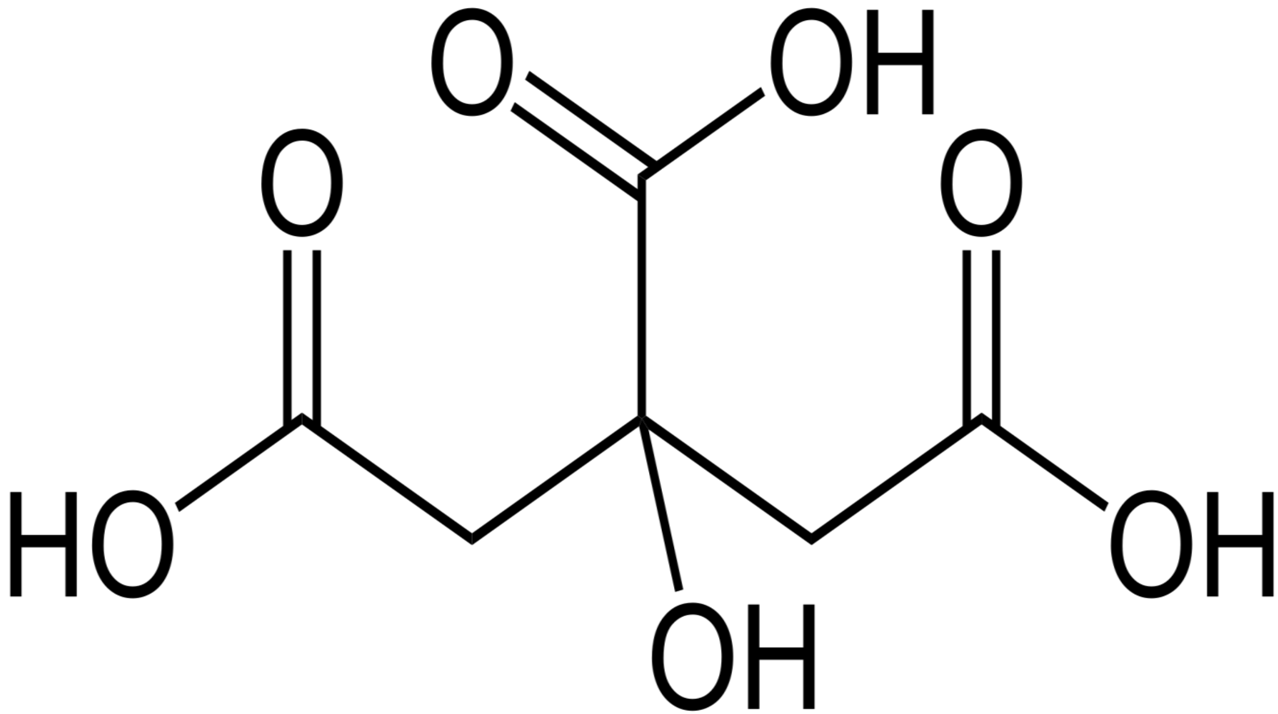
CC-11 Unit 5

Microbial production of industrial products (micro-organisms involved, media, fermentation conditions, downstream processing and uses)
Citric acid, ethanol, penicillin, glutamic acid, Vitamin B12, Enzymes (amylase, protease, lipase) Wine, beer.

Citric acid



Citric acid ($C_6H_8O_7$, 2 – hydroxy – 1,2,3 –propane tricarboxylic acid), a natural constituent and common metabolite of plants and animals, is the most versatile and widely used organic acid in the field of food (60%) and pharmaceuticals (10%).

