Organic Acid Fermentation-

Organic acids are broadly distributed in nature, and humans have used them in their natural sources since early ages. Fermentation processes that involve the production of acetic and lactic acid have been used in food preparation for centuries. Industrial production with the microbiological route was started when the acids were identified as the main product in known fermentation processes. During the first half of the twentieth century, advances in chemical synthesis offered new manufacturing procedures that became economically competitive and replaced many fermentation processes. The situation changed in the 1990s, when further developments in the biotechnology field, environmental pressures and the vertical integration of the fermentation and corn processing industries resulted in much improved economics for the biological route. A number of organic acids are obtained by fermentation using various microbes as given below.

Microbes used in Organic acid

A. Citric acid -Aspergillus niger
B. Gluconic acid -Aspergillus niger
C. Fumaric acid -Rhizopus arrhizus
D. Acetic acid -Acetobacter aceti
E. Lactic acid- Lactobacillus delbruecjii and L. bulgaris

A. Citric acid was first discovered as a constituent of lemon. Today, we know citric acid as an intermediate of ubiquitous Krebs cycle (citric acid cycle), and therefore, it is present in every living organism. In the early days, citric acid was isolated from lemons (that contain 7-9% citric acid), and today about 99% of the world's citric acid comes from microbial fermentation.

Applications of Citric Acid: 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. Besides brightening the colour, 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 serve 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 catalysed reactions. Citric acid is also used as a stabilizer of emulsions in the preparation of cheese.

5. In detergent/cleaning industry, citric acid has slowly replaced polyphosphates.

Microbial Strains for Citric Acid Production: Many microorganisms can produce citric acid. The fungus Aspergillus Niger is most commonly used for industrial production of citric acid. The other organisms (although less important) include A. clavatus, A. wentii, Penicillium luteum, Candida catenula, C. guilliermondii and Corynebacterium sp. For improved industrial production of citric acid, mutant strains of A. Niger have been developed. The strains that can tolerate high sugar concentration and low pH with reduced synthesis of undesirable byproducts (oxalic acid, isocitric acid and gluconic acid) are industrially important.

Microbial Biosynthesis of Citric Acid: Citric acid is a primary metabolic product (of primary metabolism) formed in the tricarboxylic acid (Krebs) cycle. Glucose is the predominant carbon source for citric acid production. The biosynthetic pathway for citric acid production involves glycolysis wherein glucose is converted to two molecules of pyruvate. Pyruvate in turn forms acetyl CoA and oxaloacetate which condense to finally give citrate. The major steps in the biosynthesis of citric acid are depicted in Fig. 24.1.

Carbohydrate source: A wide range of raw materials can be used for the supply of carbohydrates. These include molasses (sugar cane or sugar beet), starch (from potatoes), date syrup, cotton wastes, banana extract, sweet potato pulp, and brewery waste and pineapple waste water. A high yield of citric acid production occurs if the sugars that are rapidly metabolised are used e.g. sucrose, glucose, maltose. At present, cane molasses and beet molasses are commonly used. Temperature 27 to 330, pH 1.8-2.0,Timings for 14 days, Salts like $K2HPO4$ and $MgCl2$, Metals Cu and Fe.

