CHAPTER 9

INDUSTRIAL FERMENTATION

CHAPTER 9 OBJECTIVES

- Introduction
- Biochemical and Processing Aspects
- Food and Feed Treatment by Fermentation
- Industrial Chemicals by Fermentation
- Pharmaceutical Products by Fermentation

INTRODUCTION

- Fermentation can be defined as the alteration or production of products with the help of microorganisms.
- Fermentation has been used to conserve and alter food and feed since ancient times.
- Yogurt, salami, soy sauce, vinegar and kefir, are just a few examples of fermented food products that we still know today.
- Fermentation can be spontaneous or be induced by specifically added microorganisms.

INTRODUCTION

- An every day example of such an induced fermentation is addition of baking yeast to flour to make bread or cakes. As with the bread, fermentation can be done in a normal environment where many different microorganisms are present.
- A more sophisticated way is to exclude unwanted microorganisms by sterilization of the materials before adding a starter culture.
- The first aseptic fermentation (exclusion of unwanted microorganisms) on an industrial scale was the production of acetone, butanol, and butandiol for rubber production.
- An important milestone was the introduction of biological wastewater treatment by fermentation.

INTRODUCTION

- Traditionally, wastewater containing human or animal excrement was sprayed on the fields as fertilizer or simply discharged into rivers and lakes.
- This caused microbial pollution and was the cause of many infectious diseases, like typhus and cholera.
- During the 19th century modern industrialization started and many people migrated from the agricultural area to the big cities.
- Public hygiene became a major task. Therefore, it was a big step forward when public sewage systems and biological wastewater treatment plants were introduced.

INTRODUCTION

- Life in the big cities would be unbearable without wastewater treatment, which is perhaps the most widely used fermentation process – even today.
- Another breakthrough in fermentation and human welfare was the discovery of **penicillin**. It was the first antibiotic and the first really effective medication against bacterial infections.
- It was also the first high cost product of fermentation and it started the development of high tech fermentation reactors.
- Amino acid production by fermentation started around 1960 in Japan. Initially glutamic acid was the main product. It was sold as the sodium salt, monosodium glutamate (MSG), a flavor enhancer on Oriental cuisine.

Overview of industrial fermentation products Overview of industrial fermentation products

Category	Examples	Uses / Remarks
Food	Sour dough, soy sauce, yoghurt, kefir, cheese, pickles, salami, anchovy, sauerkraut, vinegar, beer, wine, cocoa, coffee, tea	Conservation of perishable food by formation of lactic acid and ethanol
Feed	Silage	Conservation of green plants by organic acids
Cell mass	Yeast, lactic acid bacteria, single cell protein	Used as starter cultures, animal feed
Organic solvents	Ethanol, glycerol, acetone, butanediol	Cosmetics, pharmaceuticals
Organic acids	Lactic, citric, acetic, acrylic, formic acid	Food, textiles, chemical intermediates
Amino acids	L-lysine, L-tryptophane, L-phenylalanine, glutamic acid	Food and feed additives
Antibiotics	Penicillin, streptomycin, tetramycin, tetracycline	Human and veterinary medicines
Vitamins	B12, biotin, riboflavin	Food and feed supplements
Enzymes	Amylase, cellulase, protease, lipase, lab	Food processing, tanning, detergents additives
Biopolymers	Lanthan, dextran, polyhydroxybutyrate	Food additives, medical devices, packaging
Speciality pharmaceuticals	Insulin, interferon, erythropoietin (EPO)	Human medicines
Environmental	Waste and wastewater treatment	Public hygiene
Energy	Ethanol from carbohydrates and methane from organic waste	Fuel additives or heat generation

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BIOCHEMICAL AND PROCESSING ASPECTS

- Nearly all fermentation processes follow the same principle.
- The central unit is the fermenter in which the microorganisms grow and where they produce the desired products.
- The substrate is the feed of the microorganisms; it contains also any other starting materials that are required for the process.
- The fermentation is started by adding the seed microorganisms, which are present in the starter culture.
- The starter culture is also called “inoculum”. The starter microorganisms are produced in small inoculum fermenters before being added to the main large scale production fermenters.