Source:
Wikipedia

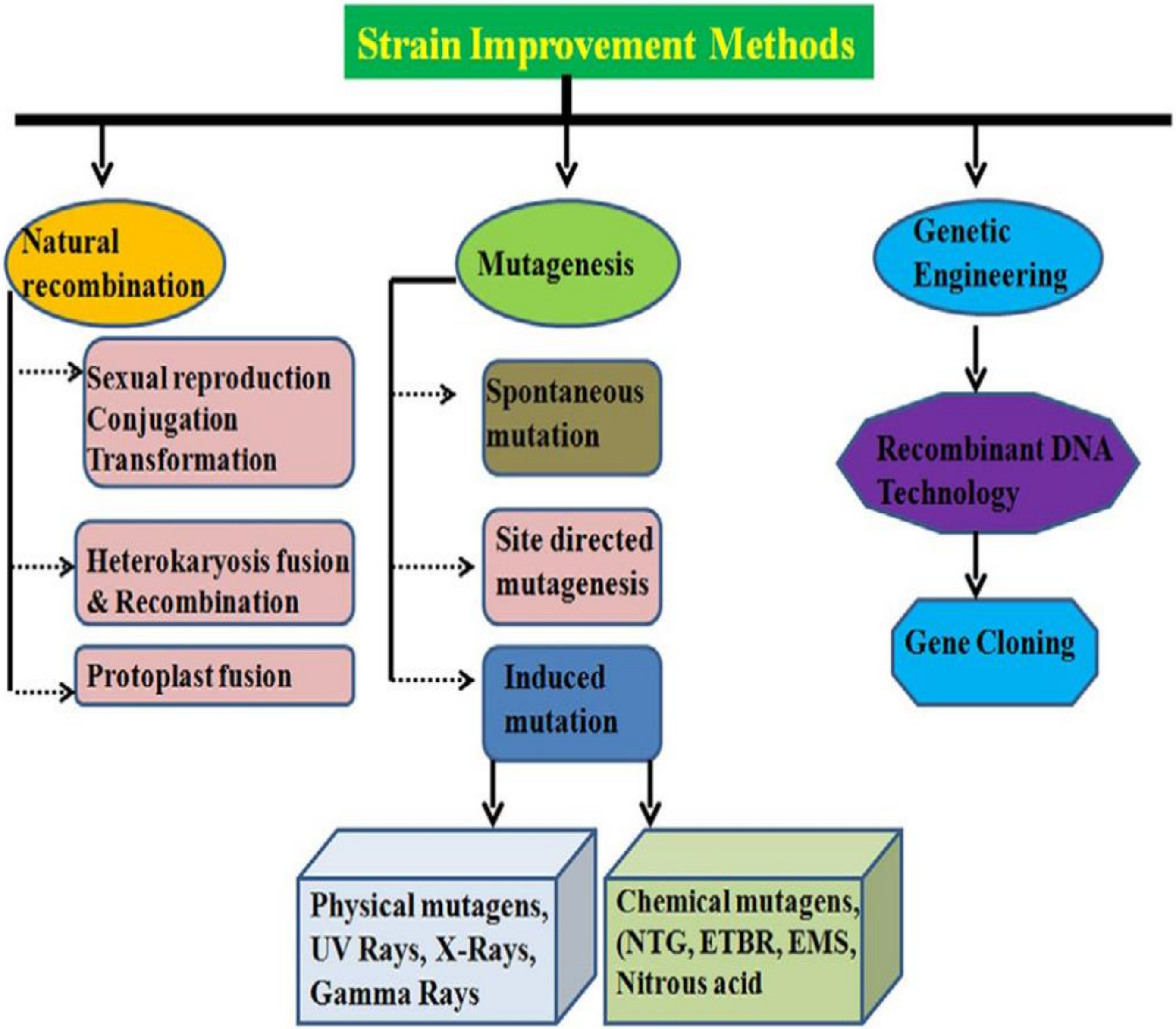
Citric acid

Historical developments

- Citric acid was first isolated by **Karls Scheels in 1874**, in England, from the lemon juice imported from Italy.
- Italian manufacturers had monopoly for its production for almost 100 years, and it was sold at high cost.
- In **1923, Wehmer** observed the presence of citric acid as a by-product of calcium oxalate produced by a culture of ***Penicillium glaucum***. Other investigations showed the isolation of two varieties of fungi belonging to genus ***Citromyces*** (namely ***Penicillium***).
- The industrial process was first open by Currie, in 1917, who found that ***Aspergillus niger*** had the capacity to accumulate significant amounts of citric acid in sugar based medium. He also showed that high concentrations of sugar favoured its production, which occurred under limitation of growth.
- The biochemical basis was only cleared in the fifties with the discovery of the glycolytic pathway and the tricarboxylic acid cycle (TCA).