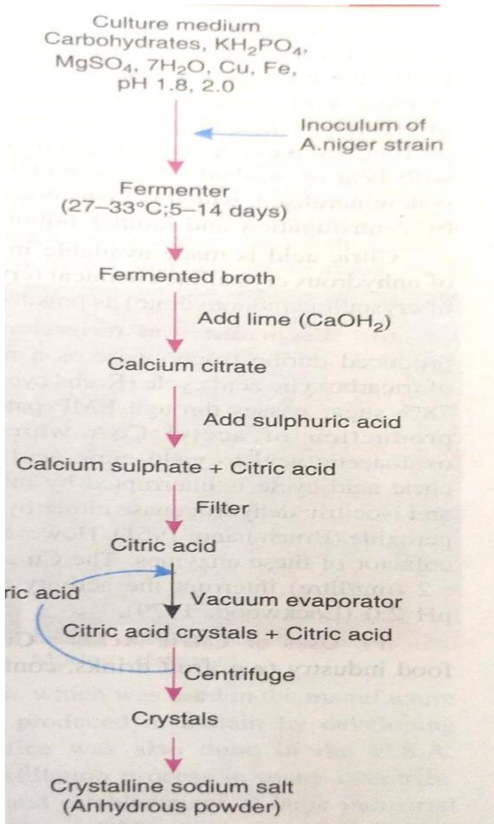

Fig. 17.2. Outline of citric acid production method.

2. Gluconic Acid: Gluconic acid can be produced by several bacteria and fungi. Glucose, on a simple direct dehydrogenation, forms D-gluconolactone which is then converted to gluconic acid.

Applications of Gluconic Acid:

1. Gluconic acid is used in the manufacture of metals, stainless steel and leather, as it can remove the calcareous and rust deposits.

2. It is used as an additive to foods and beverages.

3. Gluconic acid has pharmaceutical applications — calcium and iron therapy.

4. Sodium gluconate is used as a sequestering agent in many detergents.

5. Gluconate is used for desizing polyester or polyamide fabrics.

6. It is utilized in the manufacture of highly resistant (to frost and cracking) concrete.

Microbial Production of Gluconic Acid: Gluconic acid can be produced by a wide variety of prokaryotic and eukaryotic microorganisms.

a. Bacterial species of the genera— Gluconobacter, Acetobacter, Pseudomonas, Vibrio.

b. Fungal species of the genera—Aspergillus, Penicillium, Gliocladium.

Principle of production: The enzymatic reactions for the formation of gluconic acid in Gluconobacter suboxidans (bacteria) and Aspergillus niger (fungus) are depicted in Fig. 24.6

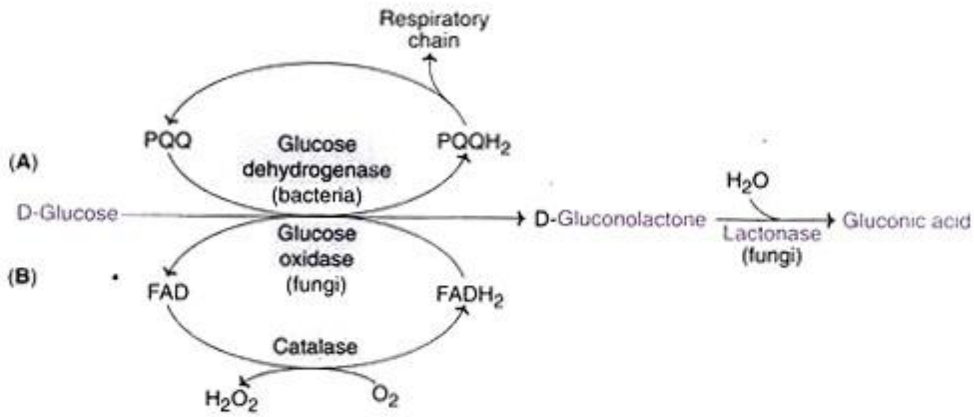

Fig. 24.6 : Biosynthesis of gluconic acid (A) in Gluconobacter suboxidans *(B) in* Aspergillus niger. *(PQQ-Pyrroloquinoline quinone)*

A. In bacteria, intracellular glucose is converted to extracellular gluconic acid. A membrane bound enzyme, glucose dehydrogenase utilizes pyrroloquinoline Quinone (PQQ) as coenzyme and converts glucose to 5-D-gluconolactone which undergoes hydrolysis (spontaneous or enzymatic) to form gluconic acid.
B. In fungal production, glucose is oxidized by the extracellular enzyme glucose oxidase to form 8-D-gluconolactone, which subsequently gets converted to gluconic acid by lactonase.

