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FACULTY OF ENGINEERING &  
TECHNOLOGY

# Production of Important Organic Acids by Fermentation

## Industrial Production of Citric Acid

- Citric acid was first discovered as a constituent of lemon.
- 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.
  - It is used in the chemical industry as an antifoam agent, and for the treatment of textiles
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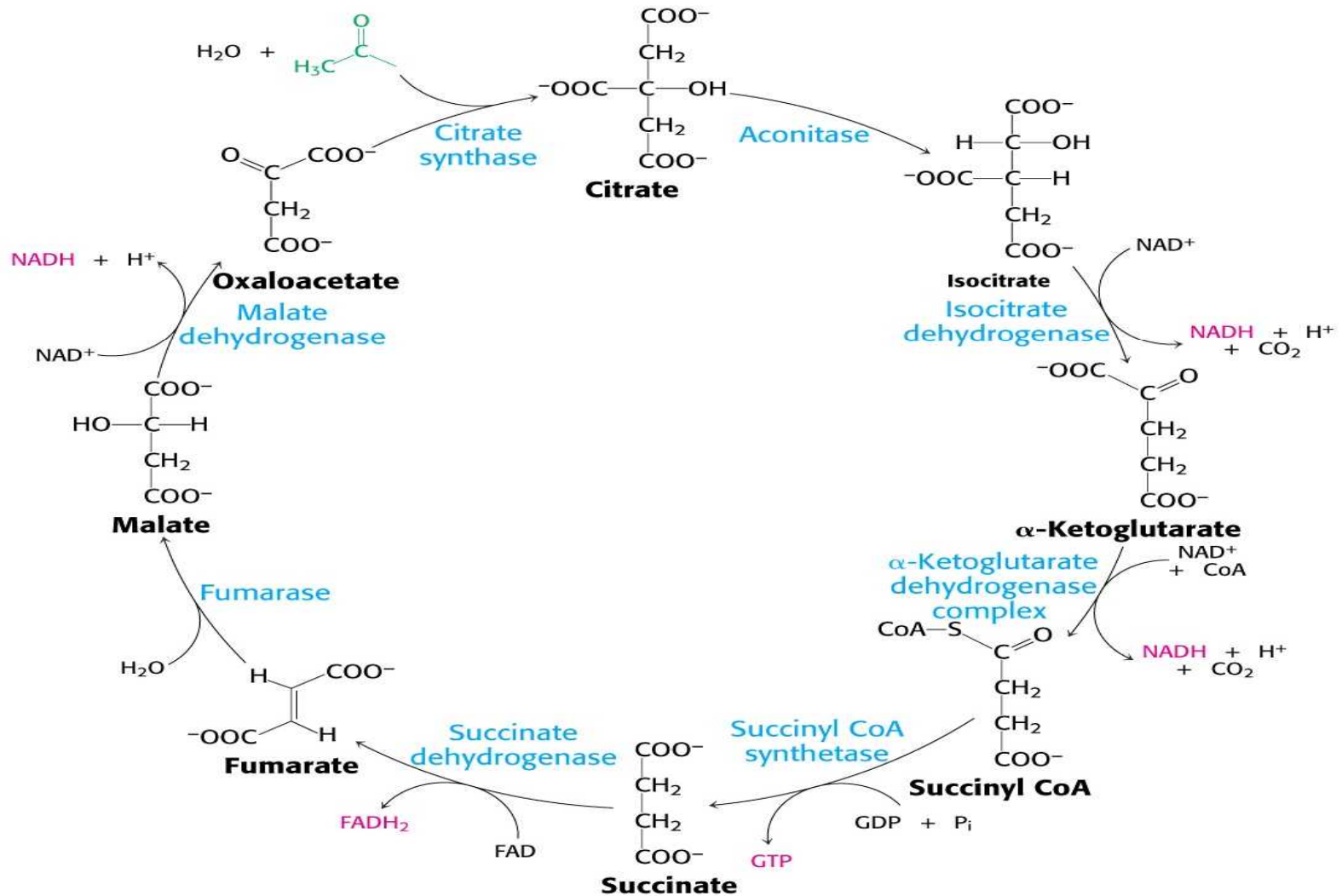
- In metal industry, pure metals are complexed with citrate and produced as metal citrates.
- In pharmaceutical industry, as trisodium citrate, it is used as a blood preservative. Citric acid is also used for preservation of ointments and cosmetic preparations. As iron citrate, it serves as a good source of iron.
- 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.
- In detergent/cleaning industry, citric acid has slowly replaced polyphosphates.

