Chapter 5 : Products of industrial fermentations

Primary and secondary metabolites

- Microbial products are often classified as primary metabolites and secondary metabolites.
- Primary metabolites include compounds linked to the synthesis of microbial cells during the growth phase. They include amino acids, nucleotides, and fermentation products such as ethanol and organic acids. Furthermore, industrially useful enzymes, either associated with microbial cells or exoenzymes, are often synthesized by microorganisms during their growth. These enzymes find many uses in food production and textile finishing.
- Secondary metabolites generally accumulate during the period of nutrient limitation or accumulation of degradation products that follows the active growth phase. These compounds have no direct link with the synthesis of cellular materials and normal growth. Most antibiotics and mycotoxins fall into this category.

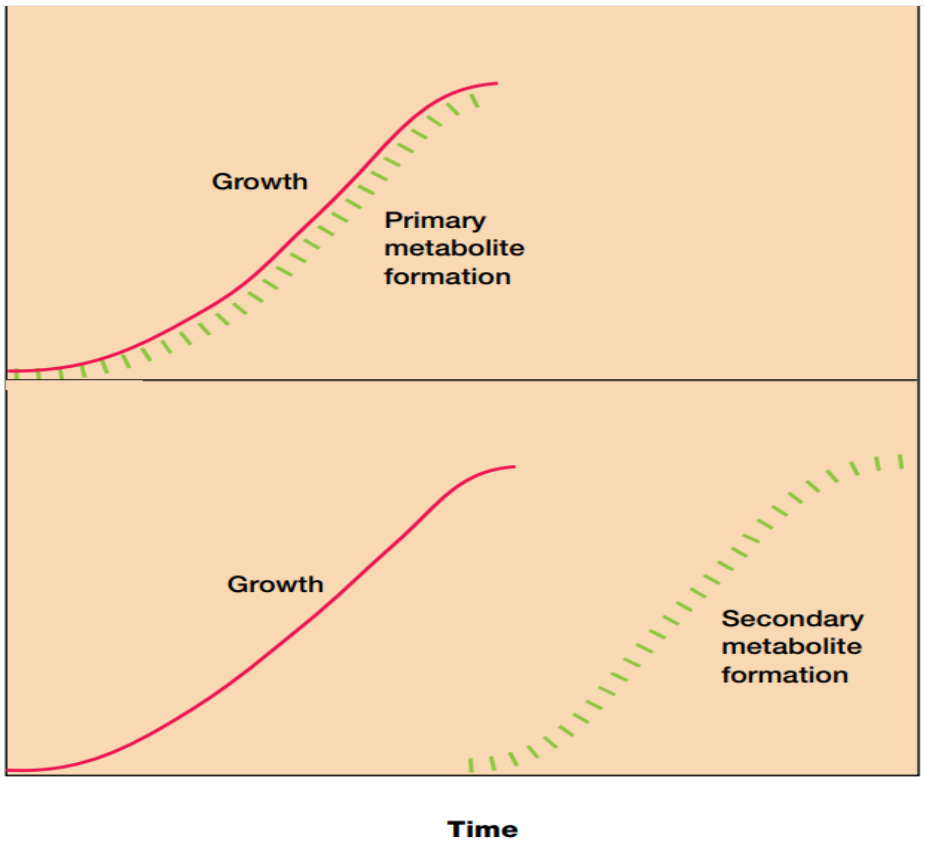


Figure : Primary and Secondary Metabolites. Depending on the particular organism, the desired product may be formed during or after growth. Primary metabolites are formed during the active growth phase, whereas secondary metabolites are formed after growth is completed.

Table: 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*

5.1. Primary metabolites obtained by microbial fermentations

5.1.1. Amino acids

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

Amino acid production is generally carried out using regulatory mutants. The production of glutamic acid and several other amino acids in significant quantities is now achieved using mutants of *Corynebacterium glutamicum*. A controlled low level of biotin and the addition of fatty acid derivatives lead to an increase in membrane permeability and the excretion of high concentrations of glutamic acid. Defective bacteria use the glyoxylate pathway to meet their needs for essential biochemical intermediates, particularly during the growth phase. After growth becomes limited due to modified nutrient availability, an almost complete conversion of isocitrate to glutamate occurs.

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

A. Main microbial strains used

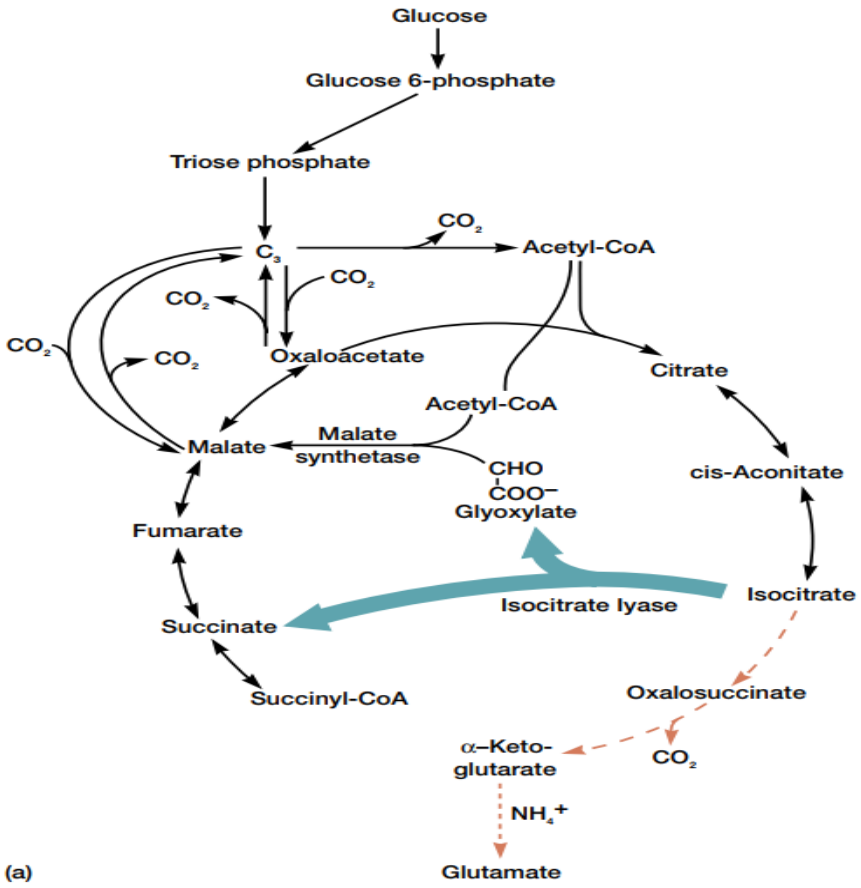
The industrial strains used for the production of glutamic acid are mutants of the species *Corynebacterium glutamicum*, *Corynebacterium callunae*, *Brevibacterium flavum*, and *Brevibacterium lactofermentum*.

