, and it is critical to choose the most efficient combination of enzymatic steps or pathway to yield the desired product. E1 → E12 = different enzymes; P = intermediary products after the addition of the first and second functional groups, and FP = final product.

1. What is combinatorial biology and what is the basic approach used in this technique?
2. What types of major products have been created using combinatorial biology?
3. Why might one want to insert a gene in a foreign cell and how is this done?
4. Why is it important to produce specific isomers of products for use in animal and human health?

Modification of Gene Expression

In addition to inserting new genes in organisms, it also is possible to modify gene regulation by changing gene transcription, fusing proteins, creating hybrid promoters, and removing feedback regulation controls. These approaches make it possible to overproduce a wide variety of products, as shown in **table 42.4.** As a further example, genes for the synthesis of the antibiotic actinorhodin have been transferred into strains producing another antibiotic, resulting in the production of two antibiotics by the same cell.

This approach of modifying gene expression also can be used to intentionally alter metabolic pathways by inactivation or deregulation of specific genes, which is the field of **pathway architecture,** as shown in **figure 42.3.** Alternative routes can be used to add three functional groups to a molecule. Some of these pathways may be more efficient than the others. Understanding pathway architecture makes it possible to design a pathway that will be most efficient by avoiding slower or energetically more costly routes. This approach has been used to improve penicillin production by ***m*etabolic *p*athway *e*ngineering (MPE).**

An interesting recent development in modifying gene expression, which illustrates **metabolic control engineering,** is that of altering controls for the synthesis of lycopene, an important antioxidant normally present at high levels in tomatoes and tomato products. In this case, an engineered regulatory circuit was designed to control lycopene synthesis in response to the internal metabolic state of *E. coli.* An artificially engineered region that controls two key lycopene synthesis enzymes is stimulated by excess glycolytic activity and influences acetyl phosphate levels, thus allowing a significant increase in lycopene production while reducing negative impacts of metabolic imbalances.

Another recent development is the use of modified gene expression to produce variants of the antibiotic erythromycin. Blocking specific biochemical steps (**figure 42.4**) in pathways for the synthesis of an antibiotic precursor resulted in modified final products. These altered products, which have slightly different structures, can be tested for their possible antimicrobial effects. In addition, by the use of this approach, it is possible to better establish the structure-function relationships of antibiotics. Procedures for using microorganisms in the production of chemical feedstocks also have been developed using this MPE approach. By turning on and off

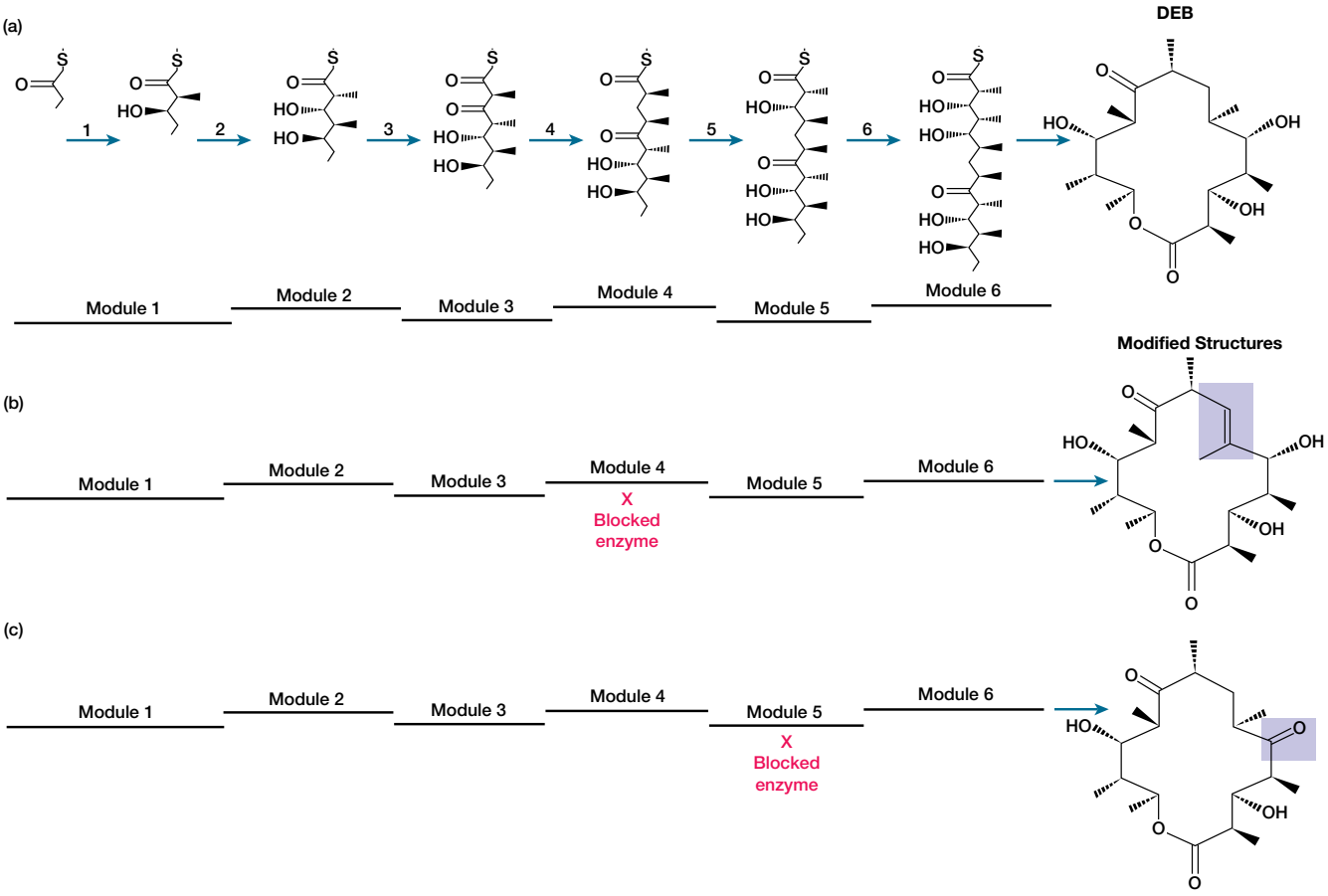


Figure 42.4 Metabolic Engineering to Create Modified Antibiotics. (**a**) Model for six elongation cycles (modules) in the normal synthesis of 6-deoxyerythonilide B (DEB), a precursor to the important antibiotic erythromycin. (**b**) Changes in structure that occur when the enoyl reductase enzyme of module 4 is blocked. (**c**) Changes in structure that occur when the keto reductase enzyme of module 5 is blocked. These changed structures (the highlighted areas) may lead to the synthesis of modified antibiotics with improved properties.

specific genes, feedstock chemicals such as 1,2-propanediol and 1,3-propanediol can be produced at high levels (**figure 42.5**). These particular chemicals are used in semimoist dog foods!

Other examples include the increased synthesis of antibiotics and cellulases, modification of gene expression, DNA amplification, greater protein synthesis, and interactive enzyme overproduction or removal of feedback inhibition. Recombinant plasminogen, for example, may comprise 20 to 40% of the soluble protein in a modified strain, a tenfold increase in concentration over that in the original strain.