Production Process for Gluconic Acid:

1. Submerged processes, by employing either A. niger or G. suboxidans, are used for producing gluconic acid.
2. The culture medium contains glucose at a concentration of 12-15% (usually obtained from corn).
3. The fermentation is carried out at pH 4.5-6.5
4. Temperature 28-30°C for a period of about 24 hours.
5. Increasing the supply of O_2 enhances gluconic acid yield.

Lactic Acid: Lactic acid occurs in two isomeric forms i.e. L (+) and D (-) isomers, and as a racemic mixture (DL-lactic acid). The isolation of lactic acid from milk was done in 1798. It was the first organic acid produced by microorganisms in 1880. Today, lactic acid is competitively produced both by microbiological and chemical methods.

Applications of Lactic Acid: There are different grades of lactic acid mainly based on the percentage of lactic acid. Microorganisms for Production of Lactic Acid:

Table 24.2 Commercial grades of lactic acid along with their applications

Grade (% lactic acid)	*Application(s)*
Technical grade (20–50%)	Ester manufacture, textile industry
Food grade (>80%)	Food additive (sour flour and dough)
Pharmaceutical grade (>90%)	Intestinal treatment (metal ion lactates)

Lactic acid producing bacteria are broadly categorized into two types.

A. **Hetero-fermentative bacteria—produce other byproducts, besides lactic acid, and therefore are not useful for industrial production of lactic acid. These bacteria are employed in food or feed preservation.**
B. **Homo-fermentative bacteria—specialised for exclusive production of lactic acid and therefore are suitable for industrial purpose.**

   Lactobacillus sp are used for lactic acid production. However, there are variations in the substrates utilised as indicated below..

L. delbrueckii, *L. leichamanni*	Glucose
L. bulgaricus, *L. helvetii*	Whey (lactose)
L. lactis	Maltose
L. amylophilus	Starch
L. pentosus	Sulfite waste liquor

Biosynthesis of lactic acid: The synthesis of lactic acid occurs through glucose oxidation by glycolysis to produce pyruvate which on reduction gives lactic acid. The reducing equivalents (NADH++H+) produced during the oxidation of glyceraldelyde 3-phosphate are utilised by .the enzyme lactate dehydrogenase to form lactate (Fig. 24.7).

Production Process for Lactic Acid:

The fermentation medium contains 12-15% of glucose, nitrogen and phosphate containing salts and micronutrients. The process is carried out at pH 5.5-6.5 and temperature 45-50°C for about 75 hours. Generally, the strains operating at higher temperature (45-60°C) are preferred, since it reduces the need for medium sterilization.

As the lactic acid is produced, it has to be removed since it is toxic to the organisms. This can achieved either by a continuous culture technique or by removal of lactic acid by electro dialysis. Theoretically, every molecule of glucose forms two molecules of lactic acid. About 90% of theoretical yield is possible in fermentation industry. L(+) Lactic acid is predominantly produced. The outline of the steps involved in the recovery lactic acid is depicted in Fig. 24.8