Citric acid (CA) producing microorganism	
Microbes	CA producing species
Bacteria	*Bacillus licheniformis, Arthrobacter paraffinens, Corynebacterium sp., Bacillus subtilis, Brevibacterium flavum, Corynebacterium sp.,*
Fungi	*Aspergillus niger, A. aculeatus, A. awamori, A. carbonarius, A. wentii, A. foetidus, Penicillium janthinelum*
Yeast	*Saccahromicopsis lipolytica, Candida tropicalis, C. Oleophila, C. Guilliermondii, C. Parapsilosis, C. Citroformans, Hansenula anamola, Yarrowia lipolytica, Torulopsis, Hansenula, Debaromyces, Torula, Pichia, Kloekera, and Zygosaccharomyces*

Source: DOI: 10.1002/fft2.66



N-methyl-*N*′-nitro-*N*-nitrosoguanidine Ethidium bromide Ethyl methanesulfonate

FACTORS AFFECTING CITRIC ACID PRODUCTION

Growth Medium and its components

Carbon source:

Citric acid accumulation is strongly affected by the nature of the carbon source. The presence of easily metabolized carbohydrates has been found essential for good production of citric acid. Sucrose was the most favourable carbon source followed by glucose, fructose and galactose.

Nitrogen source:

Citric acid production is directly influenced by the nitrogen source. Physiologically, ammonium salts are preferred, e.g. urea, ammonium sulfate, ammonium chlorure, peptone, malt extract, etc. Nitrogen consumption leads to pH decrease, which is very important point in citric acid fermentation.

Phosphorous source:

Presence of phosphate in the medium has a great effect on the yield of citric acid. Potassium dihydrogen phosphate has been reported to be the most suitable phosphorous source. Phosphate is known to be essential for the growth and metabolism of ***A. niger***. Low levels of phosphate favour citric acid production, however, the presence of excess of phosphate was shown to lead to the formation of certain sugar acids, a decrease in the fixation of CO_2, and the stimulation of growth.

Trace elements:

Trace element nutrition is probably the main factor influencing the yield of citric acid. A number of divalent metals such as zinc, manganese, iron, copper and magnesium have been found to affect citric acid production by ***A. niger***.

Copper was found to complement the ability of iron at optimum level, to enhance the biosynthesis of citric acid. Manganese deficiency resulted in the repression of the anaerobic and TCA cycle enzymes with the exception of citrate synthetase. This led to overflow of citric acid as an end product of glycolysis.

Lower alcohols:

Addition of lower alcohols enhances citric acid production from commercial glucose and other crude carbohydrate. Appropriate alcohols are methanol, ethanol, iso- propanol or methyl acetate. The optimal amount of methanol/ethanol depends upon the strain and the composition of the medium, generally optimum range being 1-3%.

Miscellaneous:

Some compounds which are inhibitors of metabolism such as calcium fluoride, sodium fluoride and potassium fluoride have been found to accelerate the citric acid production, while, potassium ferrocyanide has been found to decrease the yield. There are many compounds, which act in many ways to favour citric acid accumulation.

Process parameters

pH:

The pH of a culture may change in response to microbial metabolic activities. The most obvious reason is the secretion of organic acids such as citric, acetic or lactic acids, which will cause the pH to decrease. Changes in pH kinetics depend highly also on the microorganism