B. Culture conditions

The industrial production of glutamic acid by fermentation involves the following steps:

1. **Preparation of the fermentation medium:** A fermentation medium containing a carbon source such as glucose or sucrose is prepared. Nitrogen sources such as ammonium salts, urea, or ammonia are also added, but slowly to avoid inhibition of glutamic acid production. Sugar cane or beet molasses can also be used as a carbon source.
2. **Fermentation:** The bacterial culture, generally *Corynebacterium glutamicum*, is inoculated into the prepared fermentation medium. Fermentation takes place in stainless steel fermenters with a maximum capacity of 450 cubic meters. The culture is agitated and maintained under aerobic conditions at a specific temperature (generally between 30 and 37 °C), depending on the microorganism used. The pH of the medium is maintained between 7 and 8 to avoid excessive decreases due to glutamic acid excretion.
3. **Purification:** Once fermentation is complete (generally between 35 and 40 hours), the fermentation medium containing the produced glutamic acid is subjected to a series of purification steps. First, centrifugation is carried out to separate the microbial biomass from the supernatant containing the glutamic acid. Then, the pH of the supernatant is progressively lowered by adding hydrochloric acid to the isoelectric point of L-glutamic acid (pH = 3.2). This allows the precipitation of L-glutamic acid crystals.

4. **Recovery of crystals:** The precipitated L-glutamic acid crystals are recovered by centrifugation. They are then washed several times to remove impurities and obtain a purified product.
    - It should be noted that the specific details of the production process may vary depending on the conditions and protocols used in each glutamic acid production facility.



5.1.2. Organic acids

The production of organic acids by microorganisms is important in industrial microbiology and illustrates the effects of trace metal levels and balances on the synthesis and excretion of organic acids. Citric, acetic, lactic, fumaric, and gluconic acids are the main products.

Citric acid, also known as citrate, is an organic acid present in large quantities in lemons, hence its name. It is widely used in the food industry as a food additive for its acidifying and flavoring properties. It is found in beverages, confectionery, sour candies, and other foods. Citric acid also finds other applications outside the food industry, particularly in the medical and pharmaceutical fields.

Before the development of microbial processes, the main source of citric acid was citrus fruits. Today, most citric acid is produced by microorganisms. 5% is used in the food and beverage industry, 2% in pharmaceutical products, and the rest in other industrial applications.

Citric acid can be produced by many microorganisms, including:

1. Bacteria: *Bacillus licheniformis* and *Bacillus subtilis*.
2. Molds: *Aspergillus niger*, *Aspergillus awamori*, *Aspergillus foetidus*, and *Penicillium restrictum*.
3. Yeasts: *Candida lipolytica*, *Candida intermedia*, and *Saccharomyces cerevisiae*.

Table 2. 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	Homofementative *Lactobacillus delbrueckii*	As a carrier for calcium and as an acidifier	Purified medium used to facilitate extraction

The essence of citric acid fermentation involves limiting the amounts of trace metals such as manganese and iron to stop the growth of *Aspergillus niger* at a specific point in the fermentation. Citric acid fermentation, which was previously carried out by static surface growth, now takes place in agitated aerobic fermenters. Generally, concentrations (%) are used.

The success of this fermentation depends on the regulation and operation of the glycolytic pathway and the tricarboxylic acid cycle. 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 leads to the accumulation and excretion of citric acid by the stressed microorganism.

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

- **Bioproduction of citric acid**

The bioproduction of citric acid can be described in three main steps (inoculum preparation, fermentation, and extraction). Two types of fermentation are most commonly used, namely submerged fermentation or liquid medium fermentation (LMF) and solid-state fermentation (SSF). Each of the two techniques has advantages and disadvantages; however, the most widely used fermentation is liquid medium fermentation (LMF).

1. Liquid medium fermentation (LMF)

Currently, 99% of the total citric acid produced worldwide is obtained by fermentation. It is estimated that about 80% of this production is obtained by LMF. Indeed, this technique reduces total investment and labor costs by 2.5 to 25%. It has several advantages, such as high productivity and yield, as well as a lower risk of contamination. In general, it is carried out for 5 to 12 days.

However, the disadvantages are higher energy costs and more sophisticated control requiring more highly qualified personnel.

Three important factors intervene in the control of this production technique, which are:

- The quality of the stainless steel for the construction of the bioreactor,
- The structure of the mycelium,
- The transfer of oxygen in the reactor.

2. Solid-state fermentation (SSF)

Solid-state fermentation (SSF) is generally defined as the growth of microorganisms on humid substrates in the absence of free water. The production of citric acid by SSF (the simplest method is the Koji process) was developed in Japan. This fermentation can be carried out using different raw materials. In recent years, the technique has been of great interest because it offers many advantages for the production of citric acid and enzymes. Indeed, solid-state fermentation processes have low energy requirements, produce much less effluent, and therefore generate fewer environmental concerns. The ability to use agro-industrial residues as substrates for the bioproduction of citric acid contributes to their disposal. Furthermore, the biotransformation of agricultural waste such as banana peels.

5.1.3. Biogas

Production of biogas by the methanization process. Methanization, also known as anaerobic digestion, is a natural biological process of degradation of organic matter in the absence of oxygen (anaerobic condition), which leads to the production of biogas. Biogas is mainly composed of methane (CH_4) and carbon dioxide (CO_2).

Methanization takes place in several stages through the intervention of different microbial communities, in anaerobic digesters or methanizers. During this process, microorganisms degrade organic matter, such as food waste, sewage sludge, or agricultural residues, producing methane-rich biogas. This biogas can then be used as a renewable energy source for the production of electricity, heat, or as fuel. Methanization is an effective method for recovering organic waste and helps reduce greenhouse gas emissions while producing a source of green energy.

