Method of wine production:

Quality of the grapes is very important for the production of wines. The grapes are crushed (mechanically or by treading of feet) and the juice is extracted.

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This grape juice ready for fermentation process is technically referred to as must.

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It is a practice to add SO_2 to must to inhibit the growth of non-wine yeast and contaminating bacteria. SO_2 which can kill other organisms can be tolerated by wine fermenting yeasts. Sometimes, the must may also be subjected to partial or complete sterilization.

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The must in suitable bioreactors is inoculated with desired strains of the yeast *Saccharomyces cerevisiae*.

↓

Initially oxygen is bubbled through the fermentation medium to promote good growth of yeast cells and gradually anaerobic conditions are established.

↓

The wine production normally takes a few days (2 – 5 days). The fermentation conditions (temperature, time etc.) are actually dependent on the type of wine produced.

↓

At the end of the fermentation, wines are transferred to storage tanks (or vats) and allowed to age, which may take some months to years.

↓

Aging of wine is very important for the development of characteristic flavour and aroma. The alcohol content of wines is in the range of 10 – 16%.

Wine spoilage: Wine spoilage is most commonly caused by microorganisms especially by yeasts and bacteria.

A. **Wine defects caused by yeasts:** Yeasts represent a major cause of wine defects and spoilage.
   - ***Saccharomyces ludwigii:*** They produce high concentration of ethyl acetate which gives the wine a vinegar like taste.
   - ***Brattanomyces intermedius:*** They can spoil wine in various ways. First, the yeast forms fine, powdery precipitates which do not sediment well. Secondly, under aerobic conditions, it forms high concentrations of acetic acid and ethyl acetate by oxidation of ethanol, which gives the wine a vinegar-like taste.
   - **Pichia, Candida and Hansunela:** They are film-forming yeasts and they form a film on the surface of the wine. They are mainly found in vats which have not yet been completely filled. Growth of film yeast can lead to oxidation of ethanol and formation of acetaldehyde, acetic acids and acetic acid esters.

B. **Wine defects caused by bacteria:** Two distinct groups are generally associated with wine defects: Acetic acid bacteria and lactic acid bacteria.
   - **Acetic acid bacteria:** The acetic acid bacteria are Gram negative, strict aerobes. There are two genera in this group Acetobacter and Gluconobacter. Each genus contains only 1 species namely *Acenatobacter aceti* and *Gluconobacter oxydans*. These bacteria oxidize ethanol to acetic acid; give the wine a vinegar flavour.
   - **Lactic acid bacteria:** Lactic acid bacteria have either a homofermentive or heterofermentive metabolism, or in some strains, the metabolic capacity for both. When homofermentive lactic acid bacteria such as *Pediococcus cerevisiae* and *Lactobacillus plantarum* grow in wine and ferment glucose, they produce lactic acid. The acid causes the wine glycerol oxidation which reacts with polyphenols to form bitter compounds. Red wines are more likely to show this flavor defect.

Difference between white wine and red wine:

Features	White wine	Red wine
1. Kind of grapes	White or red grapes	Red or black grapes
2. Color	White	Red
3. Amount of tannins	Low	High
4. Removal of skin	Needed	Not needed
5. Fermentation	By wine yeast (e.g. selected strains of *S. cerevisiae* var. *elliposidus*)	By wild yeasts (e.g. *Kloeckera apiculate*, *Torulopsis stellate, Candida vini, Saccharomyces uvarum*)

6. Fermentation temperature	12 – 15^0 C	24 – 29^0 C
7. Fermentation days	1 – 4 weeks	3 – 5 days

Distilled beverages:

Distilled spirit, also called distilled liquor, alcoholic beverage (such as brandy, whisky, rum, or arrack) that is obtained by distillation from wine or other fermented fruit or plant juice or from a starchy material (such as various grains) that has first been brewed.

Examples:

1. Whiskey
2. Rum
3. Gin
4. Brandi
5. Vodka

General preparation method:

Distilled beverages are prepared by the fermentation of sugars derived directly from plant extracts and fruit juices, or indirectly from hydrolyzed grain and root starch.

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The yeast fermentation normally produces an intermediate product containing a maximum of 14% ethanol.