BIOCHEMICAL AND PROCESSING ASPECTS

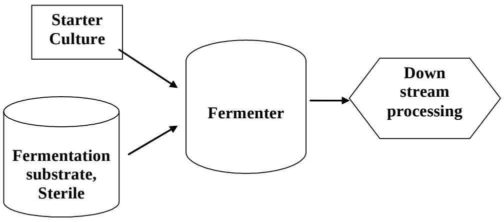


Figure 9.1. Schematic flow chart of a fermentation process of a fermentation process

BIOCHEMICAL AND PROCESSING ASPECTS Microorganisms

- Microorganisms used in fermentation are usually single cells or cell aggregates, often bacteria, sometime fungi, algae or cells of plant or animal origin.
- A bacterial cell consists of an outer cell wall lined with a cell membrane that keeps the cell content from leaking out, but allowing the transport of nutrients from the outside in and of metabolites from inside out.
- The cell liquid contains everything that the cell needs to live and to proliferate, for instance proteins, enzymes, and vitamins.
- The DNA is the carrier of most of the genetic information. Plasmids are DNA units that are independent of the chromosomal DNA.

BIOCHEMICAL AND PROCESSING ASPECTS Microorganisms

- They are important for the transfers of genetic information into other cells.
- Chemically a cell consists mainly of water and protein and a large number of minor compounds. Breaking of the cell wall (lyses) kills the organism and releases the content of the cell into the surrounding medium.
- The energy to keep the cell alive comes from absorption of light or from oxidation of organic or inorganic compounds.
- If the oxidizing agent is oxygen, the microorganisms are called **aerobic**.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

- Phototropic microorganisms use the energy of the (sun) light to convert carbon dioxide to organic matter.
- Examples are green algae and bacteria. The hydrogen comes from the organic substrates or from water and sometimes from other inorganic hydrogen compounds.
- Sulfur and nitrogen come from organic sources or from inorganic ions, such as sulfate, sulfide, nitrate or ammonium. In addition, a number of minor elements (minerals) are required to support growth.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

- Many fermentation processes use sugars as the substrate. The principle of the microbial metabolisation of glucose is described in figure 9.2.
- The first step is the cleavage of the glucose (glucolysis); it is in reality a multi-step reaction, which results in the formation of glyceraldehyde-3-phosphate.
- A series of complex enzyme induced reactions leads to pyruvate. Depending on the predominating enzymes, pyruvate reacts to L-lactic acid (with lactic dehydrogenase) or acetaldehyde and ethanol (with pyruvic decarboxylase and alcohol dehydrogenase).

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

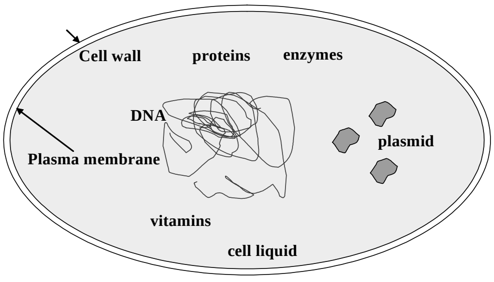


Figure 9.2. Scheme of a bacterial cell

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

Primary metabolites:

- During cell growth the nutrients of the substrate are converted to cell mass. The chemical compounds produced in this process are called “primary metabolites”.
- The cell mass itself consists mainly of proteins, but a number of primary waste products are also formed, for instance carbon dioxide, lactic acid, ethanol, etc.
- Primary metabolites are produced in parallel with the cell mass.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

Secondary metabolites:

- The formation of secondary metabolites is not directly related to cell growth.
- They are the side products of bacterial life. In nature, they are produced in low concentration, but through laboratory mutation and selection, cells can be optimized to overproduce these metabolites.
- Many antibiotics and vitamins are secondary metabolites. The formation of secondary metabolites is not directly proportional to primary metabolism and cell growth.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

Secondary metabolites:

- Primary metabolites are often released into the surrounding medium, whereas secondary metabolites tend to remain inside the cell and can be recovered only after lysis of the cell walls.
- Some metabolites are toxic; therefore any fermentation must be monitored for toxins. Two types are distinguished: exotoxins are released into the fermentation broth; endotoxins remain inside the cell and are sometimes difficult to detect.

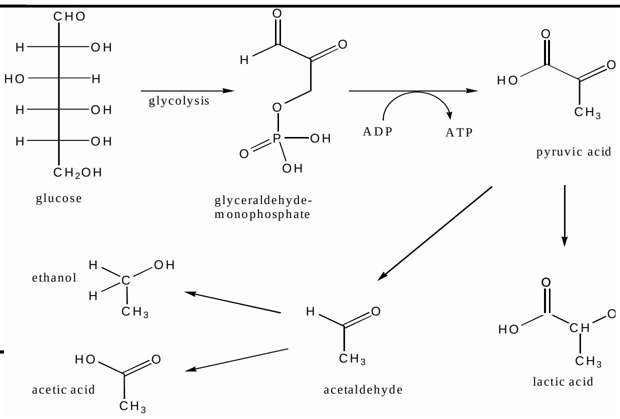


Figure 9.3 Microbial metabolism (primary) of glucose to lactate or ethanol ADP = adenosine diphosphate; ATP = adenosine triphosphate.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