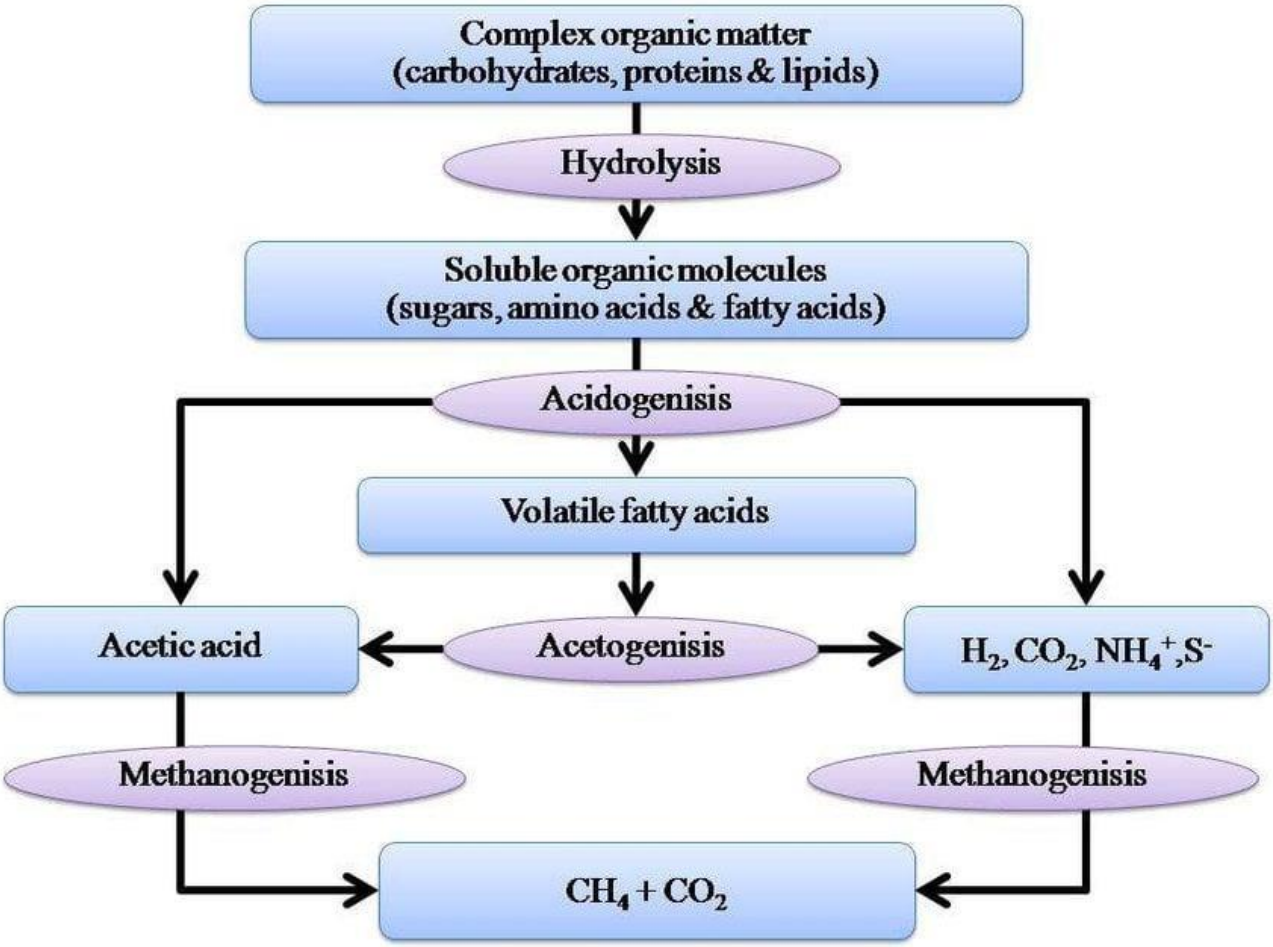


Figure: Stages of anaerobic digestion (methane fermentation process) (Rameshprabu and Natthawud, 2015)

1. **Hydrolysis phase:** Complex organic matter molecules are broken down into simpler compounds by the action of hydrolytic bacteria. This makes the organic matter more accessible for the subsequent steps.

2. **Acidogenesis phase:** The compounds resulting from hydrolysis are metabolized by acidogenic bacteria, which ferment them to produce organic acids such as acetic acid, butyric acid, and propionic acid. This step also generates by-products such as alcohol and ammonia.
3. **Acetogenesis phase:** The organic acids produced during acidogenesis are converted into acetate by acetogenic bacteria. Acetate is an essential precursor for methane production.
4. **Methanogenesis phase:** In this key step, methanogenic bacteria consume acetate and other organic compounds to produce methane (CH_4) and carbon dioxide (CO_2). It is the formation of methane that gives biogas its main energy component.

Purification of biogas (methane)

For its use as an energy source, biogas undergoes:

- Removal of solid particles and dust,
- Dehydration (to remove water),
- Removal of sulfur compounds (H_2S),
- Removal of CO_2.

5.2. Secondary metabolites obtained by microbial fermentation

5.2.1. Antibiotics

A. Background

Definition: Antibiotics are chemical substances produced by microorganisms, such as bacteria or fungi, that have the ability to inhibit the growth or kill other microorganisms, generally bacteria.
Modes of action: Antibiotics can act in different ways on target microorganisms. Some inhibit the synthesis of the bacterial cell wall, disrupt protein synthesis, or interfere with cellular metabolism. Others can alter the cell membrane or inhibit bacterial DNA replication.
Classes of antibiotics: There are many classes of antibiotics, each with a specific mode of action and activity against certain types of bacteria. Common classes of antibiotics include penicillins, cephalosporins, macrolides, quinolones, tetracyclines, and sulfonamides.
Uses: Antibiotics are used to treat bacterial infections in humans and animals. They can be administered orally, by injection, or topically, depending on the type and severity of the infection. Some antibiotics are also used prophylactically to prevent infections before surgery or in immunocompromised individuals.

The industrial production of antibiotics involves the use of microorganisms and follows different steps at different scales, ranging from laboratory production for preliminary studies to production in large-capacity fermenters, up to 500 m3. Each step requires an inoculum to seed the larger volume fermenter, generally between 1 and 15% of the production volume.

The culture of antibiotic-producing microorganisms is generally aerobic, and the production of the antibiotic itself, considered a secondary metabolite of little use to the microorganism, occurs at the end of growth.

Precursors can be added to the culture medium to influence antibiotic synthesis. For example, the addition of phenylacetate promotes the production of ampicillin from the penicillin G-producing fungus.

The production medium must first promote significant cell growth to achieve a high concentration at the time of antibiotic production. Then, it must maintain cell viability and optimize antibiotic production by providing energy sources and ensuring the desired physico-chemical conditions (pH, temperature, oxygenation).

During the antibiotic production phase, cells use energy and carbon sources that are catabolized slowly, such as lactose for penicillin production or dextrin and starch for macrolide production.

Fatty acids and their derivatives are often provided as triglycerides from oils such as soybean, peanut, corn, or rapeseed oil. Besides their role as an energy source and precursors, fatty acids exert beneficial physico-chemical actions, such as emulsion formation, foam reduction, and modification of membrane permeability.

Ammonium is the best nitrogen source to promote rapid growth of microorganisms. Ammonium salts are added to stimulate this phase, carefully monitoring the concentration to avoid a drop in production due to excessively high concentration. If ammonium salt feeding is continued, it is generally done in a semi-continuous manner.

B. Culture conditions

1. pH: pH plays a primordial role in the production of secondary metabolites. Small variations in pH can have marked effects on the productivity of the strain.

2. Temperature: If the temperature range allowing microbial growth is around 25°C, that allowing antibiotic synthesis is only 5 to 10°C. The optimal temperatures for production are situated in often narrow zones and the optima are often lower than for growth.

3. Aeration: All antibiotic productions take place under aerobic conditions. As with environmental factors, the optimal oxygen concentrations are not necessarily the same for growth and for the synthesis of secondary metabolites. For example, cephalosporin production increases whereas penicillin N production decreases if the oxygen concentration is high.