Natural Genetic Engineering

The newest approach for creating new metabolic capabilities in a given microorganism is the area of **natural genetic engineering,** which employs **forced evolution** and **adaptive mutations** (*see p. 246*). This is the process of using specific environmental stresses to "force" microorganisms to mutate and adapt, thus creating microorganisms with new biological capabilities. The mechanisms of these adaptive mutational processes include DNA rearrangements in which transposable elements and various types of recombination play critical roles, as shown in **table 42.5.**

Studies on natural genetic engineering are in a state of flux. It may be that "forced processes of evolution" are more effective than rational design in some cases. Such "environmentally directed" mutations have the potential of producing microbes with new degradative or biosynthetic capabilities.

Although there is much controversy concerning this area, the responses of microorganisms to stress provide the potential of generating microorganisms with new microbial capabilities for use in industrial microbiology and biotechnology.

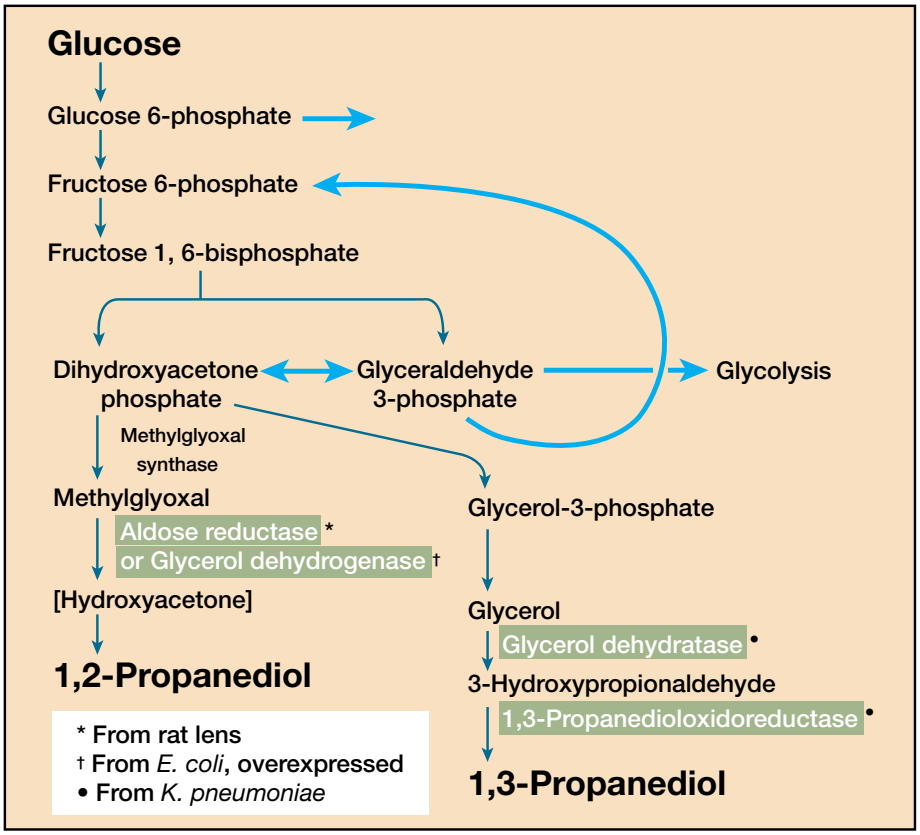


Figure 42.5 Use of Combinatorial Biology to Produce Propanediol in *E. coli.* Either an aldose reductase enzyme from rat lens or an overexpressed *E. coli* glycerol dehydrogenase enzyme and two enzymes from *Klebsiella pneumoniae,* glycerol dehydrogenase and 1,3-propanedioloxidoreductase (all green), are used to shift the intermediary metabolism of *E. coli* to the production of propanediols.

Table 42.5 Natural Genetic Engineering Systems in Bacteria

Genetic Engineering Mechanisms	DNA Changes Mediated
Localized SOS mutagenesis	Base substitutions, frameshifts
Adapted frameshifting	−1 frameshifting
Tn*5*, Tn*9*, Tn*10* precise excision	Reciprocal recombination of flanking 8/9 bp repeats; restores original sequence
In vivo deletion, inversion, fusion, and duplication formation	Generally reciprocal recombination of short sequence repeats; occasionally nonhomologous
Type II topoisomerase recombination	Deletions and fusions by nonhomologous recombination, sometimes at short repeats
Site-specific recombination (type I topoisomerases)	Insertions, excisions/deletions, inversions by concerted or successive cleavage-ligation reactions at short sequence repeats; tolerates mismatches
Transposable elements (many species)	Insertions, transpositions, replicon fusions, adjacent deletions/excisions, adjacent inversions by ligation of 3′ OH transposon ends of 5′ PO_4 groups from staggered cuts at nonhomologous target sites
DNA uptake (transformation competence)	Uptake of single strand independent of sequence, or of double-stranded DNA carrying species identifier sequence

Adapted from J. A. Shapiro. 1999. Natural genetic engineering, adaptive mutation, and bacterial evolution. In *microbial ecology of infectious disease,* E. Rosenberg, editor, 259–75. Washington, D.C.: American Society for Microbiology. Derived from Table 2, pp. 263–64.

Preservation of Microorganisms

Once a microorganism or virus has been selected or created to serve a specific purpose, it must be preserved in its original form for further use and study. Periodic transfers of cultures have been used in the past, although this can lead to mutations and phenotypic changes in microorganisms. To avoid these problems, a variety of culture preservation techniques may be used to maintain desired culture characteristics (**table 42.6**). **Lyophilization,** or freeze-drying, and storage in liquid nitrogen are frequently employed with microorganisms. Although lyophilization and liquid

Table 42.6 Methods Used to Preserve Cultures of Interest for Industrial Microbiology and Biotechnology

Method	Comments
Periodic transfer	Variables of periodic transfer to new media include transfer frequency, medium used, and holding temperature; this can lead to increased mutation rates and production of variants
Mineral oil slant	A stock culture is grown on a slant and covered with sterilized mineral oil; the slant can be stored at refrigerator temperature
Minimal medium, distilled water, or water agar	Washed cultures are stored under refrigeration; these cultures can be viable for 3 to 5 months or longer
Freezing in growth media	Not reliable; can result in damage to microbial structures; with some microorganisms, however, this can be a useful means of culture maintenance
Drying	Cultures are dried on sterile soil (soil stocks), on sterile filter paper disks, or in gelatin drops; these can be stored in a desiccator at refrigeration temperature, or frozen to improve viability
Freeze-drying (lyophilization)	Water is removed by sublimation, in the presence of a cryoprotective agent; sealing in an ampule can lead to long-term viability, with 30 years having been reported
Ultrafreezing	Liquid nitrogen at –196°C is used, and cultures of fastidious microorganisms have been preserved for more than 15 years

nitrogen storage are complicated and require expensive equipment, they do allow microbial cultures to be stored for years without loss of viability or an accumulation of mutations.

1. What types of recombinant DNA techniques are being used to modify gene expression in microorganisms?
2. Define metabolic control engineering, metabolic pathway engineering, forced evolution, and adaptive mutations.
3. Why might natural genetic engineering be useful in modern microbial biotechnology?
4. What approaches can be used for the preservation of microorganisms?

