BIOPROCESS DEVELOPMENT FOR PRODUCTION OF CITRIC ACID

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DISSERT

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THANKS AGAIN TO ALL WHO HELPED ME

INDEX PAGE

SUMMARY:

Citric acid is the most important organic acid produced in tonnage and is extensively used in food and pharmaceutical industries.

It is produced mainly by submerged fermentation using Aspergillus niger or Candida sp. from different sources of carbohydrates, such as molasses and starch based media.

Citric acid is a weak organic acid found in citrus fruits. It is a natural preservative and is also used to add an acidic (sour) taste to foods and soft drinks. In biochemistry, it is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all living things.

Citric acid is a commodity chemical produced and consumed throughout The World.It is used mainly in the food and beverage industry, primarily as an acidulate.Although it is one of the oldest industrial fermentations, its World production is still in rapid increasing.

Global production of citric acid in 2007 was over 1.6 million tones. Biochemistry of citric acid fermentation, various microbial strains, as well as various substrates, technological processes and product recovery are presented. World production and economics aspects of this strategically product of bulk biotechnology are discussed.

INTRODUCTION:

Citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid) derives its name from the Latin word *citrus*, a tree whose fruit is like the lemon. Citric acid is a tricarboxylic acid, which contains three carboxylic functional groups. It is a primary metabolic product formed in the tricarboxylic acid (or Krebs) cycle and is found in small quantities in virtually all plants and animals, being isolated from lemon juice in 1784.

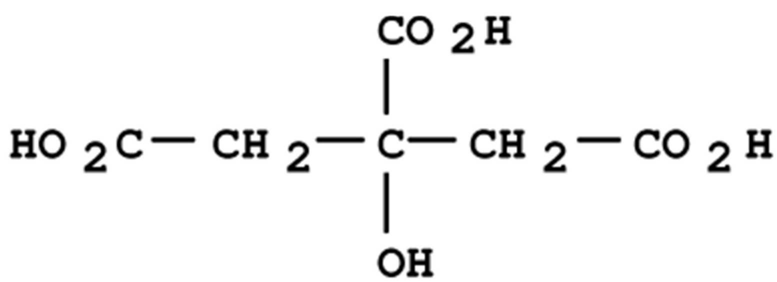


Figure 1. Chemical structure of citric acid.

Citric acid was first commercially-produced in England around 1826 from imported Italian lemons. Lemon juice remained the commercial source of citric acid until 1919, when the first industrial process using *Aspergillus niger* started in Belgium. Currently, the extraction of citric acid is limited to some small factories in Mexico and Africa.

Citric acid was synthesized from glycerol by Grimoux and Adams and later from symmetrical dicloroacetone. Other routes have been published from various synthetic materials since then, but chemical methods have so far proved uncompetitive.

Wehme was the first to demonstrate that *Citromyces* (now *Penicillium*) accumulated citric acid in a medium containing sugar and inorganic salts. Since then, many organisms have been found to accumulate citric acid: *A. niger*, *Aspergillus awamori*, *Aspergillus nidulans*, *Aspergillus fonsecaeus*, *Aspergillus luchensis*, *Aspergillus phoenicus*, *Aspergillus wentii*, *Absidia* sp., *Acremonium* sp.

Currie found that some strains of *A. niger* were able to grow in a medium containing sugars and salts at an initial pH of 2.5-3.5. Throughout their growth, these strains excreted large amounts of citric acid, which established the basis for industrial production.

Besides fungi, it is known that several yeasts produce citric acid from *n*-alkanes and carbohydrates, especially species belonging to the genera *Candida*, *Hansenula*, *Pichia*, *Debaromyces*, *Torula*, *Torulopsis*, *Kloekera*, *Zygosaccharomyces* and *Yarrowia*. During the '60s and '70s oil was cheap, and citric acid was produced industrially from this source by *Candida* sp., including *C. tropicalis*, *C. catenula*, *C. guilliermondii* and *C. intermediate* (73). Today, this production is not economical. As a disadvantage, the fermentation by yeasts led to the formation of large quantities of isocitric acid as an unwanted byproduct, so mutant strains with low aconitase activity should be used.

Although many microorganisms can be employed to produce citric acid, *A. niger* is still the main industrial producer. In fact, specific strains that are able to overproduce citric acid in different types of fermentation processes have been developed. The theoretical yield is 112 g of anhydrous citric acid per 100 g of sucrose. However, in practice, due to losses during trophophase, the yield of citric acid from these strains often does not exceed 70% of the theoretical yield on carbon source. Despite a long and successful history of producing citric acid, there is not unanimous explanation of the biochemical basis of the process.