C. Culture mode

Production processes use either batch mode or semi-continuous mode.

- **In batch mode:** Production continues until the medium is depleted of biosynthesis precursors or until unfavorable physiological conditions appear.
- **In semi-continuous mode:** Production can continue by the gradual addition of these precursor sources, which imposes rigorous control of the concentration of necessary elements.

D. Factors influencing antibiotic production

1. **Catabolic regulation by the carbon source:** For most antibiotic-producing microorganisms, the use of a rapidly assimilable carbon source, such as glucose, has a negative effect on antibiotic biosynthesis. In contrast, the use of slowly catabolizable energy sources, like starch or dextrins, promotes antibiotic production.

2. **Regulation by fatty acids:** Fatty acids play an important role in antibiotic production. They can be present in the oils used or added directly in pure form. The addition of methyl oleate (1%) in the production medium improves nigercin production by more than 700%.
3. **Regulation by the nitrogen source:** High concentrations of ammonium or rapidly metabolizable nitrogen compounds in the culture medium can suppress the biosynthesis of many antibiotics. Ammonium ions promote rapid growth, which represses genes involved in the biosynthesis of enzymes and inhibits certain enzymatic activities. During the production phases, ammonium ions must be present at limited concentrations or replaced by slowly metabolized nitrogen sources
4. **Regulation by phosphate:** High concentrations of phosphate in the culture medium can suppress the synthesis of many antibiotics by directly repressing the biosynthesis of secondary metabolism enzymes.
5. **Trace elements:** Trace elements, such as manganese, iron, and zinc, are necessary in very low concentrations (around 10-7) for the growth of organisms and play an important role in antibiotic biosynthesis.

E. Extraction and purification:

At the end of fermentation, the antibiotic is present at relatively low concentrations in a complex polyphasic mixture including cells, medium components, and numerous metabolites.

The antibiotic extraction and purification process includes several steps:

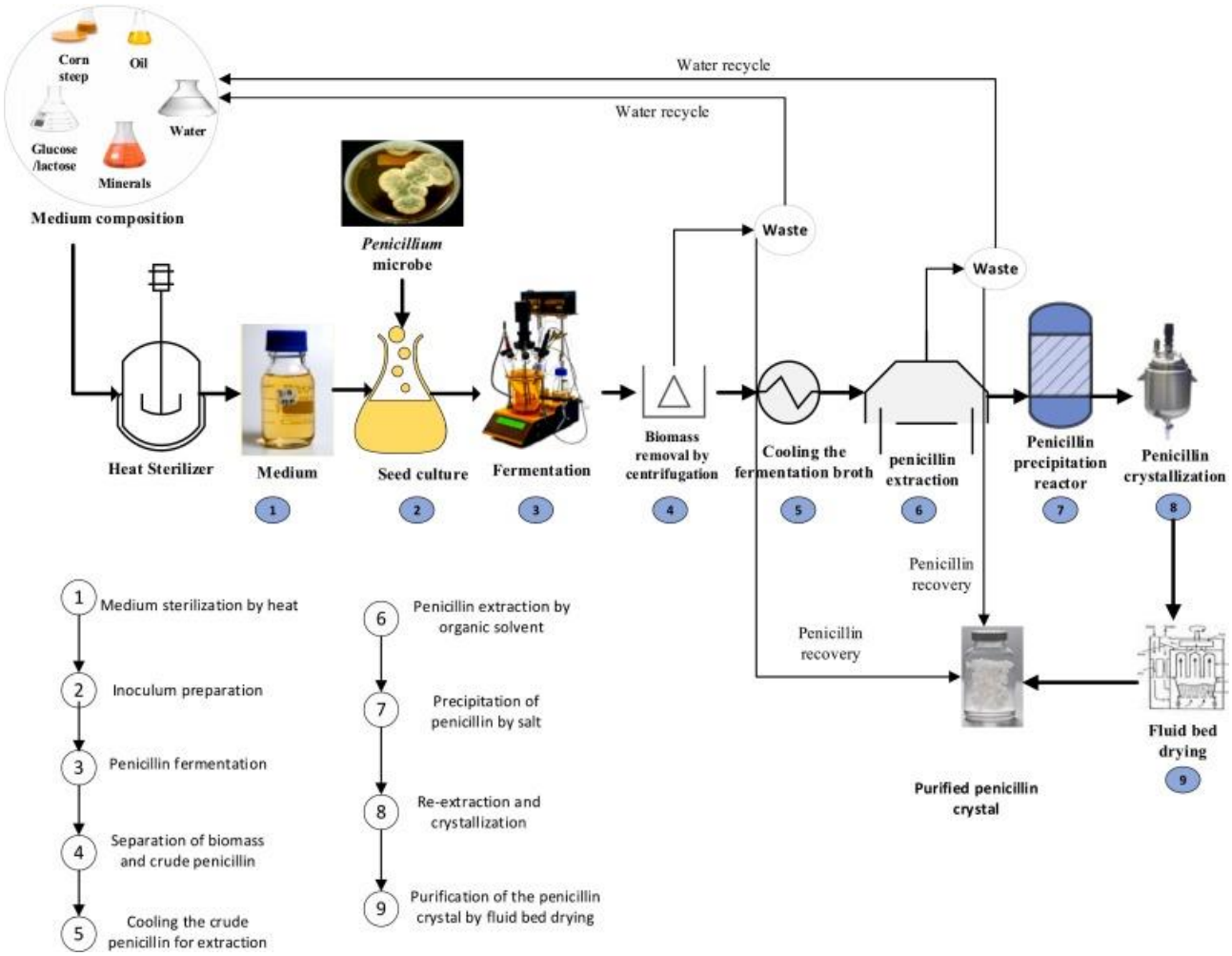
Extraction and purification: After fermentation, antibiotics are present at low concentrations in a complex mixture including cells, medium components, and other metabolites. Extraction and purification steps are necessary to isolate and purify the antibiotic. Due to the diversity of producing organisms, production media, and physicochemical properties of antibiotics, there is no standard extraction and purification protocol. It is important to quantify the antibiotic and evaluate its activity to assess the step yield and product degradation.

- **Liquid-solid separation:** This separation step can be carried out by decantation, filtration, or centrifugation. It allows the separation of the antibiotic associated with the cells from the fermentation medium, recovering the antibiotic from the obtained liquid and solid fractions.
- **Primary extraction:** If the solid-liquid phases were not separated beforehand, extraction with an appropriate solvent is carried out. Treatment with an acid or a base is often performed to obtain an

ionic form of the antibiotic. The choice of solvent is based on its ability to solubilize the antibiotic, its chemical neutrality, its selectivity for the antibiotic, its cost, and its properties facilitating its removal or reuse. Liquid-liquid extraction requires a solvent miscible with water, which can be difficult for highly hydrophilic molecules.