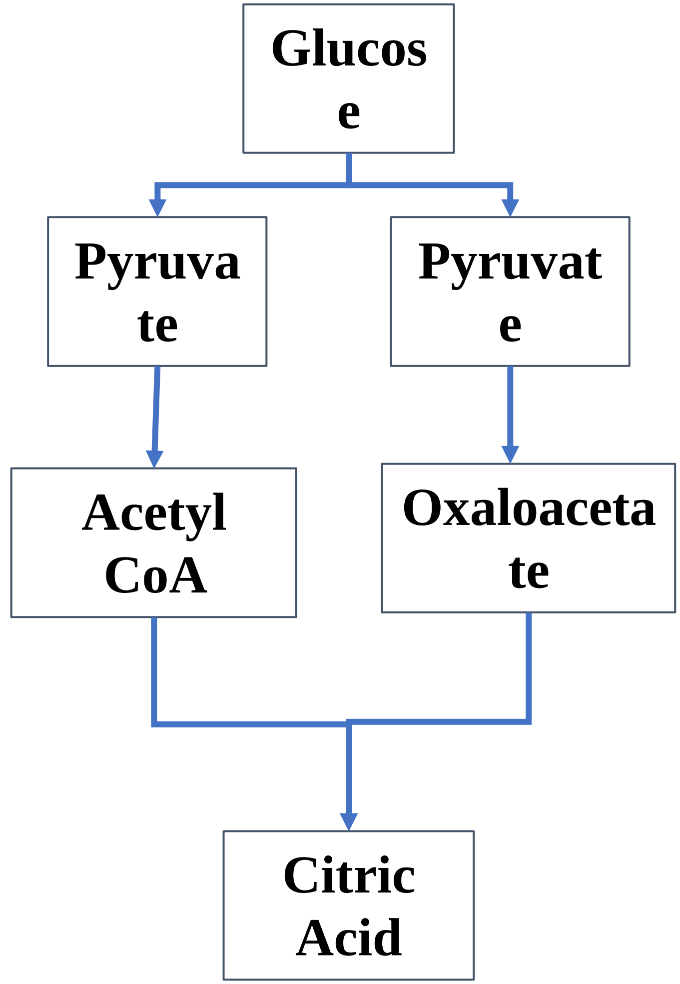
Aeration:

Aeration has been shown to have a determinant effect on citric acid fermentation. Increased aeration rates led to enhanced yields and reduced fermentation time.

Microbial biosynthesis of citric acid

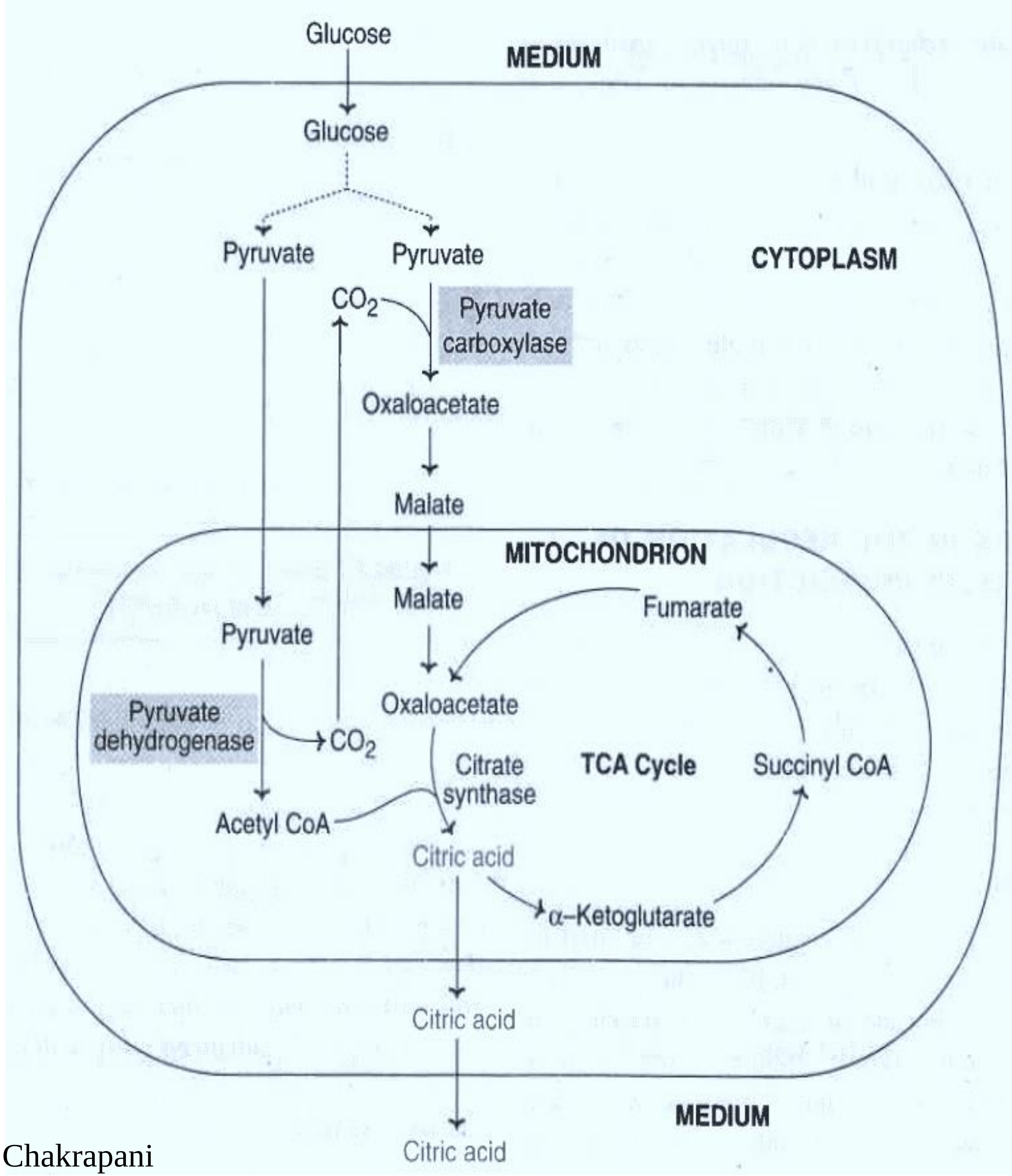
Citric acid is a primary metabolic product (of primary metabolism) formed in the tricarboxylic acid (Krebs) cycle.