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Distillation of these intermediate products results in whisky from unhoped beer and brandies from wine and cider.

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In some instances, the intermediate are not related to a product of direct beverage use and it is only the final distilled product that is of commercial interest. Several of these, including gin and vodka, can be prepared from virtually any source of ethanol. They are colorless neutral spirits with little innate flavor, but may be later colored and flavoured with plant materials such as herbs and spices.

↓

Many distilled beverages are self-flavoured, notably grape and other fruit brandies, whiskies prepared from malt and various starch sources, and rum derived from fermented sugarcane extract or molasses.

Whiskey

Whisky is distilled from fermented grain-mash containing upto 50% ethyl alcohol.

Classification:

1. **Malt whisky:** This whisky is manufactured by microbial fermentation and subsequent distillation of malted barley (barley grains are soaked in water and then germinated at 17°C).
2. **Grain whisky:** This whisky is manufactured by the microbial fermentation and subsequent distillation of a mixture of malted and un-malted barley with un-malted maize.
3. **Scotch whisky:** Malt whisky is matured (aged) in oak casks at least 3 years and then blended with grain whisky. This new product is Scotch whisky.
4. **Bourbon whisky:** This whisky is manufactured by the microbial fermentation of a grain-mash in which corn is predominant (at least 51%).
5. **Irish whisky:** This whisky is prepared from a grain-mash in which rye is predominant.
6. **Arrak (Far East) and Sake (Japan) whisky:** This whisky is prepared from microbial fermentation of rice-grains. Since the rice is starchy, its starch is hydrolyzed by enzyme amylases derived from *Aspergillus oryzae* before fermentation.

Preparation

Whiskeys are prepared from malted barley, exclusively in the case of Scottish and Irish malt whiskies or with various proportions of added cereal starch for other whiskies such as bourbon, grain and rye whisky.

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4. Production of Industrial Chemicals

Production of organic acids

The major organic acids of commercial importance produced by fermentation are:

1. Citric acid
2. Gluconic acid
3. Lactic acid
4. Acetic acid
5. Ascorbic acid and
6. Itaconic acid

Citric acid

Applications:

1. Citric acid, due to its pleasant taste and palatability, is used as a flavoring agent in foods and beverages e.g. jams, jellies, candies, desserts, frozen fruits, soft drinks, wine etc. Besides brightening the color, citric acid acts as an antioxidant and preserves the flavors of foods.
2. It is used in the chemical industry as an antifoam agent and for the treatment of textiles. In metal industry, pure metals are complexed with citrate and produced as metal citrates.
3. In pharmaceutical industry, as trisodium citrate, it is used as a blood preservative. Citric acid is also used for preservation of ointments and cosmetic preparations. As iron citrate, it serves as a good source of iron.
4. Citric acid can be utilized as an agent for stabilization of fats, oils or ascorbic acid. It forms a complex with metal ions (iron, copper) and prevents metal catalyzed reactions. Citric acid is also used as stabilized of emulsions in the preparation of cheese.
5. In detergent or cleaning industry, citric acid has slowly replaced polyphosphates.

Production process:

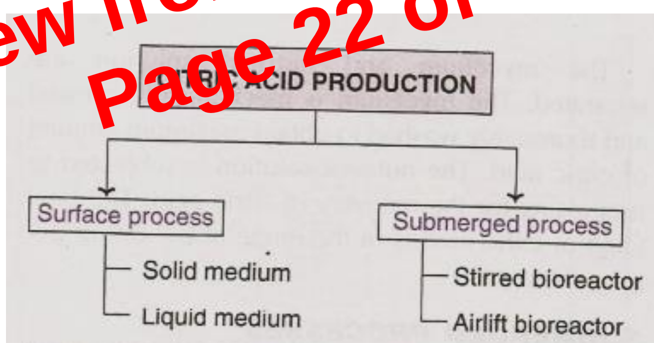

Fig. 24.3 : The industrial processes for the production of citric acid.