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## Biochemical Basis of the Production of Citric Acid

- Since it is an intermediate of Krebs cycle, so the acid can be accumulated by using one of the following methods
- **By mutation** – giving rise to mutant organisms which may only use part of a metabolic pathway, or regulatory mutants; that is using a mutant lacking an enzyme of the cycle.
- **By inhibiting** the free-flow of the cycle through altering the environmental conditions, e.g. temperature, pH, medium composition (especially the elimination of ions and cofactors considered essential for particular enzymes).
- The following are some of such environmental conditions which are applied to increase citric acid production:
  - The concentrations of iron, manganese, magnesium, zinc, and phosphate must be limited. To ensure their removal the medium is treated with ferro-cyanide or by ion exchange resins.
  - These metal ions are required as prosthetic groups in the following enzymes of the TCA:  $Mn^{++}$  or  $Mg^{++}$  by oxalosuccinic decarboxylase,  $Fe^{+++}$  is required for succinic dehydrogenase, while phosphate is required for the conversion of GDP to GTP

**Citric acid can be caused to accumulate by using a mutant lacking an enzyme of the cycle or by inhibiting the flow of the cycle**



## Biochemical Basis of the Production of Citric Acid

- The dehydrogenases, especially isocitrate dehydrogenase, are inhibited by anaerobiosis, hence limited aeration is done on the fermentation so as to increase the yield of citric acid.
- Low pH and especially the presence of citric acid itself inhibits the TCA and hence encourages the production of more citric acid; the pH of the fermentation must therefore be kept low throughout the fermentation by preventing the precipitation of the citric acid formed.
- Many of the enzymes of the TCA can be directly inhibited by various compounds and this phenomenon is exploited to increase citric acid production.
- Thus, isocitric dehydrogenase is inhibited by ferrocyanide as well as citric acid; aconitase is inhibited by fluorocitrate and succinic dehydrogenase by malonate.
- These at enzyme antagonists may be added to the fermentation.

## Fermentation for Citric Acid Production

- *Aspergillus* For a long time the production of citric acid has been based on the use of molasses and various strains of *Aspergillus niger* and occasionally *Asp. wentii*.
  - Production by *Penicillium* is available, in practice are not used because of low productivity.
  - Recently yeasts, especially *Candida spp.* (including *Candida quillermondi*) have been used to produce the acid from sugar.
  - Japanese workers described a method to produce the acid by paraffins by bacteria and yeasts. Among the bacteria were *Arthrobacter paraffineus* and *corynebacteria*; the yeasts include *Candida lipolytica* and *Candida oleiphila*.
  - Fermentation with molasses and other sugar sources can be either surface or submerged. Fermentation with paraffins however is submerged.
  - **(a) Surface fermentation**: *Surface fermentation using s niger may be done on rice bran as is the case in Japan, or in liquid solution in flat aluminium or stainless steel pans.*
  - Special strains of *Asp. niger* which can produce citric acid despite the high content of trace metals in rice bran are used. The citric acid is extracted from the bran by leaching and is then precipitated from the resulting solution as calcium citrate.
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- **(b) Submerged fermentation:** As in all other processes where citric acid is made the fermentation the fermentor is made of acid-resistant materials such as stainless steel.
- The carbohydrate sources are molasses decationized by ion exchange, sucrose or glucose.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{KH}_2\text{PO}_4$  at about 1% and 0.05-2% respectively are added (in submerged fermentation phosphate restriction is not necessary).
- The pH is never allowed higher than 3.5.
- Copper is used at up to 500 ppm as an antagonist of the enzyme aconitase which requires iron.
- 1-5% of methanol, isopropanol or ethanol when added to fermentations containing unpurified materials increase the yield; the yields are reduced in media with purified materials.
- As high aeration is deleterious to citric acid production, mechanical agitation is not necessary and air may be bubbled through. Anti-foam is added.
- The fungus occurs as a uniform dispersal of pellets in the medium.
- The fermentation lasts for five to fourteen days.



# Extraction

- The broth is filtered until clear.
- Calcium citrate is precipitated by the addition of magnesium-free  $\text{Ca}(\text{OH})_2$ .
- Since magnesium is more soluble than calcium, some acid may be lost in the solution as magnesium citrate if magnesium is added.
- Calcium citrate is filtered and the filter cake is treated with sulfuric acid to precipitate the calcium.
- The dilute solution containing citric acid is purified by treatment with activated carbon and passing through iron exchange beds.
- The purified dilute acid is evaporated to yield crystals of citric acid.
- Further purification may be required to meet pharmaceutical stipulations.