- Purification: Purification aims to specifically remove impurities compared to the antibiotic using refined techniques. The desired degree of purity depends on the application of the antibiotic. Techniques such as liquid-liquid extraction with different solvents, membrane filtration (ultra or nanofiltration), or gel filtration can be used. If these methods are insufficient, chromatography techniques may be necessary, although they are expensive.

F. Microbially fermented penicillin production process (figure).



Example of industrial production

This fermentation process concerns penicillin G and involves the following steps:

100 ml of medium containing spores of *P. chrysogenum* strains are inoculated into an Erlenmeyer flask and incubated in an oven placed on a rotary shaker. After 4 days of incubation, the contents, along with two liters of medium, are transferred to a flask containing four liters and incubated for a further two days. Then, the contents are transferred to a stainless-steel tank containing 500 ml of medium that offers favorable conditions for fungal growth. After three days of incubation, the contents are used for inoculation and are kept in a fermenter equipped with optimal conditions. The contents are filtered after six days of incubation and contain penicillin. Penicillin is extracted into

amyl acetate or butyl acetate, then transferred into an aqueous solution with a phosphate buffer. Acidify the extract and extract the penicillin again into butyl acetate. In the solvent extract, potassium acetate is added in a crystallization tank to crystallize as the potassium salt. The crystals are recovered, and the salt is then sterilized.

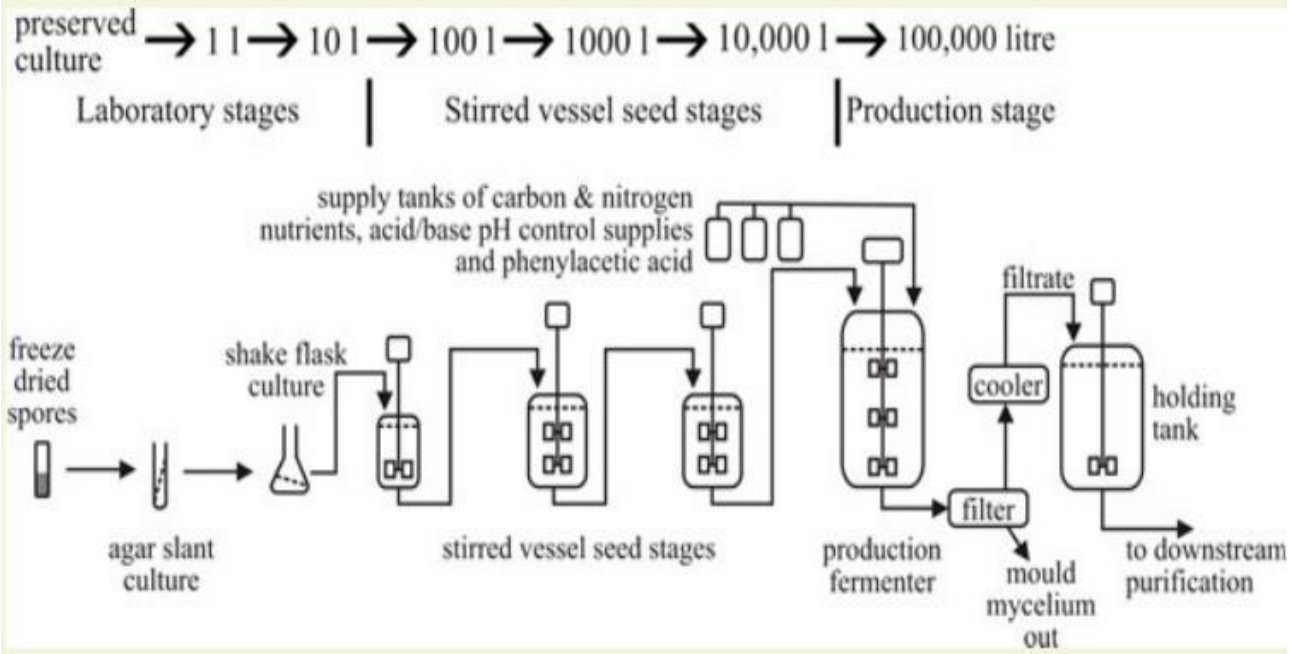


Figure: Schematic diagram of penicillin production by submerged fed-batch fermentation.

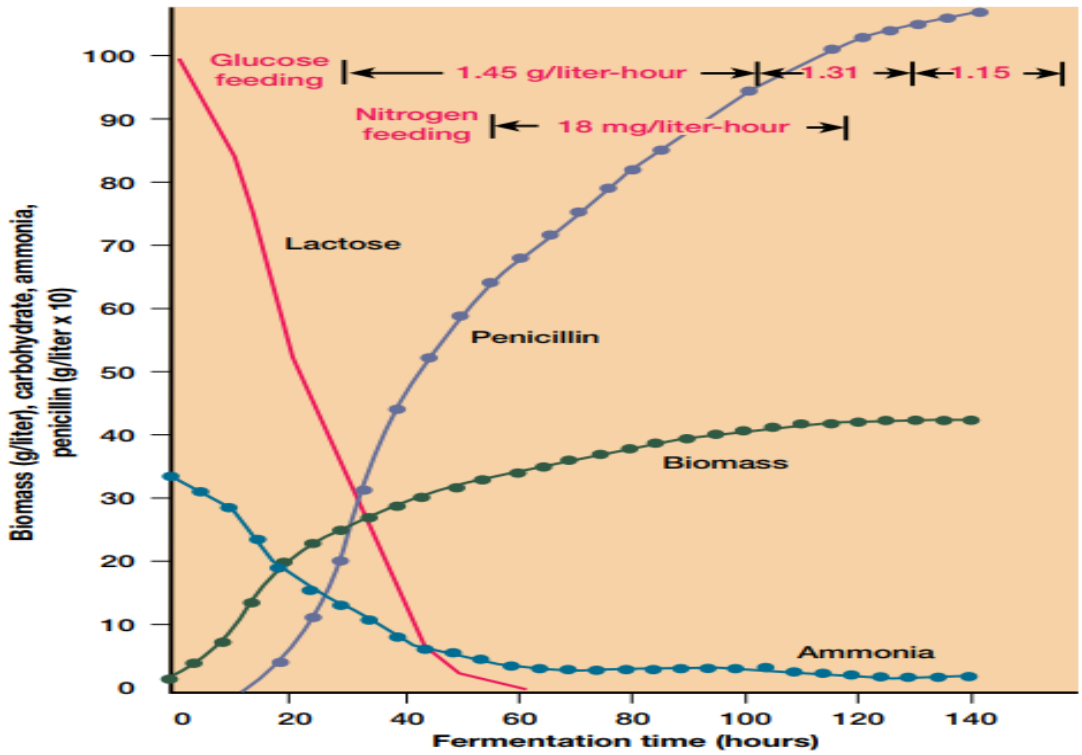


Figure 42.10 Penicillin Fermentation Involves Precise Control of Nutrients. The synthesis of penicillin begins when nitrogen from ammonia becomes limiting. After most of the lactose (a slowly catabolized disaccharide) has been degraded, glucose (a rapidly used monosaccharide) is added along with a low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

5.2.2. Vitamins

Vitamins are active, vital organic substances, essential in small quantities for the metabolism of a living organism, which cannot be synthesized in sufficient quantity by that organism. Vitamins are used in the food industry and the pharmaceutical industry. The best-known vitamin is vitamin C, it is often used in effervescent aspirin formulations and in cooked products. Vitamin B12 is a complex molecule that is synthesized only by prokaryotic microorganisms. Necessary for humans, it is provided by diet or produced by the intestinal flora.