There two processes of citric acid production. They are:

- **The surface process:** This is characterized by growing the microorganisms as a layer on the film on a surface in contact with the nutrient medium, which may be solid or liquid in nature. Thus the surface process has supported growth systems.
- **The submerged process:** In this case, the organisms are immersed in or dispersed throughout the nutrient medium. There are two types of submerged fermenters (bioreactors) – stirred bioreactors and airlift bioreactors.

They are discussed below:

A. Surface process

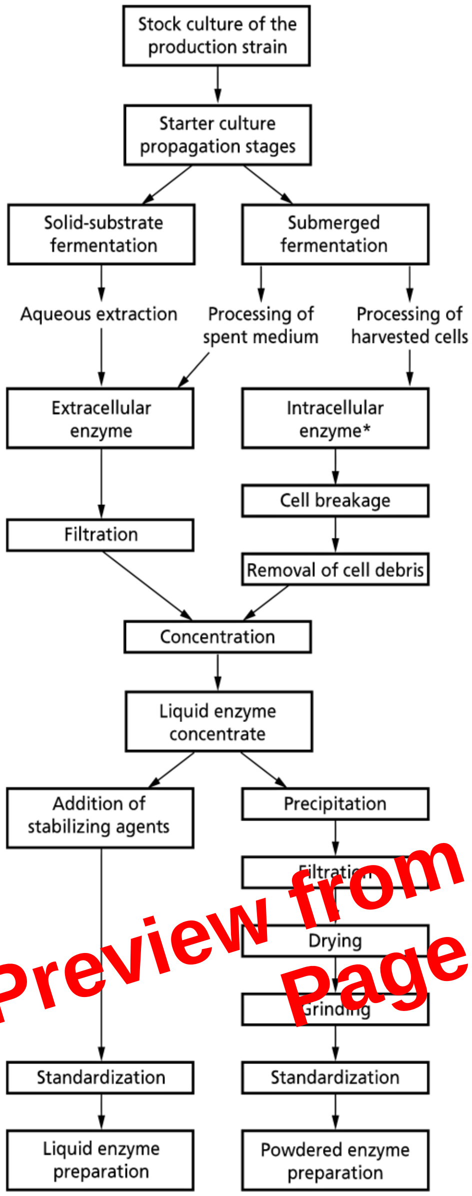

Fig. 9.3 Bulk industrial enzyme production. *Note: some intracellular enzymes, such as glucose isomerase, may not be extracted from cells but immobilized within.

Detergent enzymes

The incorporation of enzymes into detergents provides several benefits. Energy savings are made as a lower wash temperature can be used and levels of less desirable detergent chemicals can be reduced. Unlike other detergent components, enzymes do not have a negative impact on sewage treatment processes. They are totally and rapidly biodegraded to leave no harmful residues. Consequently, they are environmentally safe and are no risk to aquatic life.

The first commercial use of microbial enzymes in detergents was in 1959. A Swiss chemist Jaag, who worked for the detergent company Gebrüder Schnyder, developed a new product containing a bacterial protease, which replaced the animal trypsin that had previously been incorporated into these detergent products. From this point there was an increasing use of microbial enzymes in detergents. In the 1960s, there were further improvements through the introduction of proteases active at alkaline pH. They were relatively unaffected by other components of washing powder and functioned at the desired wash temperatures. A well-known example is subtilisin, a bacterial alkaline serine protease from *Bacillus licheniformis* and *B. subtilis*, which is used extensively in laundry detergents at levels of 0.015–0.025% (w/w). This enzyme has now been engineered to improve pH and temperature characteristics and reduce sensitivity to peroxide.

Proteases are not the only enzymes used in detergents; since the late 1980s amylases and lipases have been available for incorporation, e.g. Lipolase from Novo Nordisk. Lipolase was the first detergent enzyme to be produced through recombinant DNA technology. The lipase gene was isolated from a strain of the filamentous fungus *Humicola* and then transferred to *Aspergillus oryzae*, which is more readily cultivated in submerged fermentations. Superior fatty acid digesting enzymes have now been discovered, such as a cutinase from *Fusarium solani*, which naturally degrades the mixture of fatty acids that form plant cuticles. The gene for this enzyme has been transferred to *Saccharomyces cerevisiae* and commercial production is now being examined.