I. Glucose is the predominant carbon source for citric acid production.
II. The biosynthetic pathway for citric acid production involves glycolysis wherein glucose is converted to two molecules of pyruvate.
III. Pyruvate in turn forms acetyl CoA and oxaloacetate which condense to finally give citrate.



****Enzymatic regulation of citric acid production:**
During the synthesis of citric acid, it is seen that, there is a tenfold increase in the activity of the enzyme citrate synthase.

An outline of metabolic pathway for the biosynthesis of citric acid (TCA cycle-Tricarboxylic acid cycle).



Source: Biotechnology, U. Satyanarayana, U. Chakrapani

PRODUCTION PROCESSES FOR CITRIC ACID

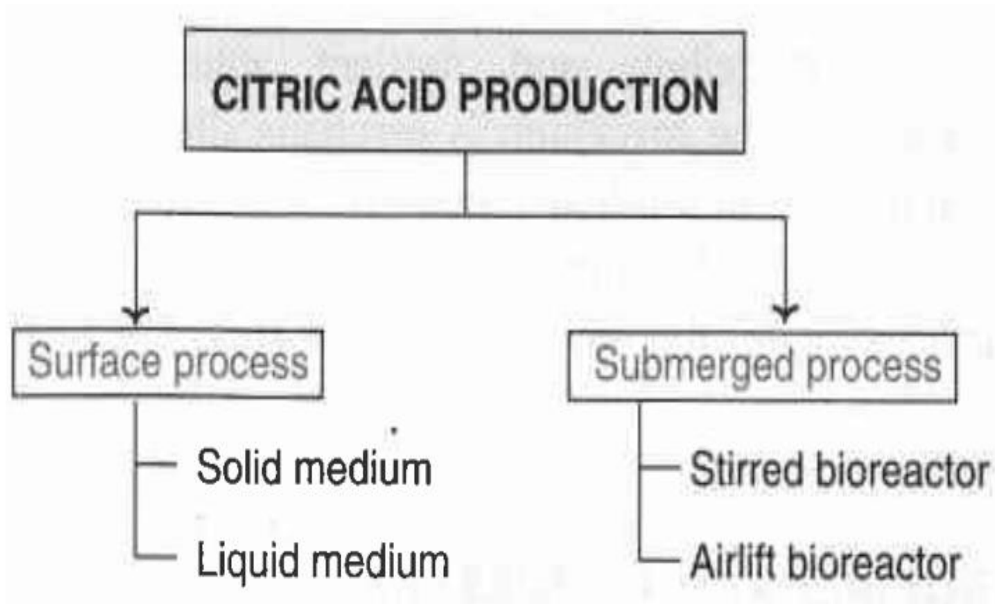
There are two processes by which citric acid can be industrially produced -the surface process and submerged process.