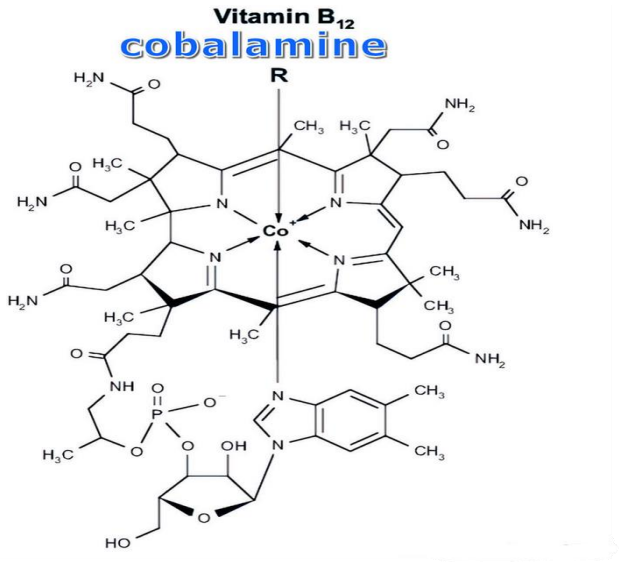
Table : Main vitamins produced by fermentation

Product	**Access route**	**Microorganisms used**
Vitamin B2 (riboflavin)	F	*Ashbya gossypii, Candida famata, Pichia miso*
Vitamin B12 (cyanocobalamin)	F	*Bacillus megaterium, Propionibacterium shermanii, Pseudomonas denitrificans*
Vitamin C (ascorbic acid)	F + C	*Acetobacter suboxydans*
Vitamin D (calciferol)	F + C	*Saccharomyces sp., Aspergillus niger*

- **Vitamin B12**

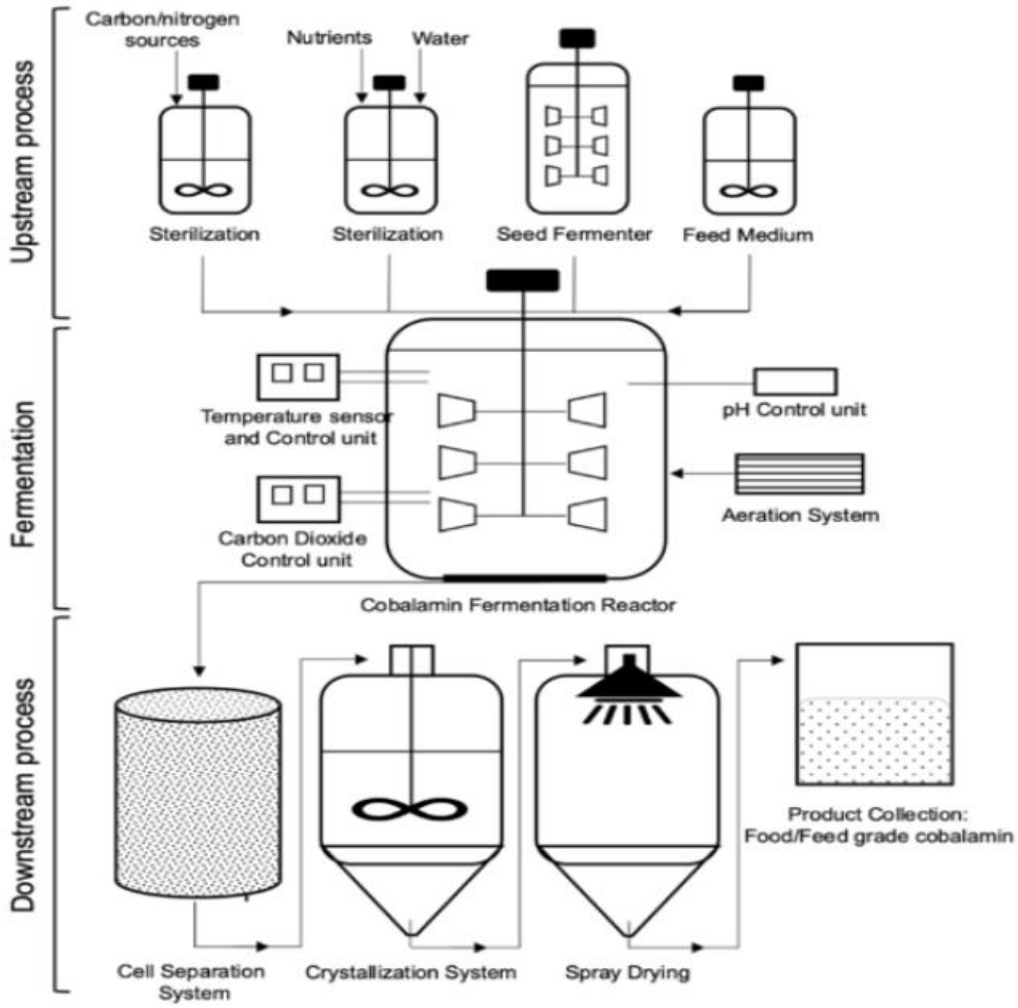
Vitamin B12, or cobalamin, is part of the B vitamin family. The cobalamin molecule contains a cobalt ion, hence its name. Vitamin B12 is currently produced industrially by microbial fermentation. The biotechnological synthesis is done through a few enzymatic steps, followed by the conversion of natural vitamin B12 into an air-stable cyanocobalamin form by reaction with cyanide.

The commercial production of vitamin B12 relies mainly on the use of two industrial strains, *Propionobacterium shermanii* and *Pseudomonas denitrificans*. These microorganisms are widely used due to their efficiency in producing vitamin B12. Although other microorganisms such as *Bacillus megaterium* can be used, they are generally considered less efficient than the first two species.