Culture Development

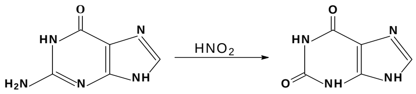
- The first step is the selection of the best culture with respect to selectivity and growth characteristics, such as pH, mechanical stress, and temperature sensitivity.
- This selection is a tedious process based on trial and error screening of a large number of strains.
- Mass screening techniques have been developed for this purpose, for example, agar plates that are doped with specific inhibitors or indicators.
- The primary screening results in several potentially useful isolates, which go into secondary screening.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

- False positives are eliminated and the best strains are selected by using a small-scale fermentation technique with shake flasks.
- Although primary and secondary screening yields the best candidate, the best natural (wild type) wild type strain is still not good enough for industrial production.
- Further development is necessary to improve the technical properties of the culture, its stability, and yield.
- The “genetic improvement” technique induces deliberate mutations in the DNA of the cells. Such mutations can be induced chemically, by ultraviolet light, or by ionizing radiation.
- This change is random that means positive or negative with respect to the intended purpose.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

- Therefore, a new selection process is needed to find the improved strains. The mutated cells are again screened; the best candidates are selected, again mutated, screened and so on, until a satisfactory strain is obtained.
- Chemical substances induce mutations by reaction with amino acids of the DNA chain. Nitrous acid (HNO_2), for example, reacts with guanine under deamination leading to xanthene.

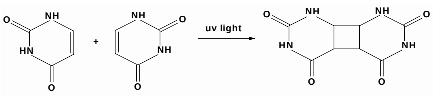


guanine xanthene

Eq. 9.1

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

- Methylation of the amino groups is also possible, e.g., with N-methyl-N´-nitro-N-nitrosoguanidine, a strong mutagen, but without lethal effects.
- A third type of mutation is the insertion of alien molecules between two amino acids and thereby altering the macroscopic structure of the DNA.
- DNA absorbs UV light with a wavelength of <260 nm, leading to photochemical reactions, for instance, the dimerisation of pyrimidine (equation 9.2). Ionizing radiation (X-rays, electron beams, gamma radiation, etc.) is less selective. It leads to a random cleavage of the DNA chains.



Eq. 9.2

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

- The most advanced method to improve the microorganisms is by changing the cells in a controlled way through genetic engineering.
- The exchange of genetic information is normally limited to cells of the same type and species.
- Membranes and other mechanisms inhibit the transfer of genes or DNA between different cell types.
- Today, it is possible to transfer genetic properties between completely different species, for instance from plants to bacteria or from bacteria to plants.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

- The principle is not difficult to understand. The DNA of a cell is cut into fragments with specific enzymes.
- The DNA fragment that is responsible for the production of the target product (e.g. insulin) is selected and transferred into the plasmid (DNA) of a microorganism (e.g. Escherichia coli = E. coli).
- The genetically engineered cells can be cultivated in fermenters like a “normal” cell. The only difference is that the genetically modified microorganisms (GMO) produce a substance (e.g. insulin) that it would never generate without modification.
- Genetic engineering of microorganisms is a key step in modern biotechnology.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

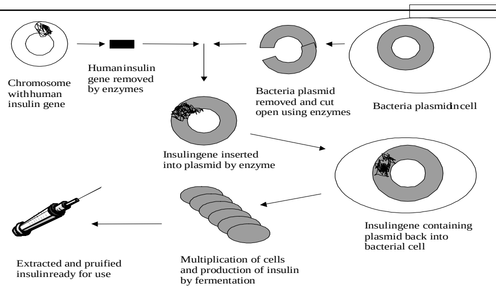


Figure 9.4: Principle of genetic engineering; example: Insulin from transgenic bacteria.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

- It makes it possible that bacteria produce valuable substances which are difficult to obtain otherwise.
- “Elicitors” are micro-organisms or chemicals that help the bacteria to produce the target product.
- For example, the production of the important pharmaceuticals morphine and codeine by *Papaver somniferum* was increased 18 fold by addition of *Verticillium dahliae*.
- Once the right culture is obtained, it must be stored under conditions that retain their genetic stability and viability.
- One method is to keep the microorganisms on an agar plates in an incubator. Agar is a substrate containing all nutrients necessary to microorganisms. It is usually sold in ready to use shallow round dishes.