42.2 Microorganism Growth in Controlled Environments

For many industrial processes, microorganisms must be grown using specifically designed media under carefully controlled conditions, including temperature, aeration, and nutrient feeding during the course of the fermentation. The growth of microorganisms under such controlled environments is expensive, and this approach is used only when the desired product can be sold for a profit. These high costs arise from the expense of development of the particular microorganism to be used in a large-scale fermentation, the equipment, medium preparation, product purification and packaging, and marketing efforts. In addition, if this is a product to be used in animal or human health care, literally millions of dollars must be spent conducting trials and obtaining regulatory approval before even a dollar of income is available to investors. Patents are obtained whenever possible to assure that investment costs can be recovered over a longer time period. Clearly products that are brought to market must have a high monetary value. The development of appropriate culture media and the growth of microorganisms under industrial conditions are the subjects of this section.

Table 42.7 Fermentation: A Word with Many Meanings for the Microbiologist

1. Any process involving the mass culture of microorganisms, either aerobic or anaerobic
2. Any biological process that occurs in the absence of O_2
3. Food spoilage
4. The production of alcoholic beverages
5. Use of an organic substrate as the electron donor and acceptor
6. Use of an organic substrate as a reductant, and of the same partially degraded organic substrate as an oxidant
7. Growth dependent on substrate-level phosphorylation

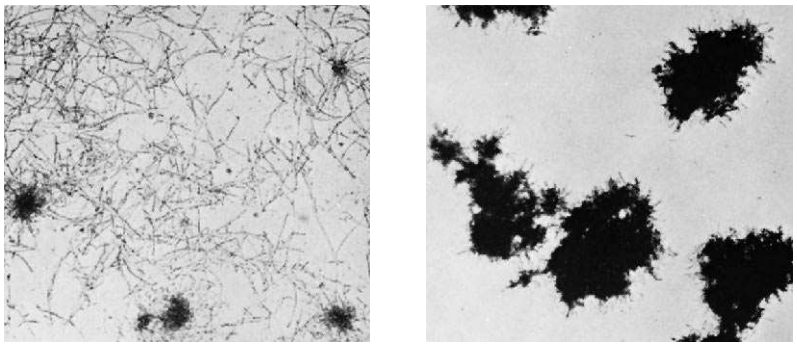
Before proceeding, it is necessary to clarify terminology. The term **fermentation,** used in a physiological sense in earlier sections of the book, is employed in a much more general way in relation to industrial microbiology and biotechnology. As noted in **table 42.7,** the term can have several meanings, including the mass culture of microorganisms (or even plant and animal cells). The development of industrial fermentations requires appropriate culture media and the large-scale screening of microorganisms. Often years are needed to achieve optimum product yields. Many isolates are tested for their ability to synthesize a new product in the desired quantity. Few are successful. Fermentation as a physiological process (pp. 179–81)

Medium Development

The medium used to grow a microorganism is critical because it can influence the economic competitiveness of a particular process. Frequently, lower-cost crude materials are used as sources of carbon, nitrogen, and phosphorus (**table 42.8**). Crude plant hydrolysates often are used as complex sources of carbon, nitrogen, and growth factors. By-products from the brewing industry frequently are employed because of their lower cost and greater availability. Other useful carbon sources include molasses and whey from cheese manufacture. Microbial growth media (pp. 104–6)

Table 42.8 Major Components of Growth Media Used in Industrial Processes

Source	Raw Material	Source	Raw Material
Carbon and energy	Molasses	Vitamins	Crude preparations of plant and animal products
	Whey		
	Grains	Iron, trace salts	Crude inorganic chemicals
	Agricultural wastes (corncobs)	Buffers	Chalk or crude carbonates
			Fertilizer-grade phosphates
Nitrogen	Corn-steep liquor	Antifoam agents	Higher alcohols
	Soybean meal		Silicones
	Stick liquor (slaughterhouse products)		Natural esters
	Ammonia and ammonium salts		Lard and vegetable oils
	Nitrates		
	Distiller's solubles		



(a) (b)

Figure 42.6 Filamentous Growth During Fermentation. Filamentous fungi and actinomycetes can change their growth form during the course of a fermentation. The development of pelleted growth by fungi has major effects on oxygen transfer and energy required to agitate the culture. (**a**) Initial culture. (**b**) after 18 hours growth.

The levels and balance of minerals (especially iron) and growth factors can be critical in medium formulation. For example, biotin and thiamine, by influencing biosynthetic reactions, control product accumulation in many fermentations. The medium also may be designed so that carbon, nitrogen, phosphorus, iron, or a specific growth factor will become limiting after a given time during the fermentation. In such cases the limitation often causes a shift from growth to production of desired metabolites.

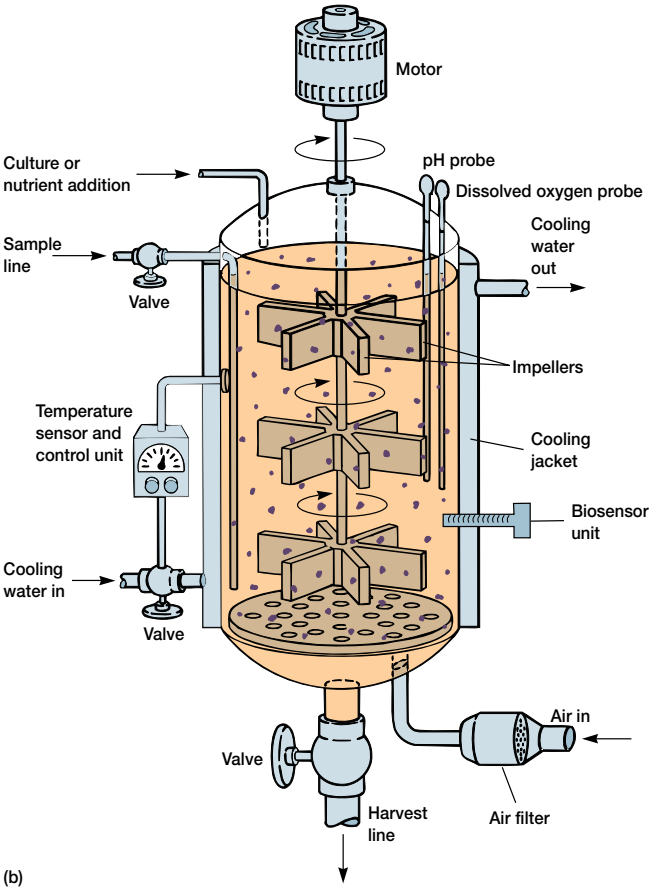
Growth of Microorganisms in an Industrial Setting

Once a medium is developed, the physical environment for microbial functioning in the mass culture system must be defined. This often involves precise control of agitation, temperature, pH changes, and oxygenation. Phosphate buffers can be used to control pH while also functioning as a source of phosphorus. Oxygen limitations, especially, can be critical in aerobic growth processes. The O_2 concentration and flux rate must be sufficiently high to have O_2 in excess within the cells so that it is not limiting. This is especially true when a dense microbial culture is growing. When filamentous fungi and actinomycetes are cultured, aeration can be even further limited by filamentous growth (**figure 42.6**). Such filamentous growth results in a viscous, plastic medium, known as a **non-Newtonian broth,** which offers even more resistance to stirring and aeration. To minimize this problem, cultures can be grown as pellets or flocs or bound to artificial particles.

It is essential to assure that these physical factors are not limiting microbial growth. This is most critical during **scaleup,** where a successful procedure developed in a small shake flask is modified for use in a large fermenter. One must understand the microenvironment of the culture and maintain similar conditions near the individual cell despite increases in the culture volume. If a successful transition can be made from a process originally developed in a 250 ml Erlenmeyer flask to a 100,000 liter reactor, then the process of scaleup has been carried out properly.