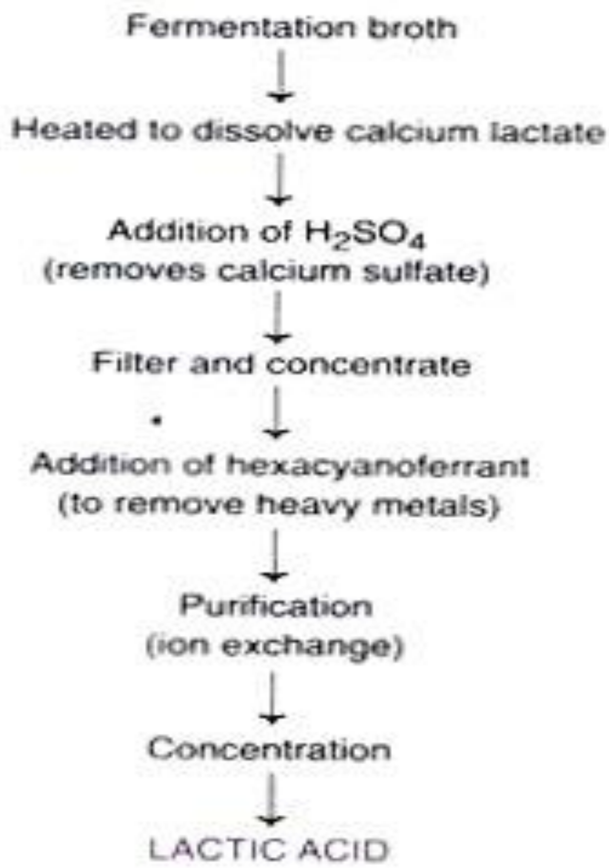

Fig. 24.8 : Flow chart for recovery of lactic acid from fermentation broth.

Acetic Acid: The production of acetic acid, in the form of vinegar (used as a refreshing drink), from alcoholic liquids has been known for centuries.

Microorganisms Used for Production of Acetic Acid: The commercial production of acetic acid is carried out by a special group of acetic acid bacteria, which are divided into two genera.

A. Gluconobacter that oxidizes ethanol exclusively to acetic acid.

B. Acetobacter that oxidizes ethanol first to acetic acid, and then to CO_2 and H_2O. These over-oxidizers are Gram-negative and acid tolerant e.g. A. aceti, A. peroxidans, A. pasteurianus.

Biosynthesis of acetic acid: Acetic acid is a product of incomplete oxidation of ethanol. Ethanol is first oxidized by alcohol dehydrogenase to acetaldehyde which then gets hydrated to form acetaldehyde hydrate. The latter is then acted upon by acetaldehyde dehydrogenase to form acetic acid

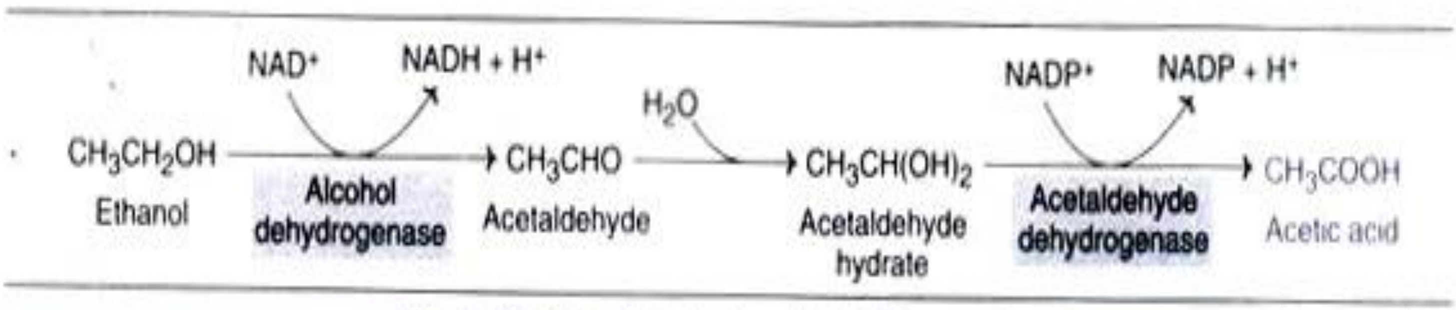

Fig. 24.9 : Biosynthesis of acetic acid from ethanol.

Production Process for Acetic Acid: For every molecule of ethanol oxidised, one molecule of acetic acid is produced. Thus, high- yielding strains can produce 11-12% acetic acid from 12% alcohol. For optimal production, adequate supply of oxygen is very essential. Insufficient O_2, coupled with high concentration of alcohol and acetic acid result in the death of microorganisms. Surface fermentation or submerged fermentation processes can be carried out to produce acetic acid. Trickling generation process, a type of surface fermentation, is very commonly used.

Recovery:

The acetic acid produced is clarified by filtration and then subjected to decolourization by $K_4(FeCN)_6$.