BIOCHEMICAL AND PROCESSING ASPECTS
Microorganisms

- Maintenance of microorganisms on agar plates requires continuing attention by skilled personnel. Another common method is lyophilization (freeze drying).
- The cell suspension is shock frozen and the water is removed by evaporation at low temperature under reduced pressure.
- Freeze dried microorganisms can be stored for a long time with minimum maintenance, but only robust cell types survive the procedure.
- A third method is cryopreservation of the cells at very low temperature. Cell suspensions in aqueous glycerol or DMSO are shock frozen and stored in liquid nitrogen or dry ice.

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OBJECTIVES

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- Industrial Chemicals by Fermentation
- Pharmaceutical Products by Fermentation

FOOD AND FEED TREATMENT BY FERMENTATION
FOOD CONSERVATION

- Lactic acid produced by bacteria protects the food from deterioration by inhibiting the growth of mold and other microorganisms.
- Most vitamins and nutrients of the food are preserved during fermentation.
- Three examples are discussed in more detail below: The production of sauerkraut, soy sauce, and milk products.

FOOD AND FEED TREATMENT BY FERMENTATION
FOOD CONSERVATION

- The early sailors used sauerkraut to fight scurvy but, a disease that is caused by vitamin C deficiency.
- Sauerkraut is the German name for fermented white cabbage produced in a batch process following a traditional recipe.
- The cabbage heads are cut into 1-3 mm wide strips and placed in large concrete tanks in intermittent layers with salt. The liquor of the previous batch is added as the starter culture.
- The tank is sealed and remains undisturbed for 4-6 weeks.

FOOD AND FEED TREATMENT BY FERMENTATION
FOOD CONSERVATION

- *Soy sauce* is a dark brown salty liquid with a peculiar aroma and a meaty taste. It is produced from salt, water, wheat and soybeans, originally in the batch mode.
- Today's processes are continuous and much faster than the traditional batch fermentation. They allow the production of 100 million liters per year in one factory.
- The heart of the manufacturing process is a complex sequence of fermentation steps in which the carbohydrates are converted to ethanol and lactic acid, and the proteins are broken down to peptides and amino acids.

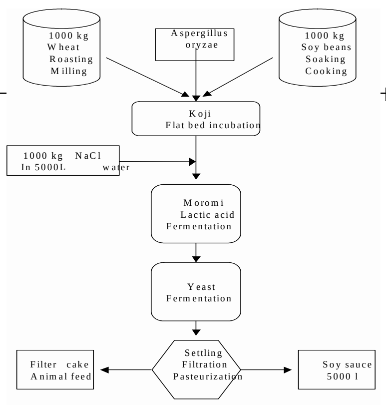

Figure 9.9: Schematic flow chart for the production of soy sauce

FOOD AND FEED TREATMENT BY FERMENTATION
FOOD CONSERVATION

- The principle product of milk fermentation is the same in all processes, namely that lactic acid is produced by fermentation.
- At acidic pH the casein cells break up and precipitate. Depending on the target, they are either separated (e.g. to produce cheese) or re-homogenized to stay in the product (e.g. yogurt) (Figure 9.10).
- They can spoil the food by misguided fermentation and the production of substances with annoying odor or bad taste, such as butyric acid, hydrogen sulfate, or aromatic amines.

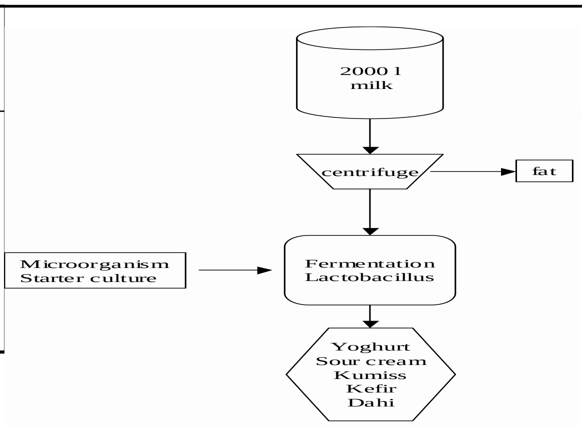

Figure 9.10: Schematic flow chart for the production of milk products

FOOD AND FEED TREATMENT BY FERMENTATION
Single Cell Protein (SCP)

- After the process of fermentation is over, the exhausted bacteria can be separated from the broth by filtration.
- This cell mass has a number of names, such as “microbial biomass” or “single cell protein” (SCP). Microbial biomass is a side product of all fermentation processes but in some cases it is actually the sole target product.
- Bacterial cells have a high content of protein, but are low in fat and cholesterol. This explains the names “single cell protein” (SCP) or “microbial protein”.