Starch processing enzymes and related carbohydrases

Microbial enzymes have proved to be of immense value in the processing of starch, a polysaccharide composed of amylose (linear α-1,4-linked glucose units) and amylopectin (branched polymer with both α-1,4 and α-1,6 linkages). **α-Amylase** (1,4-α-D-glucan glucanohydrolase) is one of the most important of these industrial enzymes. This endo-enzyme acts randomly on α-1,4 linkages, and ultimately generates glucose, maltose and

5. Production of Pharmaceuticals

Antibiotics

Antibiotics are the chemical substances that can kill microorganisms or inhibit their growth, and are therefore, used to fight infections in humans or animals. Most of the antibiotics are produced by microorganisms (i.e. product of one organism that can kill other organism). Certain semisynthetic antibiotics are the chemically modified natural antibiotics.

Penicillins:

Production process of penicillins:

Penicillin production is an aerobic process and therefore, a continuous supply of O_2 to the growing culture is very essential. The pH is maintained around 6.5 and the optimal temperature is in the range of 25 – 27^0 C. penicillin production is usually carried out by submerged process.

↓

The medium used for fermentation consists of corn steep liquor and carbon source (usually lactose).

↓

An addition of yeast extract, soy meal or whey is done for a good supply of nitrogen. Sometimes ammonium sulfate is added for the supply of nitrogen.

↓

Phenylacetic acid (or phenoxyacetic acid) which serves as a precursor for penicillin biosynthesis is continuously fed.

↓

Further, continuous feeding of sugar is advantageous for a good yield of penicillin.

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Recovery: After completion of fermentation, the broth containing about 1% of penicillin is processed for extraction. The mycelium is removed by filtration. Penicillin is recovered by solvent. The penicillin-containing solvent is treated with activated carbon to remove impurities and pigments. Penicillin can be recovered by adding potassium or sodium acetate. The yield of penicillin is around 90%.

Cephalosporins: The pharmaceutical uses of penicillins are associated with allergic reactions in some individuals. To overcome these allergic problems, cephalosporins were developed. They have improved stability against beta-lactamases, and are more active against Gram-negative bacteria. Cephalosporins are broad-spectrum antibiotics with low toxicity.

Production process:

The culture media consists of corn steep liquor and soy flour-based media in a continuous feeding system. The other ingredients of the media include sucrose, glucose and ammonium salts. Methionine is added as a source of sulfur.

↓

The fermentation is carried out at temperature 25 – 28^0 C and pH 6 – 7. The growth of microorganisms substantially increases with good O_2 supply, although during production phase, O_2 consumption declines.

↓

Cephalosporin C from the culture broth can be recovered by ion-exchange resins, and by using column chromatography.

↓

Cephalosporin C can be precipitated as zinc, sodium or potassium salt and isolated.

Steroids

Vaccines

5. Methane is unsuitable for use as a fuel in automobiles. This is because it is very difficult to convert the gaseous methane into liquid state.

Despite the limitations stated above, production of methane from a wide range of biodegradable materials (particularly the wastes) is still attractive. This is due to the fact that the biomass used for methane generation is renewable, in contrast to the permanent depletion of naturally produced methane (in the gas and oil fields).

Biofuels: Biofuels are liquid fuels that have been developed from other materials such as plants or animal waste matter. These fuels are generally in the form of alcohols, esters, ethers and other chemicals produced from biomass. The principle fuel used as a petrol substitute for road transport vehicles is bioethanol. Bioethanol fuel is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. The main sources of sugar required to produce ethanol come from fuel or energy crops. These crops are grown specially for energy use and include corn, maize and wheat crops.

Ethanol (CH_3CH_2OH) is a clear, colorless liquid, it is biodegradable, low in toxicity and causes little environmental pollution if split. Ethanol burns to produce CO_2 and water.

Biodiesel is an alternative fuel similar to conventional or fossil diesel. Biodiesel can be produced from straight vegetable oil, animal oil/fats, tallow and waste cooking oil. The process used to convert these oils to biodiesel is called transesterification.

Why biofuels?