Microorganisms can be grown in culture tubes, shake flasks, and stirred fermenters or other mass culture systems. Stirred fermenters can range in size from 3 or 4 liters to 100,000 liters or larger, depending on production requirements (**figure 42.7**). A typical industrial stirred fermentation unit is illustrated in figure 42.7*b*. This unit requires a large capital investment and skilled operators. All required steps in the growth and harvesting of products must be carried out under aseptic conditions. Not only must the medium be sterilized but aeration, pH adjustment, sampling, and process monitoring must be carried out under rigorously controlled conditions. When required, foam control agents must be added, especially with high-protein media. Computers are commonly used to monitor outputs from probes that determine microbial biomass, levels of critical metabolic

(a)



(b)

Figure 42.7 Industrial Stirred Fermenters. (**a**) Large fermenters used by a pharmaceutical company for the microbial production of antibiotics. (**b**) Details of a fermenter unit. This unit can be run under aerobic or anaerobic conditions, and nutrient additions, sampling, and fermentation monitoring can be carried out under aseptic conditions. Biosensors and infrared monitoring can provide real-time information on the course of the fermentation. Specific substrates, metabolic intermediates, and final products can be detected.

products, pH, input and exhaust gas composition, and other parameters. Such information is needed for precise process and product control. Environmental conditions can be changed or held constant over time, depending on the goals for the particular process.

Frequently a critical component in the medium, often the carbon source, is added continuously—**continuous feed**—so that the microorganism will not have excess substrate available at any given time. An excess of substrate can cause undesirable metabolic waste products to accumulate. This is particularly important with glucose and other carbohydrates. If excess glucose is present at the beginning of a fermentation, it can be catabolized to yield ethanol, which is lost as a volatile product and reduces the final yield. This can occur even under aerobic conditions.

Besides the traditional stirred aerobic or anaerobic fermenter, other approaches can be used to grow microorganisms. These alternatives, illustrated in **figure 42.8,** include lift-tube fermenters (figure 42.8*a*), which eliminate the need for stirrers that can be fouled by filamentous fungi. Also available is solid-state fermentation (figure 42.8*b*), in which the substrate is not diluted in water. In various types of fixed- (figure 42.8*c*) and fluidized-bed reactors (figure 42.8*d*), the microorganisms are associated with inert surfaces as biofilms (*see pp. 620–22*), and medium flows past the fixed or suspended particles.

Dialysis culture units also can be used (figure 42.8*e*). These units allow toxic waste metabolites or end products to diffuse away from the microbial culture and permit new substrates to diffuse through the membrane toward the culture. Continuous culture techniques using chemostats (figure 42.8*f*) can markedly improve cell outputs and rates of substrate use because microorganisms can be maintained in a continuous logarithmic phase. However, continuous maintenance of an organism in an active growth phase is undesirable in many industrial processes.

Microbial products often are classified as primary and secondary metabolites. As shown in **figure 42.9, primary metabolites** consist of compounds related to the synthesis of microbial cells in the growth phase. They include amino acids, nucleotides, and fermentation end products such as ethanol and organic acids. In addition, industrially useful enzymes, either associated with the microbial cells or exoenzymes, often are synthesized by microorganisms during growth. These enzymes find many uses in food production and textile finishing.

Secondary metabolites usually accumulate during the period of nutrient limitation or waste product accumulation that follows the active growth phase. These compounds have no direct relationship to the synthesis of cell materials and normal growth. Most antibiotics and the mycotoxins fall into this category.

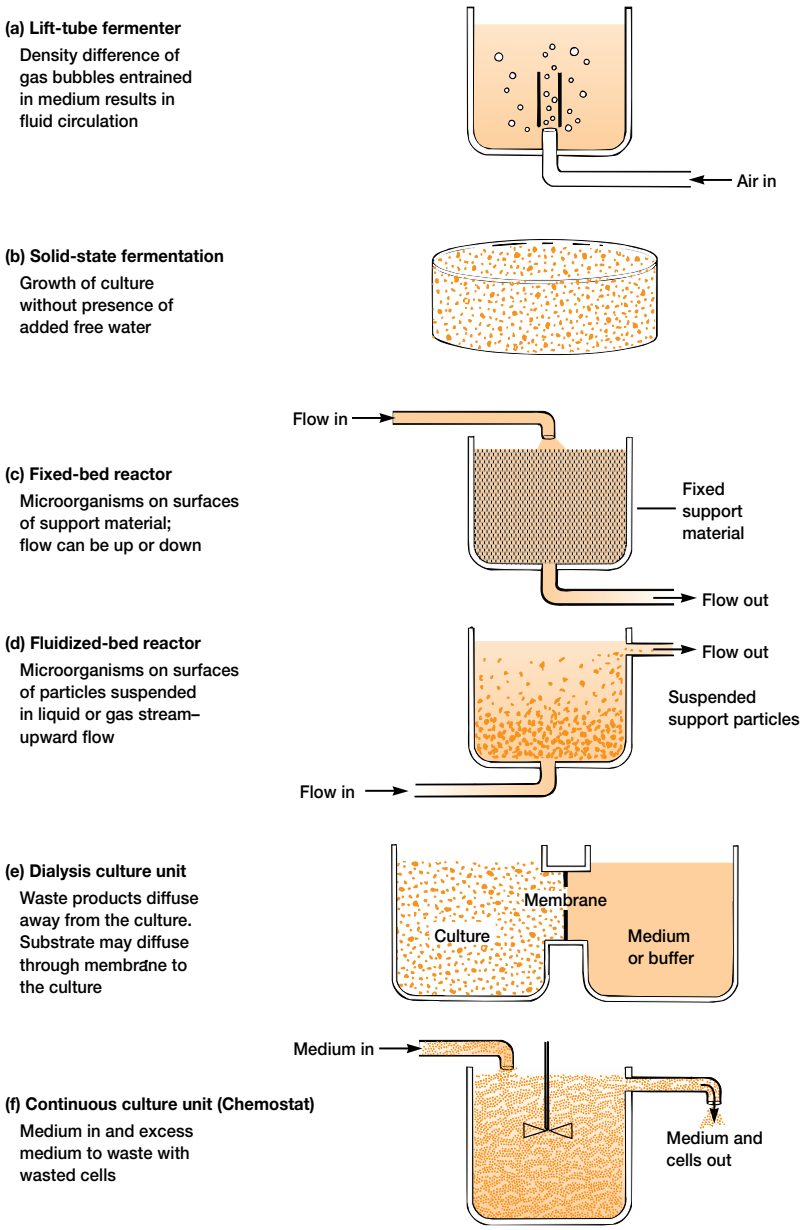


Figure 42.8 Alternate Methods for Mass Culture. In addition to stirred fermenters, other methods can be used to culture microorganisms in industrial processes. In many cases these alternate approaches will have lower operating costs and can provide specialized growth conditions needed for product synthesis.

1. How is the cost of media reduced during industrial operations? Discuss the effect of changing balances in nutrients such as minerals, growth factors, and the sources of carbon, nitrogen, and phosphorus.
2. What factors increase the costs of microbial products, such as antibiotics, used in animal and human health?
3. What are non-Newtonian broths, and why are these important in fermentations?
4. Discuss scaleup and the objective of the scaleup process.
5. What parameters can be monitored in a modern, large-scale industrial fermentation?
6. Besides the aerated, stirred fermenter, what other alternatives are available for the mass culture of microorganisms in industrial processes? What is the principle by which a dialysis culture system functions?