FOOD AND FEED TREATMENT BY FERMENTATION
Single Cell Protein (SCP)

- Large quantities of organic material are available from the production of pulp and paper, sugar, canned food, etc. In some countries agricultural crops like sugar cane, maize or sorghum are used as feedstock for SCP production.
- The technology for protein production from chemicals exists and may be applied.
- Several processes were developed: Shell had originally introduced a process that used methane (natural gas) as the feedstock for SCP production.
- The microorganisms are cultured in an aqueous medium at temperatures of 42-45°C and at a pH value of 6.8 under semi-sterile conditions.

FOOD AND FEED TREATMENT BY FERMENTATION
Single Cell Protein (SCP)

- The mass balance equation shows that large volumes of oxygen are needed and that carbon dioxide and heat must be removed from the reactor.

3 kg O_2 + 1.2 kg CH_4 →

1 kg cells + 1.2 kg CO_2 + 2 kg water + 13.2 kcal/mol

- Several types of microorganisms are needed for an optimized continuous process.
- Methylococcus species metabolize the methane; Pseudomonas, Nordica, Moraxella species are present to convert other hydrocarbons and side-products.

FOOD AND FEED TREATMENT BY FERMENTATION
Single Cell Protein (SCP)

- The process developed by BP uses a continuous stirred tank reactor under sterile conditions. The SCP is harvested by centrifugation and then spray-dried.
- The mass balance equation shows that less heat is generated and that a little less oxygen is needed than for the methane process.

1.12 kg paraffin + 2.56 kg O_2 →

0.13 kg CO_2 + 1.08 kg H_2O + 8 kcal

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Industrial Chemicals by Fermentation
Ethanol

- Ethanol is a primary alcohol with many industrial uses. It can be produced from sugar containing feedstock by fermentation.
- Alcoholic fermentation is one of the oldest and most important examples of industrial fermentation.
- Traditionally, this process has been used to produce alcoholic beverages, but today it also plays an outstanding role in the chemical and automotive industry.

Industrial Chemicals by Fermentation
Ethanol

- The largest potential use of ethanol is as car fuel either neat or as an octane booster and oxygenate in normal gasoline.
- In the USA, it is heavily promoted as a replacement of MTBE (methyl-t-butylether).
- Ethanol is also an important solvent and starting material for cosmetics and pharmaceuticals and is also widely used as a disinfectant in medicine.
- Ethanol is produced from carbohydrate materials by yeasts in an extra-cellular process.
- The overall biochemical reaction is represented by equation 9.5.

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + \text{energy} \qquad \text{Eq. 9.5}$$

Industrial Chemicals by Fermentation
Ethanol

- Sugar containing plant material can be used without chemical pretreatment either directly as mash or after extraction with water.
- Examples are fruits, sugar beets, sugar cane, wheat sorghum, etc. Starch containing agricultural commodities or waste products is pretreated with enzymes.
- Cellulose materials, such as wood, are cooked with acid to break up the polymeric carbohydrate bonds and to produce monomeric or dimeric sugars.

Industrial Chemicals by Fermentation
Ethanol

1. ***Feedstock preparation***

- Sugarcane or sorghum must be crushed to extract their simple sugars.
- Starches are converted to sugars in two stages, liquefaction and saccharification, by adding water, enzymes, and heat (enzymatic hydrolysis).

Industrial Chemicals by Fermentation
Ethanol

2. ***Fermentation***

- The mash is transferred to the fermentation tank and cooled to the optimum temperature (around 30 °C).
- Care has to be taken to assure that no infection (other organisms that compete with the yeast for the glucose) occurs.
- Then the appropriate proportion of yeast is added. The yeast will begin producing alcohol up to a concentration of 8-12 percent and then become inactive as the alcohol content becomes too high.

Industrial Chemicals by Fermentation
Ethanol

3. Separation

- The mash is now ready for distillation. A simple one step "stripper" distillation separates the liquid from the solids.
- The residue of this distillation is a slurry consisting of the microbial biomass and water, called stillage.
- It is removed to prevent clogging problems during the next step, fractionated distillation.
- It is often used to produce secondary products, such as animal feed additives or seasonings or it is converted to methane and burned as an energy source.

Industrial Chemicals by Fermentation
Ethanol

4. Distillation

- Distillation separates the ethanol from the water in a rectifying column.
- The product is 96 % ethanol. It cannot be further enriched by distillation because of azeotrope formation, but must be dehydrated by other means.

5. Dehydration

- Anhydrous ethanol is required for blending gasoline.
- It can be obtained by additional dehydration, e.g. with molecular sieves or carrier assisted distillation.