Industrial production of vit B12 by *Pseudomonas denitrificans*

- The industrial process for producing vitamin B12 uses *Pseudomonas denitrificans* in 120 m3 fermenters, at a temperature of 30°C and pH values of 6.0 to 7.0. Sucrose is the carbon source and yeast extract is used as the nitrogen source with mineral salts. The process duration is about 6 to 7 days.
- During the growth phase, the fermentation is aerated and controlled by adjusting dissolved oxygen and CO2 concentrations, which allows obtaining a cobalamin yield greater than 150 mg/L.
- Improvements have been made using a multi-step dissolved oxygen concentration (DOC) control strategy, increasing the vitamin B12 yield by about 20% (70 mg/L). The dissolved oxygen concentration is progressively reduced during fermentation.
- Another improvement consists of controlling the CO2 fraction, which increases vitamin B12 production by about 10%.
- After fermentation, a mixture of adenosylcobalamin, methylcobalamin, and hydroxocobalamin undergoes chemical processes, including cyanation.
- The extraction of vitamin B12 is done by heating the broth or cell suspension to 80-120 °C for 10-30 minutes at a pH of 6.5-8.5.
- Conversion to cyanocobalamin is achieved by treating the heated broth or cell suspension with cyanide or potassium thiocyanate.
- After clarification of the solution, vitamin B12 is precipitated by adding auxiliaries such as tannic acid or cresol, reaching a purity of about 80%.
- Further purification is often carried out using organic solvents such as cresol, carbon tetrachloride, and water/butanol, as well as adsorption on ion exchangers or activated charcoal.
- Finally, vitamin B12 is crystallized by adding organic solvents, which yields a high-quality product recommended for food and pharmaceutical applications.



5.2.3. Polysaccharides

Polysaccharides are molecules composed of multiple simple sugar units, called monosaccharides, linked together by glycosidic bonds. They can be formed by a single type of monosaccharide, which classifies them as homopolysaccharides. Alternatively, they can be composed of several different types of monosaccharides, which qualifies them as heterogeneous heteropolysaccharides.

Industrial polysaccharides are compounds used in various applications and can come from different sources. Some are extracted from plants, such as cellulose, pectin, gum arabic, and starch, while others are derived from algae, such as agar-agar, alginate, and carrageenan. Microorganisms, particularly bacteria and fungi, are also capable of synthesizing industrial polysaccharides.

According to their location in the microbial cell, three types of polysaccharides of microbial origin are distinguished.

- **Intracellular polysaccharides** are difficult to extract because they are located inside the cell.
- **Cell wall polysaccharides**, like chitin present in the fungal wall, are also difficult to extract.
- Finally, **extracellular polysaccharides**, also called exopolysaccharides (EPS), are not covalently linked to the cell wall. They can be secreted outside the cell as loose slime or form a capsule around the cell, called slime or capsular polysaccharides (CPS).

Microbial exopolysaccharides fulfill several physiological functions for microorganisms. They can protect the microorganism against desiccation, allow it to escape the immune system, act as a barrier against viruses and chemical agents, facilitate the attachment of the microorganism to different surfaces, and serve as an energy reserve. These properties make extracellular polysaccharides of microbial origin materials of interest in many industrial fields.

3. Main biopolymers produced by fermentation

- Polysaccharides constitute the majority of biopolymers used as stabilizers, film-forming agents, etc. They are used to modify the flow characteristics of liquids and as gelling agents.
- Biopolymers include dextrans, alginate polysaccharides, and polyesters derived from *Pseudomonas oleovorans*.
- Microfibril celluloses, scleroglucans, and xanthan polymers are also used in different fields.
- Cyclodextrins are cyclic oligosaccharides used for various applications. They can increase drug solubility, reduce bitterness, and mask chemical odors. Cyclodextrins can also be used as selective adsorbents to remove cholesterol or protect spices from oxidation.

Table 3: Main biopolymers produced by fermentation

Product	Access route	Microorganisms used
Alginate	F	*Azotobacter vinelandii*
Levane	F	*Zymomonas mobilis*
Phosphomannane	F	*Hansenula holstii*
Polyhydroxybutyrate	F	*Alcaligenes eutrophus*
Pullulane	F	*Aurebasidium pullulans*
Scleroglucane	F	*Sclerotium rolfisii*
Xanthane	F	*Xantomonas campestris*
Hyaluronic acid	F	*Streptococcus zooepidemicus*

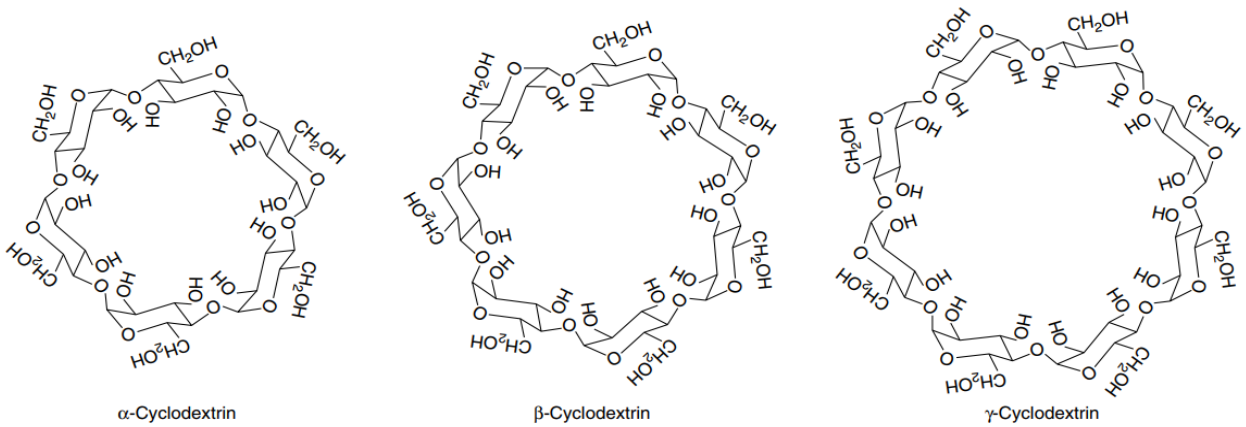


Figure 42.13 **Cyclodextrins.** The basic structures of cyclodextrins produced by *Thermoanaerobacter* are illustrated here. These unique oligopolysaccharides have many applications in medicine and industry.

Example of industrial production of a polysaccharide: xanthan gum

Xanthan gum is a heteropolysaccharide obtained by aerobic fermentation of sugars by bacteria of the genus *Xanthomonas*, including *Xanthomonas campestris*, *Xanthomonas carotae*, *Xanthomonas malvacearum*, and *Xanthomonas phaseoli*. Many of them are phytopathogens.

Xanthan gum has a complex structure. It consists of a main chain of D-glucose units linked β (1→4). This chain has side branches every two glucoses, formed by a trisaccharide composed of α-D-mannose, β-D-glucuronic acid, and a terminal β-D-mannose.

This complex structure gives xanthan gum unique rheological properties, including its ability to form viscoelastic solutions, increase viscosity, and stabilize emulsions. These properties make it a valuable ingredient in many industrial applications, such as food, pharmaceuticals, cosmetics, and coatings.