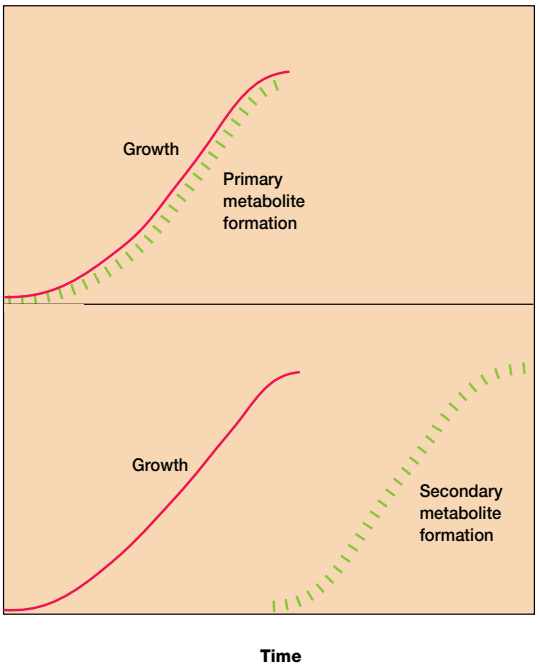


Figure 42.9 Primary and Secondary Metabolites. Depending on the particular organism, the desired product may be formed during or after growth. Primary metabolites are formed during the active growth phase, whereas secondary metabolites are formed after growth is completed.

42.3 Major Products of Industrial Microbiology

Industrial microbiology has provided products that have impacted our lives in many direct and often not appreciated ways. These products have profoundly changed our lives and life spans. They include industrial and agricultural products, food additives, medical products for human and animal health, and biofuels (**table 42.9**). Particularly, in the last few years, nonantibiotic compounds used in medicine and health have made major contributions to the improved well-being of animal and human populations. Only major products in each category will be discussed here.

Antibiotics

Many antibiotics are produced by microorganisms, predominantly by actinomycetes in the genus *Streptomyces* and by filamentous fungi (*see table 35.2*). In this chapter, the synthesis of several of the most important antibiotics will be discussed to illustrate the critical role of medium formulation and environmental control in the production of these important compounds. Antibiotics in medicine (chapter 35)

Penicillin

Penicillin, produced by *Penicillium chrysogenum,* is an excellent example of a fermentation for which careful adjustment of the medium composition is used to achieve maximum yields. Rapid production of cells, which can occur when high levels of glucose are used as a carbon source, does not lead to maximum antibiotic

Table 42.9 Major Microbial Products and Processes of Interest in Industrial Microbiology and Biotechnology

Substances	Microorganisms
Industrial Products	
Ethanol (from glucose)	*Saccharomyces cerevisiae*
Ethanol (from lactose)	*Kluyveromyces fragilis*
Acetone and butanol	*Clostridium acetobutylicum*
2,3-butanediol	*Enterobacter, Serratia*
Enzymes	*Aspergillus, Bacillus, Mucor, Trichoderma*
Agricultural Products	
Gibberellins	*Gibberella fujikuroi*
Food Additives	
Amino acids (e.g., lysine)	*Corynebacterium glutamicum*
Organic acids (citric acid)	*Aspergillus niger*
Nucleotides	*Corynebacterium glutamicum*
Vitamins	*Ashbya, Eremothecium, Blakeslea*
Polysaccharides	*Xanthomonas*
Medical Products	
Antibiotics	*Penicillium, Streptomyces, Bacillus*
Alkaloids	*Claviceps purpurea*
Steroid transformations	*Rhizopus, Arthrobacter*
Insulin, human growth hormone, somatostatin, interferons	*Escherichia coli, Saccharomyces cerevisiae,* and others (recombinant DNA technology)
Biofuels	
Hydrogen	Photosynthetic microorganisms
Methane	*Methanobacterium*
Ethanol	*Zymomonas, Thermoanaerobacter*

yields. Provision of the slowly hydrolyzed disaccharide lactose, in combination with limited nitrogen availability, stimulates a greater accumulation of penicillin after growth has stopped (**figure 42.10**). The same result can be achieved by using a slow continuous feed of glucose. If a particular penicillin is needed, the specific precursor is added to the medium. For example, phenylacetic acid is added to maximize production of penicillin G, which has a benzyl side chain (*see figure 35.7*). This "steering" process is used to maximize the production of desired compounds. The fermentation pH is maintained around neutrality by the addition of sterile alkali, which assures maximum stability of the newly synthesized penicillin. Once the fermentation is completed, normally in 6 to 7 days, the broth is separated from the fungal mycelium and processed by absorption, precipitation, and crystallization to yield the final product. This basic product can then be modified by chemical procedures to yield a variety of **semisynthetic penicillins.**

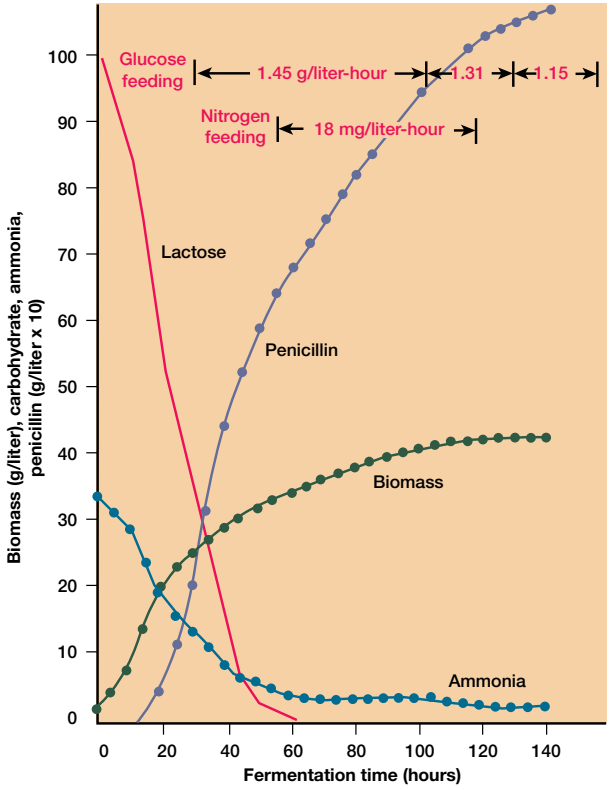


Figure 42.10 Penicillin Fermentation Involves Precise Control of Nutrients. The synthesis of penicillin begins when nitrogen from ammonia becomes limiting. After most of the lactose (a slowly catabolized disaccharide) has been degraded, glucose (a rapidly used monosaccharide) is added along with a low level of nitrogen. This stimulates maximum transformation of the carbon sources to penicillin.

Streptomycin

Streptomycin is a secondary metabolite produced by *Streptomyces griseus,* for which changes in environmental conditions and substrate availability also influence final product accumulation. In this fermentation a soybean-based medium is used with glucose as a carbon source. The nitrogen source is thus in a combined form (soybean meal), which limits growth. After growth the antibiotic levels in the culture begin to increase (**figure 42.11**) under conditions of controlled nitrogen limitation.