Production of Vinegar: Vinegar is an aqueous solution containing about 4% by volume acetic acid and small quantities of alcohol, salts, sugars and esters. It is widely used as a flavoring agent for processed liquid foods such as sauces and ketchups. The starting materials for vinegar production are wine, whey, malt (with low alcohol content). Vinegar production can be carried out either by surface process (trickling generator) or by submerged process.

1. Surface process: The fermentation material is sprayed over the surface which trickles through the shavings that contain the acetic acid producing bacteria. The temperature is around 30°C on the upper part while it is around 35°C on the lower part. Vinegar is produced in about 3 days.
2. Submerged process: The fermentation bioreactors are made up of stainless steel. Aeration is done by a suction pump from the top. The production rate in the submerged process is about 10 times higher than the surface process.

L-Ascorbic Acid: L-Ascorbic acid is the commonly used chemical name for the water soluble vitamin C. This vitamin forms a redox system and participates in several biological processes. It is intimately involved in the biosynthesis of collagen, the most abundant protein in the human body. Vitamin C also protects the body against carcinogenic nitrosamines and free radicals. The deficiency of ascorbic acid causes scurvy.

Applications of Ascorbic Acid:

Because of the wide range of physiological and beneficial functions of ascorbic acid, its commercial production assumes significance. Vitamin C is mainly used in food and pharma¬ceutical industries.

Industrial Production of Ascorbic Acid:

Ascorbic acid is commercially produced by a combination of several chemical steps, and one reaction of biotransformation brought out by microorganisms. This process is referred to as Reichstein-Grussner synthesis (Fig. 24.10B). D-Glucose is first converted to D-sorbitol. Oxidation of D-sorbitol to L-sorbose is carried out by Acetobacter xylinum or A. suboxydans (The enzyme being sorbitol dehydrogenase).

A submerged bioreactor fermentation process is ideal for this reaction. It takes about 24 hours at temperature 30-35°C. Sorbose by a couple of chemical reactions can be finally converted to L-ascorbic acid. Normally, about 100 g of ascorbic acid is produced from 200 g of glucose in Reichstein-Grussner synthesis.

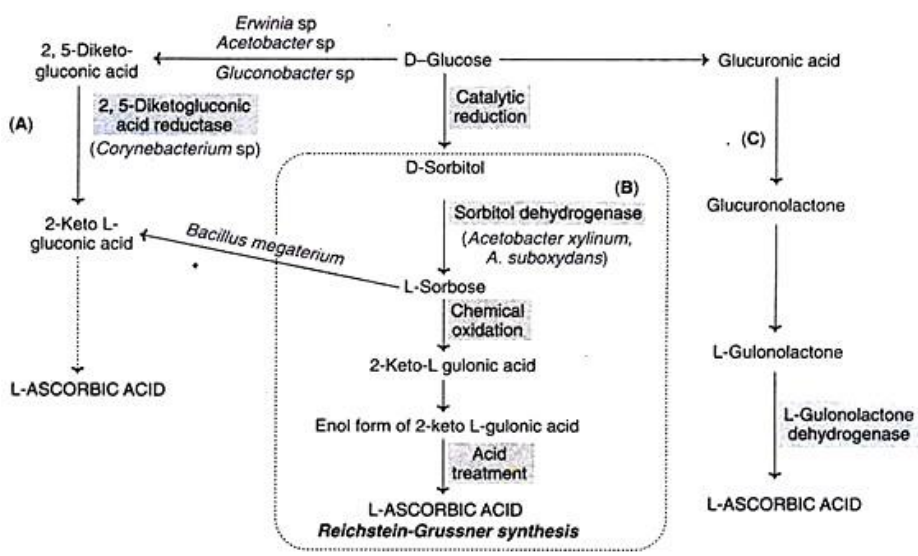

Fig. 24.10 : Pathways for the commercial production of ascorbic acid. (A) Two-step fermentation process (B) Reichstein-Grussner synthesis (C) Production via L-gulonolactone.