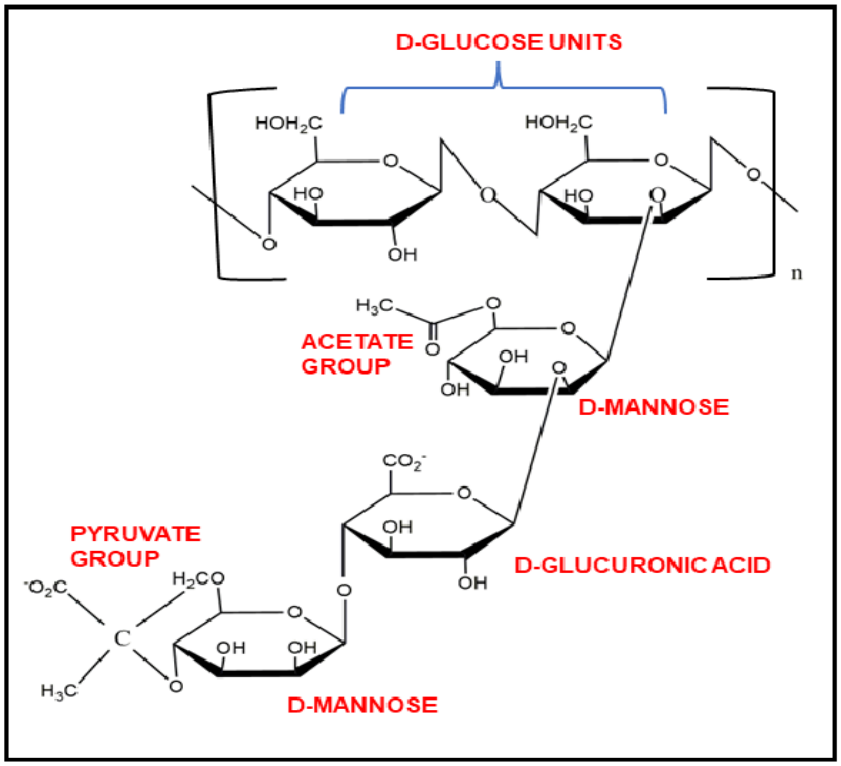


Figure 1. Chemical structure of xanthan gum and its functional groups.

Production

For industrial production of xanthan gum, the first step is to culture the bacterium *Xanthomonas campestris*. This culture is then used to inoculate pilot-scale fermenters with a capacity of 50 to 200 m3. The culture medium used includes a carbon source, such as D-glucose, sucrose, or molasses, added at a concentration of 30 to 40 g/L. A nitrogen source is also added, with a carbon/nitrogen ratio of about 10:1, to promote high yield of xanthan gum. Common nitrogen sources include casein, soy hydrolysate, ammonium salts, peptone, corn steep liquor, and yeast extract. Traces of MgCl2 and a phosphate buffer solution are also present in the culture medium.

The culture is agitated at a temperature between 28 and 30 °C and maintained at a pH of 7.0. The bacterium begins to produce xanthan gum during the exponential growth phase, and fermentation is generally complete after about 3 days of incubation. At the end of fermentation, the culture is heated to 100-110 °C for 10 minutes to eliminate bacteria and improve the rheological properties of the xanthan gum.

Purification of xanthan gum is done by filtration, followed by precipitation in isopropanol. Then, a centrifugation step is carried out to obtain the xanthan gum as a precipitate. After drying, the precipitate is ground to obtain a xanthan gum powder. The minimum expected yield is about 25 g/L to 50 g/L of culture medium used.

5.3. Enzymes

Enzymes are biological catalysts of a protein nature that play an essential role in the synthesis of many molecules. Their main property is to facilitate and accelerate the specific chemical reactions with which they are associated.

Enzymes can be extracted from plant or animal tissues or produced by certain microorganisms. They are valued for their remarkable efficiency and specificity, as they only react with specific molecular compounds. This specificity makes it possible to precisely target desired chemical reactions, thus avoiding undesirable effects.

The use of enzymes presents many advantages. Their catalytic properties allow chemical reactions to be carried out more efficiently, faster, and under less severe temperature and pressure conditions compared to traditional chemical methods. This can lead to cost reduction and energy savings, while minimizing waste and promoting a more environmentally friendly approach.

Enzymes are widely used in various industrial sectors, such as the food industry, pharmaceutical industry, chemical industry, paper industry, textile industry, etc. Their growing use in these fields contributes to the implementation of more sustainable and environmentally friendly processes, often replacing more polluting and less effective chemical methods.

Industrial production of enzymes by fermentation

- A distinction is made between extraction enzymes (from plant or animal organisms) and fermentation enzymes (microorganisms).
- The typical industrial process for enzyme production is deep-tank aerobic culture using a microorganism that produces a large quantity of an extracellular enzyme. The main advantages of fermentation enzymes compared to extraction enzymes:
    - Large-scale production in fermenters,
    - Production independent of geographical and seasonal constraints,
    - Cheap raw materials,
    - Easy genetic manipulation – hyperproductive mutants,
    - Easier purification in the case of extracellular enzymes.

- The microorganisms used for enzyme production can be eukaryotes such as yeasts and fungi, or prokaryotes such as Gram-positive or Gram-negative bacteria.

Enzymes	Producing microorganisms
α-amylase	*Aspergillus niger, Bacillus subtilis, Aspergillus oryzae...*
Pectinases	*Aspergillus niger, Rhizopus*
cellulase	*Aspergillus niger, Penicillium, Trichoderma harzianum*
Proteases	*Aspergillus niger, Bacillus subtilis*
Lipases	*Trichoderma harzianum, Aspergillus niger, Penicillium, Mucor, Pseudomonas*
L-Asparaginase	*Cladosporium sp.*

- ✓ The desired characteristics in a strain producing an industrial enzyme are: high growth rate, high enzyme productivity, high enzyme specific activity, reduced regulation, resistance to catabolic repression, few secondary products, limited sporulation, reduced nutritional requirements, extracellular enzyme, morphology adapted to reactor culture. As it is difficult to find all these characteristics in the same organism, the majority of strains used for industrial enzyme production have been improved by:
    - o Mutagenesis (chemical agents or UV irradiation) allowing rapid attainment of useful characteristics, or
    - o Genetic engineering allowing microorganisms to produce enzymes from higher organisms by inserting the corresponding gene into the microorganism. Chymosin (E.C. 3.4.23.4) has been cloned by different groups into several easy-to-cultivate microorganisms like yeast to industrially produce enzyme preparations that coagulate milk.
- ✓ Production media must be rich (complete), providing all the nutrients (carbon, nitrogen, phosphorus, trace elements, vitamins...). Furthermore, the substrates for the desired enzyme must be added, for example: starch for α-amylase and cellulose for cellulase.
- ✓ Fermentation to produce an enzyme is done on a very large scale. When the enzyme is extracellular, a fermenter up to 450 m3 can be used. In the case of intracellular enzymes, the need