The field of antibiotic development continues to expand. At present, 6,000 antibiotics have been described, with 4,000 of these derived from actinomycetes. About 300 new antibiotics are being discovered per year.

Amino Acids

Amino acids such as lysine and glutamic acid are used in the food industry as nutritional supplements in bread products and as flavor-enhancing compounds such as monosodium glutamate (MSG).

Amino acid production is typically carried out by means of **regulatory mutants,** which have a reduced ability to limit synthesis of an end product. The normal microorganism avoids overproduction of biochemical intermediates by the careful regulation of cellular metabolism. Production of glutamic acid and several other amino acids in large quantities is now carried out using mutants of

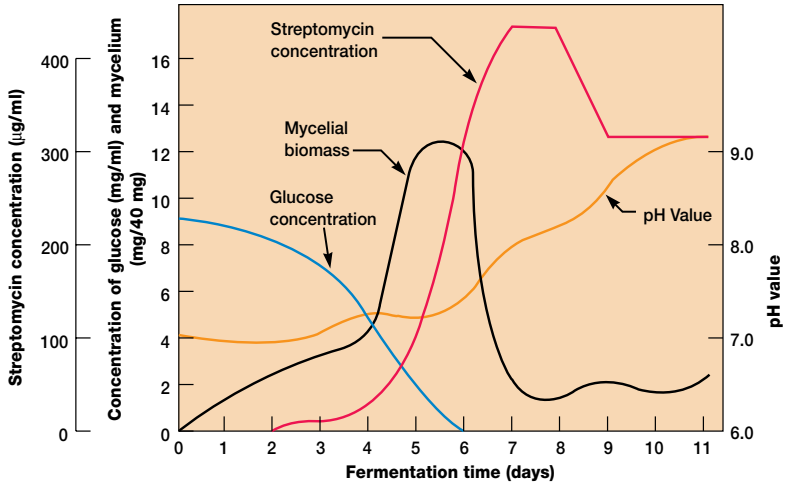


Figure 42.11 Streptomycin Production by *Streptomyces griseus*. Depletion of glucose leads to maximum antibiotic yields.

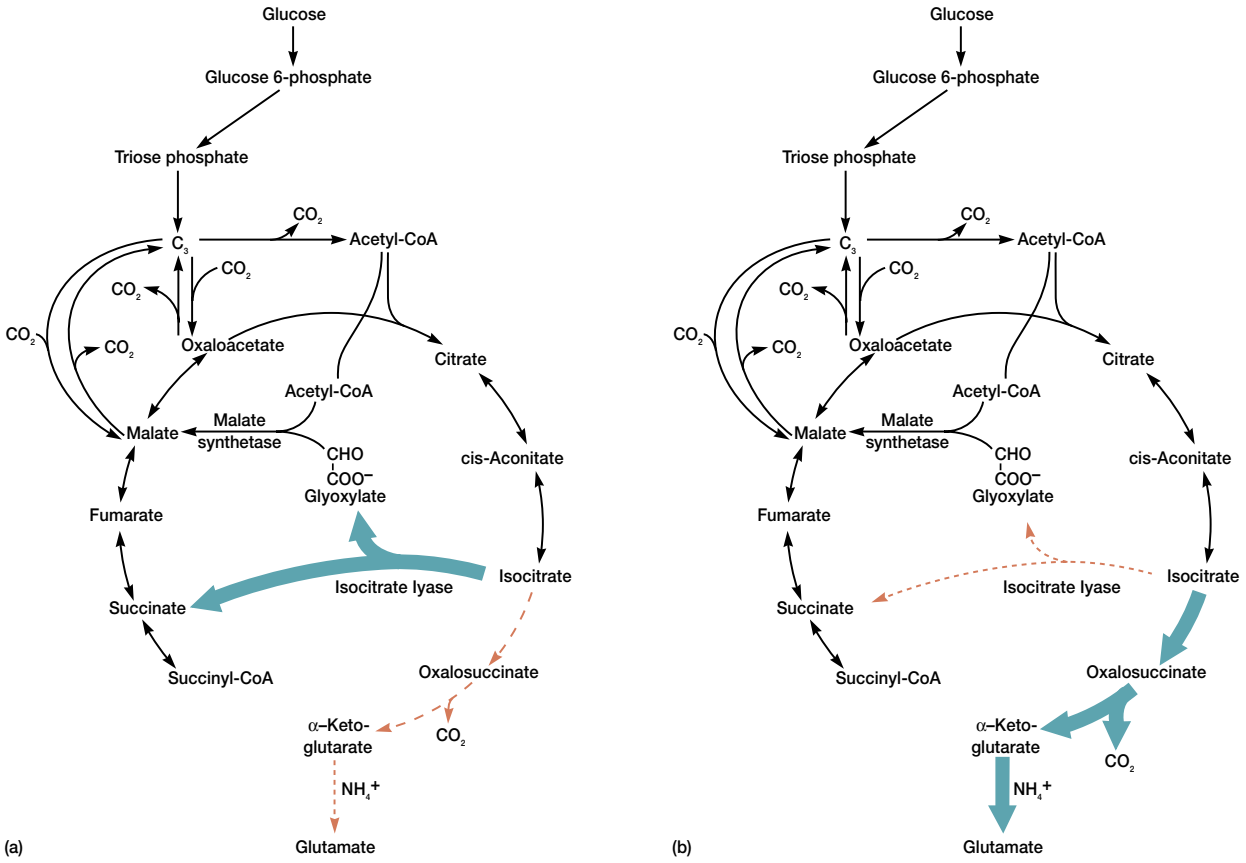


Figure 42.12 Glutamic Acid Production. The sequence of biosynthetic reactions leading from glucose to the accumulation of glutamate by *Corynebacterium glutamicum.* Major carbon flows are noted by bold arrows. (**a**) Growth with use of the glyoxylate bypass to provide critical intermediates in the TCA cycle. (**b**) After growth is completed, most of the substrate carbon is processed to glutamate (note shifted bold arrows). The dashed lines indicate reactions that are being used to a lesser extent.

Corynebacterium glutamicum that lack, or have only a limited ability to process, the TCA cycle intermediate α-ketoglutarate (*see appendix II*) to succinyl-CoA as shown in **figure 42.12.** A controlled low biotin level and the addition of fatty acid derivatives results in increased membrane permeability and excretion of high concentrations of glutamic acid. The impaired bacteria use the glyoxylate pathway (*see section 10.6*) to meet their needs for essential biochemical intermediates, especially during the growth phase. After growth becomes limited because of changed nutrient availability, an almost complete molar conversion (or 81.7% weight conversion) of isocitrate to glutamate occurs.

Lysine, an essential amino acid used to supplement cereals and breads, was originally produced in a two-step microbial process. This has been replaced by a single-step fermentation in which the bacterium *Corynebacterium glutamicum,* blocked in the synthesis of homoserine, accumulates lysine. Over 44 g/liter can be produced in a 3 day fermentation.

Although not used extensively in the United States, microorganisms with related regulatory mutations have been employed to produce a series of 5′ purine nucleotides that serve as flavor enhancers for soups and meat products.

Organic Acids

Organic acid production by microorganisms is important in industrial microbiology and illustrates the effects of trace metal levels and balances on organic acid synthesis and excretion. Citric, acetic, lactic, fumaric, and gluconic acids are major products (**table 42.10**). Until microbial processes were developed, the major source of citric acid was citrus fruit from Italy. Today most citric acid is produced by microorganisms; 70% is used in the food and beverage industry, 20% in pharmaceuticals, and the balance in other industrial applications.