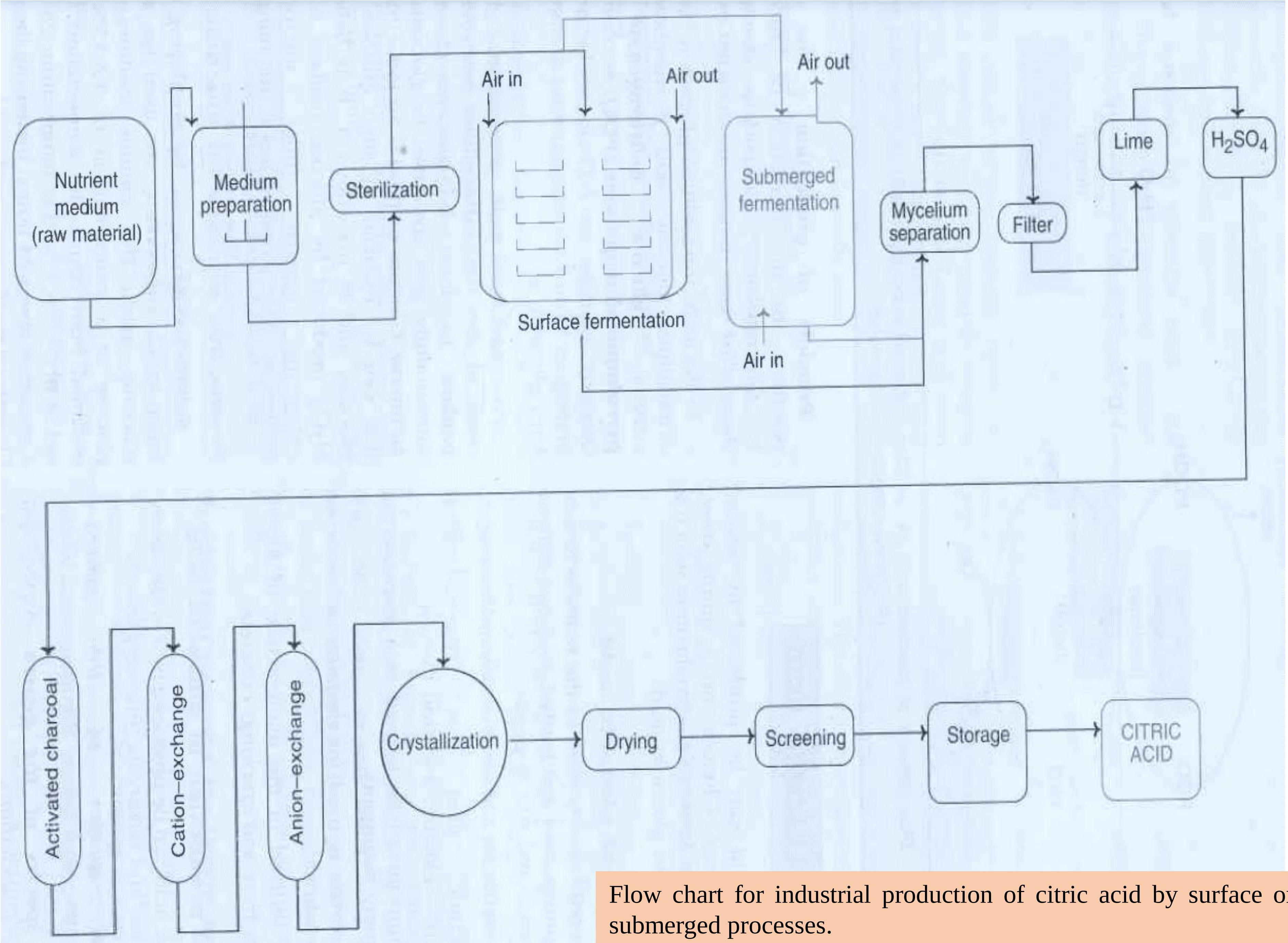
1. **The surface process :**

This is characterized by growing the microorganisms as a layer or a 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.

2. 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.





Flow chart for industrial production of citric acid by surface or submerged processes.

SURFACE PROCESSES

Solid surface fermentation

Surface processes using solid substrates are particularly carried out in less developed areas of some Asian countries.

The solid substrates such as wheat bran or pulp from sweet potato starch are used, as culture media.

↓

The pH of the medium is adjusted to 4-5, and then sterilized.