. Itaconic Acid: Itaconic acid is used in plastic industry, paper industry and in the manufacture of adhesives. Itaconic acid can be commercially produced by Aspergillus itoconicus and A. terreus. The biosynthesis of itaconic acid occurs by way of Krebs cycle. The metabolite cis-aconitic acid (formed from citric acid) undergoes decarboxylation catalysed by the enzyme cis-aconitic decarboxylase (Fig. 24.11).

Itaconic acid is oxidised to itatartaric acid by itaconic acid oxidase. This enzyme has to be inhibited for a maximum yield of itaconic acid. This can be achieved by adding calcium. Batch submerged fermentation process is commonly used for itaconic acid production. The yield is around 75% of the theoretical calculation when the medium contains 15% sucrose.

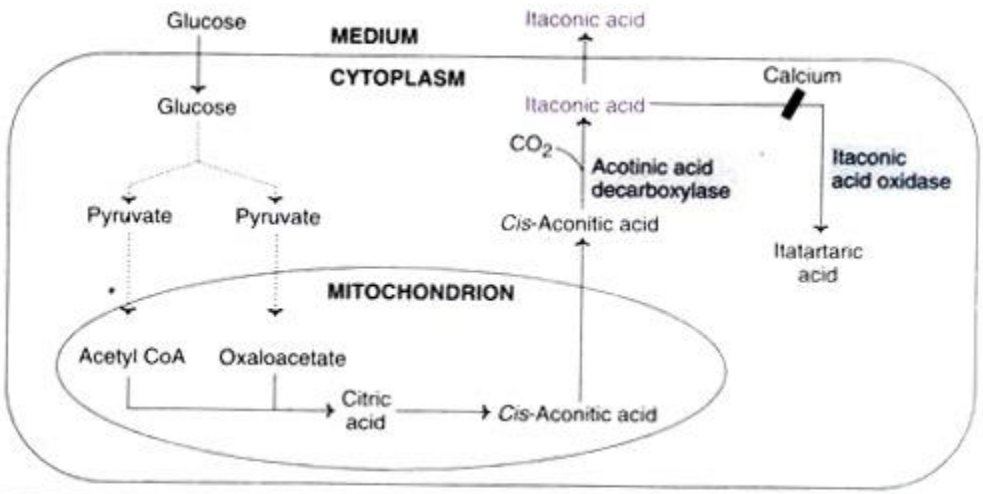

Fig. 24.11 : Biosynthesis of itaconic acid—an outline of metabolic pathway.

Uses of organic acid

1. Acetic acid id used in food industry and research. Acetic acid is also known as ethanoic acid, ethylic acid, vinegar acid, and methane carboxylic acid; it has the chemical formula of CH_3COOH. Acetic acid is a byproduct of fermentation, and gives vinegar its characteristic odor. Vinegar is about 4-6% acetic acid in water.
2. Fumaric acid is used as a food acidulant in beverages and baking powders. Furthermore, fumaric acid is a pharmaceutically active substance that is used to treat psoriasis or multiple sclerosis [42]. Originally, fumaric acid was isolated from plants belonging to the genus Fumaria, from which its name is derived.
3. Gluconic acid is used in medicals as calcium, iron, potassium gluconate and in cleaning of bottles. Gluconic acid is used in the manufacture of metal, leather, and food. It has been accredited with the capability of inhibiting bitterness in foods. Sodium gluconate is permitted in food and it has GRAS (generally recognized as safe) status.
4. Lactic acid is used in food industries, in dye mordant, in tanning decalcifying skins, in medical asiron and calcium lactase
5. Citric acid is commonly used as a food additive for natural flavoring and as a preservative. It is also used in cosmetics, for medical purposes, as an antioxidant and in cleaning products. In addition to its use as a cleaner, citric acid is used for pickling and canning foods, a rinse for

fresh fruits and vegetables, adding flavor to dishes, and crafting projects like soap and bath bombs.