The essence of citric acid fermentation involves limiting the amounts of trace metals such as manganese and iron to stop *As-*

Table 42.10 Major Organic Acids Produced by Microbial Processes

Product	Microorganism Used	Representative Uses	Fermentation Conditions
Acetic acid	*Acetobacter* with ethanol solutions	Wide variety of food uses	Single-step oxidation, with 15% solutions produced; 95–99% yields
Citric acid	*Aspergillus niger* in molasses-based medium	Pharmaceuticals, as a food additive	High carbohydrate concentrations and controlled limitation of trace metals; 60–80% yields
Fumaric acid	*Rhizopus nigricans* in sugar-based medium	Resin manufacture, tanning, and sizing	Strongly aerobic fermentation; carbon-nitrogen ratio is critical; zinc should be limited; 60% yields
Gluconic acid	*Aspergillus niger* in glucose-mineral salts medium	A carrier for calcium and sodium	Uses agitation or stirred fermenters; 95% yields
Itaconic acid	*Aspergillus terreus* in molasses-salts medium	Esters can be polymerized to make plastics	Highly aerobic medium, below pH 2.2; 85% yields
Kojic acid	*Aspergillus flavus-oryzae* in carbohydrate-inorganic N medium	The manufacture of fungicides and insecticides when complexed with metals	Iron must be carefully controlled to avoid reaction with kojic acid after fermentation
Lactic acid	Homofermentative *Lactobacillus delbrueckii*	As a carrier for calcium and as an acidifier	Purified medium used to facilitate extraction

pergillus niger growth at a specific point in the fermentation. The medium often is treated with ion exchange resins to ensure low and controlled concentrations of available metals. Citric acid fermentation, which earlier was carried out by means of static surface growth, now takes place in aerobic stirred fermenters. Generally, high sugar concentrations (15 to 18%) are used, and copper has been found to counteract the inhibition of citric acid production by iron above 0.2 ppm. The success of this fermentation depends on the regulation and functioning of the glycolytic pathway and the tricarboxylic acid cycle (*see section 9.4*). After the active growth phase, when the substrate level is high, citrate synthase activity increases and the activities of aconitase and isocitrate dehydrogenase decrease. This results in citric acid accumulation and excretion by the stressed microorganism.

In comparison, the production of gluconic acid involves a single microbial enzyme, glucose oxidase, found in *Aspergillus niger. A. niger* is grown under optimum conditions in a corn-steep liquor medium. Growth becomes limited by nitrogen, and the resting cells transform the remaining glucose to gluconic acid in a single-step reaction. Gluconic acid is used as a carrier for calcium and iron and as a component of detergents.

Specialty Compounds for Use in Medicine and Health

In addition to the bulk products that have been produced over the last 30 to 40 years, such as antibiotics, amino acids, and organic acids, microorganisms are used for the production of nonantibiotic specialty compounds. These include sex hormones, antitumor agents, ionophores, and special compounds that influence bacteria, fungi, amoebae, insects, and plants (**table 42.11**). In all cases, it is necessary to produce and recover the products under carefully controlled conditions to assure that these medically important compounds reach the consumer in a stable, effective condition.

1. Approximately how many new antibiotics are being discovered per year? What portion of these are derived from actinomycetes?
2. What is the principal limitation created to stimulate citric acid accumulation by *Aspergillus niger?*
3. What types of nutrient limitations are often used in carrying out a successful fermentation? Consider carbon and nitrogen sources.
4. What critical limiting factors are used in the penicillin and streptomycin fermentations?
5. Give some important specialty compounds that are produced by the use of microorganisms.

Biopolymers

Biopolymers are microbially produced polymers used to modify the flow characteristics of liquids and to serve as gelling agents. These are employed in many areas of the pharmaceutical and food industries. The advantage of using microbial biopolymers is that production is independent of climate, political events that can limit raw material supplies, and the depletion of natural resources. Production facilities also can be located near sources of inexpensive substrates (e.g., near agricultural areas). Bacterial exopolysaccharides (p. 61)

At least 75% of all polysaccharides are used as stabilizers, for the dispersion of particulates, as film-forming agents, or to promote water retention in various products. Polysaccharides help maintain the texture of many frozen foods, such as ice cream, that are subject to drastic temperature changes. These polysaccharides must maintain their properties under the pH conditions in the particular food and be compatible with other polysaccharides. They should not lose their physical characteristics if heated.

Biopolymers include (1) dextrans, which are used as blood expanders and absorbents; (2) *Erwinia* polysaccharides that are in

Table 42.11 Nonantibiotic Specialty Compounds Produced by Microorganisms

Compound Type	Source	Specific Product	Process/Organism Affected
Polyethers	*Streptomyces cinnamonensis*	Monensin	Coccidiostat, rumenal growth promoter
	S. lasaliensis	Lasalocid	Coccidiostat, ruminal growth promoter
	S. albus	Salinomycin	Coccidiostat, ruminal growth promoter
Avermectins	*S. avermitilis*		Helminths and arthropods
Statins	*Aspergillus terreus*	Lovastatin	Cholesterol-lowering agent
	Penicillium citrinum + actinomycete[a]	Pravastatin	Cholesterol-lowering agent
Enzyme inhibitors	*S. clavaligerus*	Clavulanic acid	Penicillinase inhibitor
	Actinoplanes sp.	Acarbose	Intestinal glucosidase inhibitor (decreases hyperglycemia and triglyceride synthesis)
Bioherbicide	*S. hygroscopicus*	Bialaphos	
Immunosuppressants	*Tolypocladium inflatum*	Cyclosporin A	Organ transplants
	S. tsukabaensis	FK-506	Organ transplants
	S. hygroscopicus	Rapamycin	Organ transplants
Anabolic agents	*Gibberella zeae*	Zearalenone	Farm animal medication
Uterocontractants	*Claviceps purpurea*	Ergot alkaloids	Induction of labor
Antitumor agents	*S. peuceticus* subsp. *caesius*	Doxorubicin	Cancer treatment
	S. peuceticus	Daunorubicin	Cancer treatment
	S. caespitosus	Mitomycin	Cancer treatment
	S. verticillus	Bleomycin	Cancer treatment

[a]Compactin, produced by *Penicillium citrinum,* is changed to pravastatin by an actinomycete bioconversion.

Based on: A. L. Demain. 2000. Microbial biotechnology. *Tibtech* 18:26–31; A. L. Demain. 2000. Pharmaceutically active secondary metabolites of microorganisms. *App. Microbiol. Biotechnol.* 52:455–463; G. Lancini; A. L. Demain. 1999. Secondary metabolism in bacteria: Antibiotic pathways regulation, and function. In *Biology of the prokaryotes,* J. W. Lengeler, G. Drews, and H. G. Schlegel, editors, 627–51. New York: Thieme.