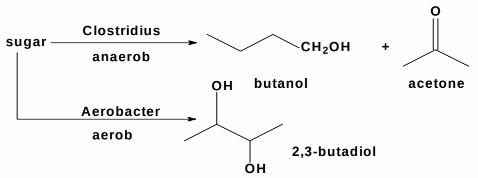
Industrial Chemicals by Fermentation
Other industrial alcohols

- Fermentation by aerobic bacteria, such as Aerobacter, produces butane-2,3-diol with concentrations up to 10 %.
- In the early 20th century the diol was an important product, since it could be converted to but-1,3-diene, which was polymerised to give synthetic rubber.
- At that time, natural rubber supplies were limited and the synthesis of butadiene from petrochemicals not yet develop.

Industrial Chemicals by Fermentation
Other industial alcohols

- ABE (acetone, butanol, and ethanol) fermentation has a long history of commercial use and perhaps the greatest potential for an industrial comeback.
- Acetone, butanol and ethanol can all be isolated from this remarkable metabolic system; carbon dioxide and hydrogen are additional products.
- The solvents were used as paint solvents in the expanding automobile industry.

Industrial Chemicals by Fermentation
Other industrial alcohols



Eq. 9.6

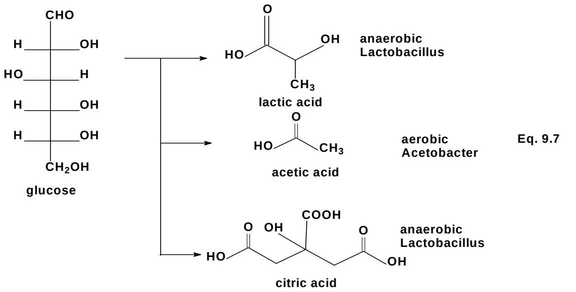
Industrial Chemicals by Fermentation
Organic acids

- The formation of lactic acid and its role as a food preservative was already discussed in connection with food fermentations, where it is produced in small concentrations.
- It is also possible to isolate it as a neat acid to convert the acid to the corresponding esters.
- Ethyl and butyl esters are good solvents for polymers and resins. Ethyl lactate, for instance, is used in the electronics industry to remove salts and fat from circuit boards, it is also a component in paint strippers.
- Ethyl and butyl esters are approved food additives. This illustrates their low toxicity.

Industrial Chemicals by Fermentation
Organic acids

- Acetic acid is produced by oxidation of ethanol by Acetobacter organisms.
- It is either used in diluted form as vinegar or distilled to give neat (100 % pure) acetic acid. For many centuries, acetic acid was produced only via the fermentation route.
- Since the advancement of the petrochemical industry, it is also produced synthetically, at least for industrial use.
- By changing the fermentation conditions to aerobic using Aspergillus Niger microorganisms it is possible to produce citric acid from sugar containing feedstock.
- These three examples show how versatile fermentation is and how minor modifications lead to different products (eq. 9.7).

Industrial Chemicals by Fermentation
Organic acids



Industrial Chemicals by Fermentation
Amino acids

- L-Glutamic acid or its salt monosodium glutamate (MSG) is used as an additive to human food to enhance the taste.
- Although seaweed had been used in Asia to enhance food flavor for over 1,000 years, it was not until 1908 that the essential component responsible for the flavor phenomenon was identified as glutamic acid.
- From 1910 until 1956, monosodium glutamate was extracted from sea weed, a slow and costly method.
- In 1956, Ajinomoto, a Japanese company, succeeded in producing glutamic acid by means of fermentation.
- Today, L-glutamic acid or MSG is generally made by microbial fermentation using genetically modified bacteria.

Industrial Chemicals by Fermentation
Amino acids

- Amino acids can be produced as mixtures or as single compounds.
- Special microbial strains are responsible for the production of single amino acids. Scheme 9-5 shows a schematic flow chart of the L-lysine production.
- The medium contains glucose as the carbon source, ammonium sulphate, urea or ammonia as nitrogen sources, and other nutrients, such as minerals and vitamins.

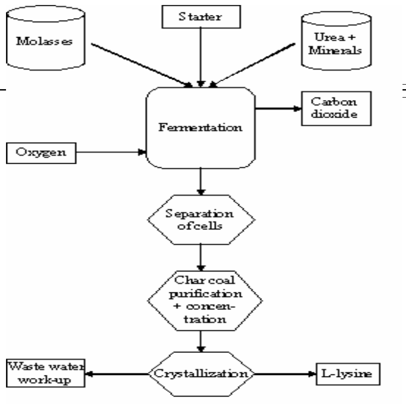


Figure 9.12: Production of L-lysine as an example for industrial amino acids

Industrial Chemicals by Fermentation
Vitamins

- Vitamins are produced by fermentation of sugar containing starting materials and special additives by bacteria or yeast.
- They are produced inside the cell and not released into the fermentation broth.
- The process parameters are similar to those described for the other examples; the difference being the additives, which are essential components of the vitamins.
- Vitamin A1 (retinal) is produced from β-carotene, which can be obtained by fermentation of corn, soybean meal, kerosene, thiamin and α-ionone.
- The dry-mass after fermentation contains 120 –150 g product/kg.