↓

Now the inoculum in the form of spores of *A. niger* is spread as layers (3-6 cm thickness) and incubated at 28^{o}C.

↓

The growth of the organisms can be accelerated by the addition of α-amylase.

↓

Solid-state fermentation takes about 80 to 100 hours for maximal production of citric acid.

↓

At the end of the process, citric acid can be extracted into hot water and isolated

Liquid surface fermentation

1. The nutrient supply for surface fermentation normally comes from beet molasses.
2. The fermentation is usually carried out in aluminium trays filled with sterile nutrient medium.
3. The inoculum in the form of spores is sprayed over the medium. A sterile air is passed for supplying O_2, as well as cooling.
4. The temperature is maintained around 30^oC during fermentation.
5. As the spores germinate (that occurs within 24 hours of inoculation), a layer of mycelium is formed over the medium.
6. The pH of the nutrient medium falls to less than 2, as the mycelium grows in size and forms a thick layer on the surface of the nutrient solution.
7. The fermentation is stopped after 7-15 days.
8. The mycelium and nutrient solution are separated. The mycelium is mechanically pressed and thoroughly washed to obtain maximum amount of citric acid.
9. The nutrient solution is subjected to processing for the recovery of citric acid.
10. The final yield of citric acid is in the range of 0.7-0.9 of per gram of sugar.

SUBMERGED PROCESSES

1. Around 80% of the world's supply of citric acid is produced by submerged processes.
2. This is the most preferred method due to its high efficiency and easy automation.
3. The disadvantages of submerged fermentation are - adverse influence of trace metals and other impurities, variations in O_2, tension, and advanced control technology that requires highly trained personnel.
4. Two types of bioreactors are in use- stirred tanks and aerated towers.
5. The vessels of the bioreactors are made up of high-quality stainless steel.
6. The sparging of air occurs from the base of the fermenter.

RECOVERY OF CITRIC ACID

1. The recovery starts with the filtration of the culture broth and washing of mycelium (which may contain about 10% of citric acid produced).
2. Oxalic acid is an unwanted byproduct and it can be removed by precipitation by adding lime at pH < 3.
3. The culture broth is then subjected to pH 7.2 and temperature 70-90 ^{o}C for precipitating citric acid.
4. For further purification, citric acid is dissolved in sulfuric acid (calcium sulfate precipitate separates).
5. The final steps for citric acid recovery are - treatment with activated charcoal, cation and anion-exchangers and crystallization.
6. Citric acid monohydrate formed below 36 ^{o}C is the main commercial product. Above 40 ^{o}C, citric acid crystallizes in an anhydrous form.
7. The degree of purity of citric acid produced depends on the purpose for which it is required.
8. For instance, pure forms of citric acid are needed for use in food preparations, while for industrial use it can be crude form.

Applications of Citric Acid

Industry	Applications
Beverages	Provides tartness and complements fruits and berries flavors. Increases the effectiveness of antimicrobial preservatives. Used in pH adjustment to provide uniform acidity.
Jellies, Jams and Preserves	Provides tartness. pH adjustment.
Candy	Provides tartness. Minimizes sucrose inversion. Produces dark color in hard candies. Acts as acidulant.
Frozen fruit	Lowers pH to inactivate oxidative enzymes. Protects ascorbic acid by inactivating trace metals
Dairy products	As emulsifier in ice creams and processed cheese; acidifying agent in many cheese products and as an antioxidant.
Fats and oils	Synergist for other antioxidants, as sequestrant.
Pharmaceuticals	As effervescent in powders and tablets in combination with bicarbonates. Provides rapid dissolution of active ingredients. Acidulant in mild astringent formulation. Anticoagulant.
Cosmetics and toiletries	pH adjustment, antioxidant as a metallic–ion chelator, buffering agent.
Industrial applications	Sequestrant of metal ions, neutralizant, buffer agent
Metal cleaning	Removes metal oxides from surface of ferrous and nonferrous metals, for preperational and operational cleaning of iron and copper oxides
Others	In electroplating, copper plating, metal cleaning, leather tanning, printing inks, bottle washing compounds, floor cement, textiles, photographic reagents, concrete, plaster, refractories and moulds, adhesives, paper, polymers, tobacco, waste treatment, etc.