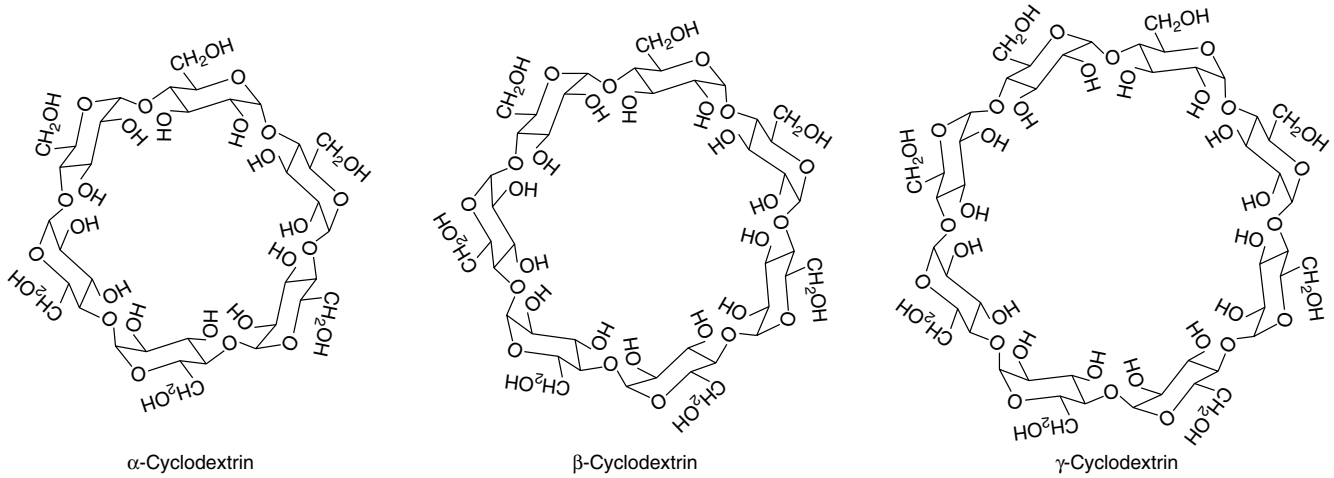


Figure 42.13 **Cyclodextrins.** The basic structures of cyclodextrins produced by *Thermoanaerobacter* are illustrated here. These unique oligopolysaccharides have many applications in medicine and industry.

paints; and (3) polyesters, derived from *Pseudomonas oleovorans,* which are a feedstock for specialty plastics. Cellulose microfibrils, produced by an *Acetobacter* strain, are used as a food thickener. Polysaccharides such as scleroglucan are used by the oil industry as drilling mud additives. Xanthan polymers enhance oil recovery by improving water flooding and the displacement of oil. This use of xanthan gum, produced by *Xanthomonas campestris,* represents a large potential market for this microbial product.

The cyclodextrins have a unique structure, as shown in **figure 42.13.** They are cyclic oligosaccharides whose sugars are joined by α-1,4 linkages. Cyclodextrins can be used for a wide variety of purposes because these cyclical molecules bind with

substances and modify their physical properties. For example, cyclodextrins will increase the solubility of pharmaceuticals, reduce their bitterness, and mask chemical odors. Cyclodextrins also can be used as selective adsorbents to remove cholesterol from eggs and butter or protect spices from oxidation.

Biosurfactants

Many surfactants that have been used for commercial purposes are products of synthetic chemistry. At the present time there is an increasing interest in the use of biosurfactants. These are especially important for environmental applications where biodegradability is a major requirement. Biosurfactants are used for emulsification, increasing detergency, wetting and phase dispersion, as well as for solubilization. These properties are especially important in bioremediation, oil spill dispersion, and enhanced oil recovery (EOR).

The most widely used microbially produced biosurfactants are glycolipids. These compounds have distinct hydrophilic and hydrophobic regions, and the final compound structure and characteristics depend on the particular growth conditions and the carbon source used. Good yields often are obtained with insoluble substrates. These biosurfactants are excellent dispersing agents and have been used with the *Exxon Valdez* oil spill.

Bioconversion Processes

Bioconversions, also known as **microbial transformations** or **biotransformations,** are minor changes in molecules, such as the insertion of a hydroxyl or keto function or the saturation/desaturation of a complex cyclic structure, that are carried out by nongrowing microorganisms. The microorganisms thus act as **biocatalysts.** Bioconversions have many advantages over chemical procedures. A major advantage is stereochemical; the biologically active form of a product is made. In contrast, most chemical syntheses produce racemic mixtures in which only one of the two isomers will be able to be used efficiently by the organism. Enzymes also carry out very specific reactions under mild conditions, and larger water-insoluble molecules can be transformed. Unicellular bacteria, actinomycetes, yeasts, and molds have been used in various bioconversions. The enzymes responsible for these conversions can be intracellular or extracellular. Cells can be produced in batch or continuous culture and then dried for direct use, or they can be prepared in more specific ways to carry out desired bioconversions.

A typical bioconversion is the hydroxylation of a steroid (**figure 42.14**). In this example, the water-insoluble steroid is dissolved in acetone and then added to the reaction system that contains the pregrown microbial cells. The course of the modification is monitored, and the final product is extracted from the medium and purified.

Biotransformations carried out by free enzymes or intact nongrowing cells do have limitations. Reactions that occur in the absence of active metabolism—without reducing power or ATP being available continually—are primarily exergonic reactions (*see section 8.3*). If ATP or reductants are required, an energy source such as glucose must be supplied under carefully controlled nongrowth conditions.

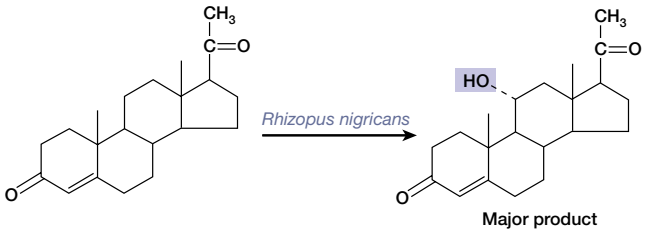


Figure 42.14 Biotransformation to Modify a Steroid. Hydroxylation of progesterone in the 11α position by *Rhizopus nigricans.* The steroid is dissolved in acetone before addition to the pregrown fungal culture.

When freely suspended vegetative cells or spores are employed, the microbial biomass usually is used only once. At the end of the process, the cells are discarded. Cells often can be used repeatedly after attaching them to ion exchange resins by ionic interactions or immobilizing them in a polymeric matrix. Ionic, covalent, and physical entrapment approaches can be used to immobilize microbial cells, spores, and enzymes. Microorganisms also can be immobilized on the inner walls of fine tubes. The solution to be modified is then simply passed through the microorganism-lined tubing; this approach is being applied in many industrial and environmental processes. These include bioconversions of steroids, degradation of phenol, and the production of a wide range of antibiotics, enzymes, organic acids, and metabolic intermediates. One application of cells as biocatalysts is the recovery of precious metals from dilute-process streams.

1. Discuss the major uses for biopolymers and biosurfactants.
2. What are cyclodextrins and why are they important additives?
3. What are bioconversions or biotransformations? Describe the changes in molecules that result from these processes.

42.4 Microbial Growth in Complex Environments

Industrial microbiology and biotechnology also can be carried out in complex natural environments such as waters, soils, or high organic matter–containing composts. In these complex environments, the physical and nutritional conditions for microbial growth cannot be completely controlled, and a largely unknown microbial community is present. These applications of industrial microbiology and biotechnology usually are lower cost, larger volume processes, where no specific commercial microbial product is created. Examples are (1) the use of microbial communities to carry out biodegradation, bioremediation, and environmental maintenance processes; and (2) the addition of microorganisms to soils or plants for the improvement of crop production. Both of these applications will be discussed in this section.