Biofuels production and consumption ensures that the natural carbon cycle to be 100% achieved which completely eliminates the continuous increase in CO_2 rates in the atmosphere which in turns will have the greatest effect on the environment and a way to end global warming. For example, a crop of plants used to produce a barrel of biofuel will absorb exactly the same amount of CO_2 as emitted from burning the barrel produced.

Types of biofuels: On the basis of generation, biofuels are of the following types:

1. **First generation biofuels:**
   These include the following:
   - Bioalcohol
   - Biodiesel
   - Vegetative oil
   - Biogas
   - Syngas
   - Solid biofuels

In the first generation biofuels, bioalcohol and biodiesel are the main two types of biofuels.

2. **Second generation biofuels: Second-generation biofuels**, also known as **advanced biofuels**, are fuels that can be manufactured from various types of non-food biomass. Example: Cellulosic ethanol.

Uses of biofuels:

1. **Cars and trucks:** Diesel cars and trucks can run on biodiesel.
2. **Aircraft:** Recent testing has shown to viability of biofuel use in the aviation industry and use of biofuels to power aircraft is expected to substantially increase in the next decade.
3. **Off road equipment:** A large percentage of off-road equipment such as vehicles used in agriculture, mining, forestry, construction and power and heat production – use diesel fuel, making its equipment suitable for biodiesel use.
4. **Small engines:** Small engines like those found in lawn and mowers and chainsaws, can use ethanol blend up to 10% without problems.

Advantages of biofuel:

1. **Biodegradable:** It is biodegradable and non-toxic.
2. **Cost effective:** It is cost effective than gasoline and other fossil fuels.
3. **Economic stimulation:** Because biofuels are produced locally, biofuel manufacturing plants can employ hundreds of workers, creating new jobs in rural areas.
4. **Easy to recycle:** Whereas oil is a limited resource that comes from specific materials, biofuels can be manufactured from a wide range of material including crop waste, manure and other byproducts. This makes it an efficient step in recycling.
5. **Renewability:** It takes a very long time for fossil fuels to be produced, but biofuels are much more easily renewable as new crops are grown and waste material is collected.
6. **Security:** Biofuels can be produced locally, which decreases the nation's dependence upon foreign energy.
7. **Lower carbon emissions:** When biofuels are burned, they produce significantly less carbon output and fewer toxins, making them a safer alternative to preserve atmospheric quality and lower air pollution.
8. Biofuels can be used directly in unmodified diesel engine.
9. **High flashpoint:** As it has high flashpoint, it is safer to store and transport.
10. Simple to make
11. It can be used purely or blended in any ratio with petroleum diesel.

Disadvantages of biofuel:

1. **Produce carbon emissions:** Several studies have been conducted that the process to produce fuel including the machinery necessary to cultivate the crops and the plants has hefty carbon emissions.
2. **High cost:** To refine biofuels to more efficient energy outputs and to build the necessary manufacturing plants to increase biofuel quantities, a high initial investment is often required.
3. **Food prices:** As demand for food crops such as corn grows for biofuel production, it could also raise prices for necessary staple food crops.
4. **Food shortages:** There is concern that using valuable cropland to grow fuel crops could have an impact on the cost of food and could possibly lead to food shortages.
5. **Water use:** Massive quantities of water are required for proper irrigation of biofuel crops as well as manufacture the fuel, which could strain local and regional water crisis.

Future problems:

1. Some countries have limited water resources.
2. A suitable climate is needed to grow most crops.
3. Crops grown for biodiesel use land for food crops.
4. There is not enough food waste to produce large amounts of biodiesel.

Bioethanol

Bioethanol: Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produces in sugar or starch crops such as corn or sugarcane.

Cellulosic biomass derived from non- food sources such as trees and grasses, is also being developed as a feedstock for ethanol production. It is used to substitute petrol for the road transport vehicles. It is one of the widely used alternative automotive fuels in the world (Brazil and USA are the largest ethanol producers). It is much more environmental friendly and have low toxicity level.

Sources: Ethanol is produced from sugar crops, starches and cellulose. It is produced by fermentation process of materials containing sugar.

Applications of bioethanol:

Ethanol can be used:

- as a transport fuel to replace gasoline
- as a fuel for power generation by thermal combustion
- as a fuel for fuel cells by thermochemical reaction