Industrial Chemicals by Fermentation
Vitamins

- Vitamin B2 (riboflavin) is produced by yeast from glucose, urea and mineral salts in an aerobic fermentation.
- Vitamin B12 (cyanocobalamine) is produced by bacteria from glucose, corn and cobalt salts in anaerobic (3 days) and then an aerobic fermentation (also 3 days).
- The starting point for synthesis of Vitamin C is the selective of oxidation of the sugar compound D-sorbit to L-sorbose using Acetobacter suboxidans bacteria. L-sorbose is then converted to L-ascorbic acid, better known as Vitamin C.
- Vitamin D2 is formed by photochemical cleavage of ergosterin, which is a side product of many fermentation processes. Microorganisms usually contain up to 3 % of ergosterin.

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Pharmaceutical Products by Fermentation
Pharmaceuticals by direct fermentation

- Although most research is devoted to the biological and pharmacological problems, the key step in the actual production of biotech pharmaceuticals is fermentation.
- This is demonstrated by the examples, penicillin, insulin, interferon, and erythropoietin (EPO) – to name just a few.
- Penicillin changed the world! It was the first highly efficient antibiotic pharmaceutical that allowed an effective treatment of bacterial infections.
- Penicillin was discovered in 1928 by Alexander Fleming by chance. He observed that the growth of a bacteria culture was inhibited by a fungus Penicillum notatum.

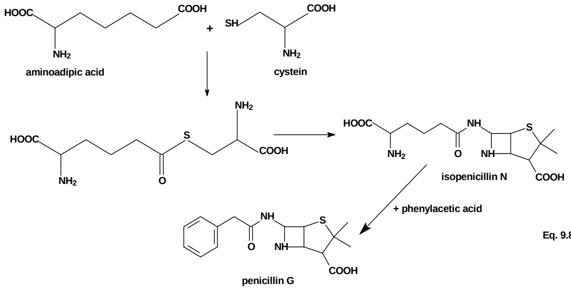
Pharmaceutical Products by Fermentation
Pharmaceuticals by direct fermentation

- Penicillin did not only change the medical world, but also the fermentation technology. The naturally growing (wild type) Penicillum notatum produced penicillin with a yield of 10 mg/l.
- To enhance the penicillin production further, the old method of growing the Penicillum mold on the surface of the medium in liter-sized flasks was replaced by fermentation in large aerated tanks.
- This allowed the mold to grow throughout the entire tank and not just on the surface of the medium.
- Today, penicillin and other antibiotics are produced in large scale fermenters holding several hundred cubic meters of medium and the yield has increased 5000 fold to 50 g/l.

Pharmaceutical Products by Fermentation
Pharmaceuticals by direct fermentation

- Equation 8 shows a simplified scheme of the biosynthesis of penicillin. It starts with the amino acids L-α-aminoadipic acid and L-cysteine from penicillin N in a complex reaction sequence.
- When phenyl acetic acid is added to the fermentation medium, the side chain of the molecule is modified and the resulting product is called penicillin G.

Pharmaceutical Products by Fermentation
Pharmaceuticals by direct fermentation



Eq. 9.8

Pharmaceutical Products by Fermentation
Pharmaceuticals via Biotransformation

- Biotransformations are chemical reactions that are induced by enzymes in the cells.
- Sometimes it is possible to isolate the enzymes and to carry out the chemical reaction in a separate reactor in the absence of living cells.
- Starting materials are single chemical compounds or mixtures of related compounds, which are converted to the product with high selectivity.
- Many biotransformations are difficult to achieve by conventional synthesis. A classical example is the synthesis of chiral molecules.

Pharmaceutical Products by Fermentation
Pharmaceuticals via Biotransformation

- A compound is chiral, when can occur in two forms that are mirror images of each other.
- Classical synthesis produces both enantiomers in a 1 to 1 ratio. They cannot be separated by normal physical means.
- Nature is, however, more selective. Here only single enantiomers are formed. This can be utilized to separate D,L enantiomers of amino acids.
- The enzyme L-amylase produces selectively the L-amino acid from a mixture of the DL-acylamino acids.

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$$\underset{\text{D,L acylamino acid}}{\text{R-CH(NH-CO-CH}_3\text{)-COOH}} \longrightarrow \underset{\text{L-amino acid}}{\text{R-CH(NH}_2\text{)-COOH}} + \underset{\text{acetic acid}}{\text{CH}_3\text{COOH}} \quad \text{Eq. 9.9}$$

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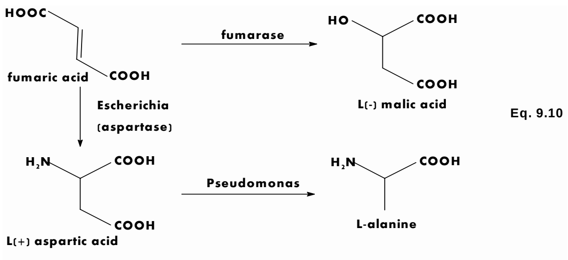
- The same compound is converted to the amino acid L(+)-aspartic acid by Escherichia bacteria that contain the enzyme aspartase. If Pseudomonas bacteria are added, another amino acid L-alanine is formed.



Pharmaceutical Products by Fermentation
Biopolymers

- Many membranes, proteins, and nucleotides that are present in living organisms are polymers.
- Industrial biopolymers are still niche products, but they are gaining rapidly in importance, since they have advantages in special applications.
- Here are a few examples: Water-soluble carbohydrate (= polysaccharide) polymers modify the properties of aqueous systems. They can thicken, emulsify, stabilize, flocculate, swell, and suspend, or to form gels, films and membranes.

Pharmaceutical Products by Fermentation
Biopolymers

- Other important aspects are that polysaccharides come from natural, renewable sources, that they are bio-compatible and biodegradable.
- For example, Xanthan gum is a water soluble hetero-polysaccharide with a very high molecular weight (> 1 million) produced by the bacterium Xanthomonas campestris.
- It is used in food processing as a stabilizer for sauces and dressings.
- Biopolymers are also used in adhesives, water color, printing inks, cosmetics, and in the pharmaceutical industry.
- Polylactides are made from lactic acid and are use for orthopedic repair materials.

Pharmaceutical Products by Fermentation
Biopolymers

- The butyrate/ octanoate copolymer and butyrate / hexanoate / decanoate terpolymer have properties similar to those of higher-grade LLDPE (linear low density polyethylene) and higher-grade PET (polyethylene terephthalate).
- They can be molded or converted into films, fibers, and non-woven fabrics. The biopolymer is produced by low-cost fermentation or from waste streams substrates.
- Polyhydroxyalkanoic acids (PHAs) have been extensively researched since the 1970s because of the potential applications.
- The most successful PHA products are the polyhydroxybutyrates (PHBs).

Environmental Biotechnology

- Modern biological waste water treatment plants use a combination of aerobic and anaerobic fermentation reactors to remove organic matter from the waste water.
- In the aerobic part the microorganisms feed on the organic matter in the waste water and convert it to microbial biomass and carbon dioxide.
- In the anaerobic part the microbial biomass of the aerobic part is digested by a second type of microorganism that produces methane as it grows.
- The anaerobic microorganisms die immediately when they come into contact with air. That means that they are not infectious and do not present a risk to humans and the environment, when they are released.

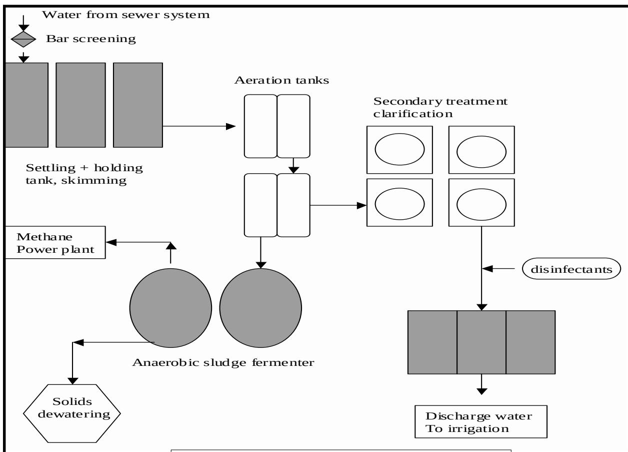


Figure 9.14: Wastewater treatment plant

CHAPTER 9
OBJECTIVES

- Introduction
- Biochemical and Processing Aspects
- Food and Feed Treatment by Fermentation
- Industrial Chemicals by Fermentation
- Pharmaceutical Products by Fermentation