DISCUSSION:

Citric acid was first commercially-produced in England around 1826 from imported Italian lemons (lemons contain 7-9% citric acid). Lemon juice remained the commercial source of citric acid until 1919, when the first industrial process using *Aspergillus niger* started in Belgium. Currently, the extraction of citric acid is limited to some small factories in Mexico and Africa.

Microorganism used for Citric Acid Production

- A large number of micro-organisms have been employed to produce citric acid. These include bacteria, fungi, and yeasts.
  But *A.Niger* and *saccharomycopsis* sp. are employed for commercial production because it has several advantages.

Advantages of using this micro-organism are:

- Its ease of handling.
- Its ability to ferment a variety of cheap raw materials.
- It provides high yields.

MICRO-ORGANISMSUSED FOR CITRIC ACID PRODUCTON

YEASTS: Candida tropicalis

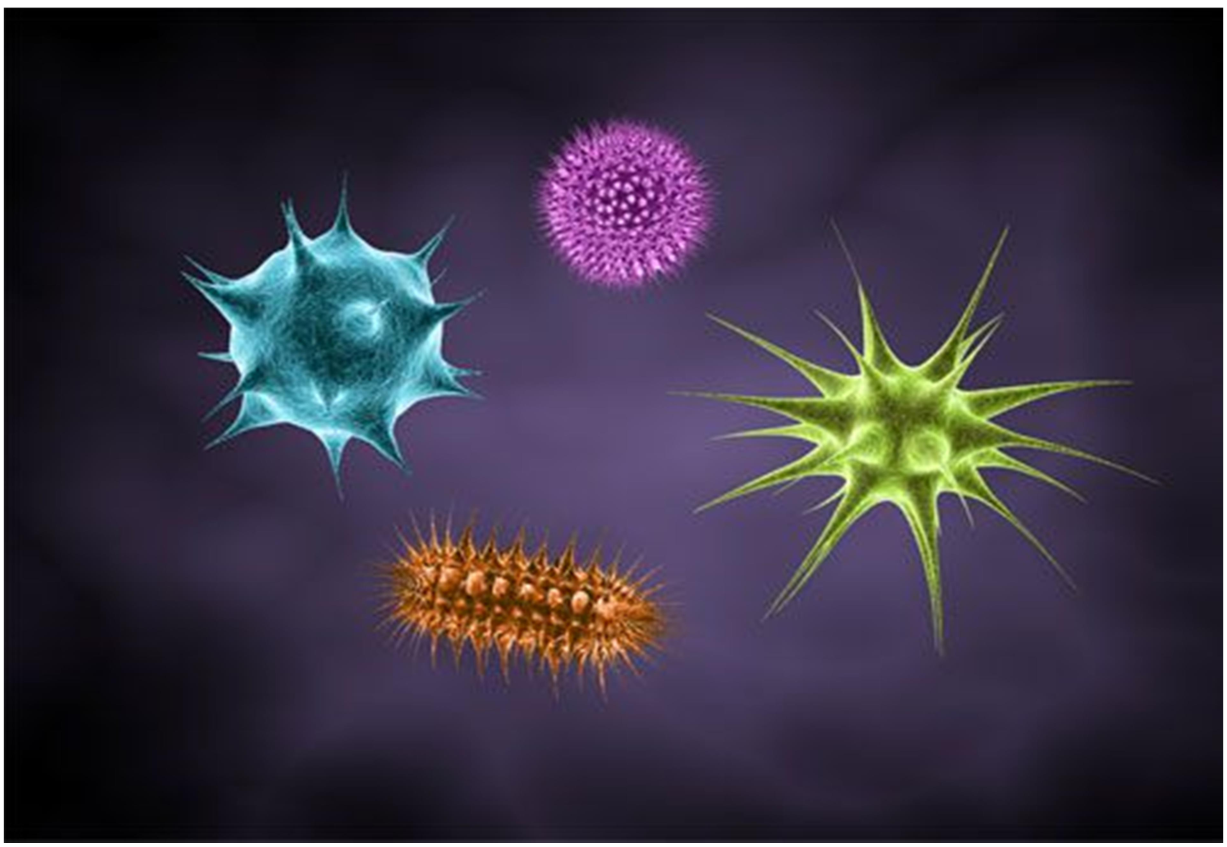
C.oleophila

BACTERIA: Bacillus licheniformis

Arthrobacter paraffines

FUNGI: Aspergillus nagger

A.carbonarius



RAW MATERIALS

Raw materials that are used for citric acid production are divided into two groups:

Group 1:

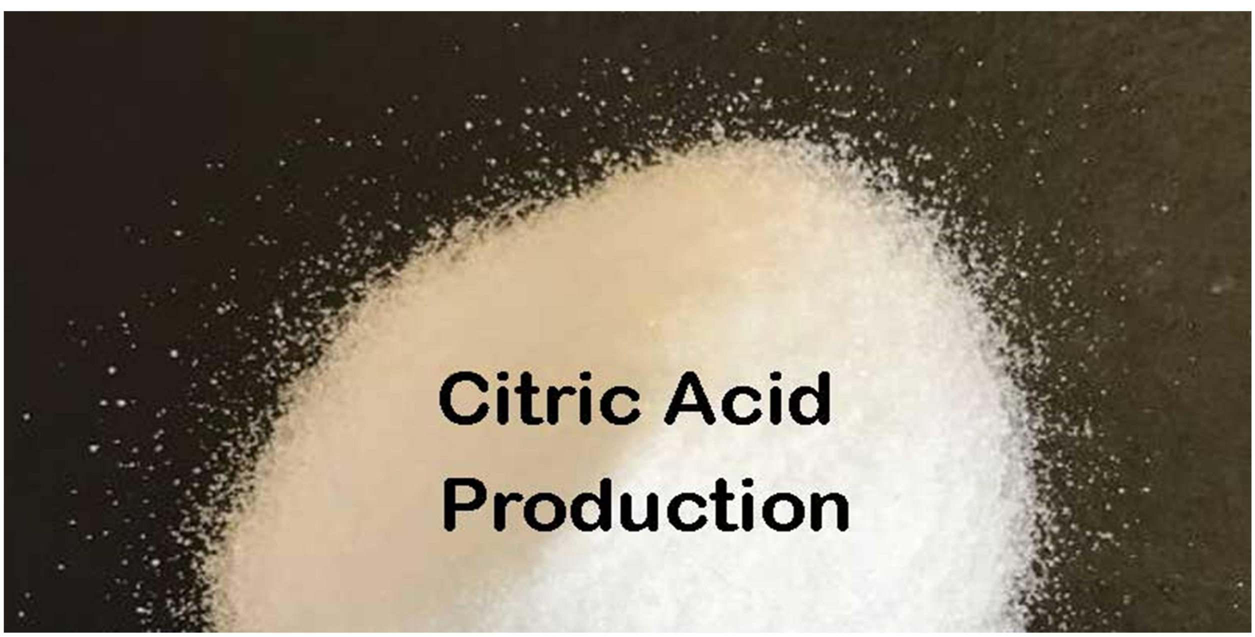
- Substances with low ash content

e.g. cane or beet sugar, dextrose syrups, and crystallized dextrose

Group 2:

- Materials which have high ash content.

e.g. cane and beet molasses, crude unfiltered starch hydro-lysates.



CITRIC ACID CYCLE

Steps of Citric Acid Cycle

- The citric acid cycle is also called the tricarboxylic acid cycle (TCA).

The steps include in the citric acid cycle are:

1. Formation of Citrate
2. Formation of Isocitrate via cis-Aconitate
3. Oxidation of Isocitrate to a-Ketoglutarate and CO_2
4. Oxidation of a-Ketoglutarate to Succinyl-CoA and CO_2
5. Oxidation of Succinate to Fumarate
6. Hydration of Fumarate to Malate
7. Oxidation of Malate to Oxaloacetate

Accumulation of citric acid

By mutation:

- Giving rise such a mutant organism that can carry an incomplete citric acid cycle for its accumulation

By inhibiting enzymes:

- By altering environmental conditions (pH, Temperature)
- Treat medium with ferrocyanide or ion exchange versions so that the enzymes involved in the TCA cycle inhibit except citrate synthase.

CITRIC ACID PRODUCTION TECHNIQUES

The industrial citric acid production can be carried in three different ways:

- Surface fermentation
- Submerged fermentation
- Solid-state fermentation

SURFACE FERMENTATION PROCESS

- Molasses substrate(15-20% of sucrose, added nutrients) acidified with, phosphoric acid to a pH 6.0-6.5 and heated at T 110c for 15 to 45 min.
- Potassium hexacyanoferrate is added to the hot substrate, to precipitate or complex trace metals (Fe, Mn, Zn) and to act in excess as a metabolic inhibitor restricting growth and promoting acid production.
- Inoculation is performed in two ways, as a suspension of conidia added to the cooling medium, or as a dry conidium mixed with sterile air and spread as an aerosol over the trays.
- The temperature is kept constant at 30 degrees during the fermentation by means of air current
- Within 24 hours after inoculation, the germinating spores start forming a 2-3 cm cover blanket of mycelium floating on the surface of the substrate. As a result of the uptake of ammonium ions, the pH of the substrate falls to 2.0.
- The fully developed mycelium floats as a thick white layer on the nutrient solution. The fermentation process stop after 8-14 days.
- Recovery of mycelium to extract citric acid.

- These all steps followed during the surface fermentation to produce citric acid

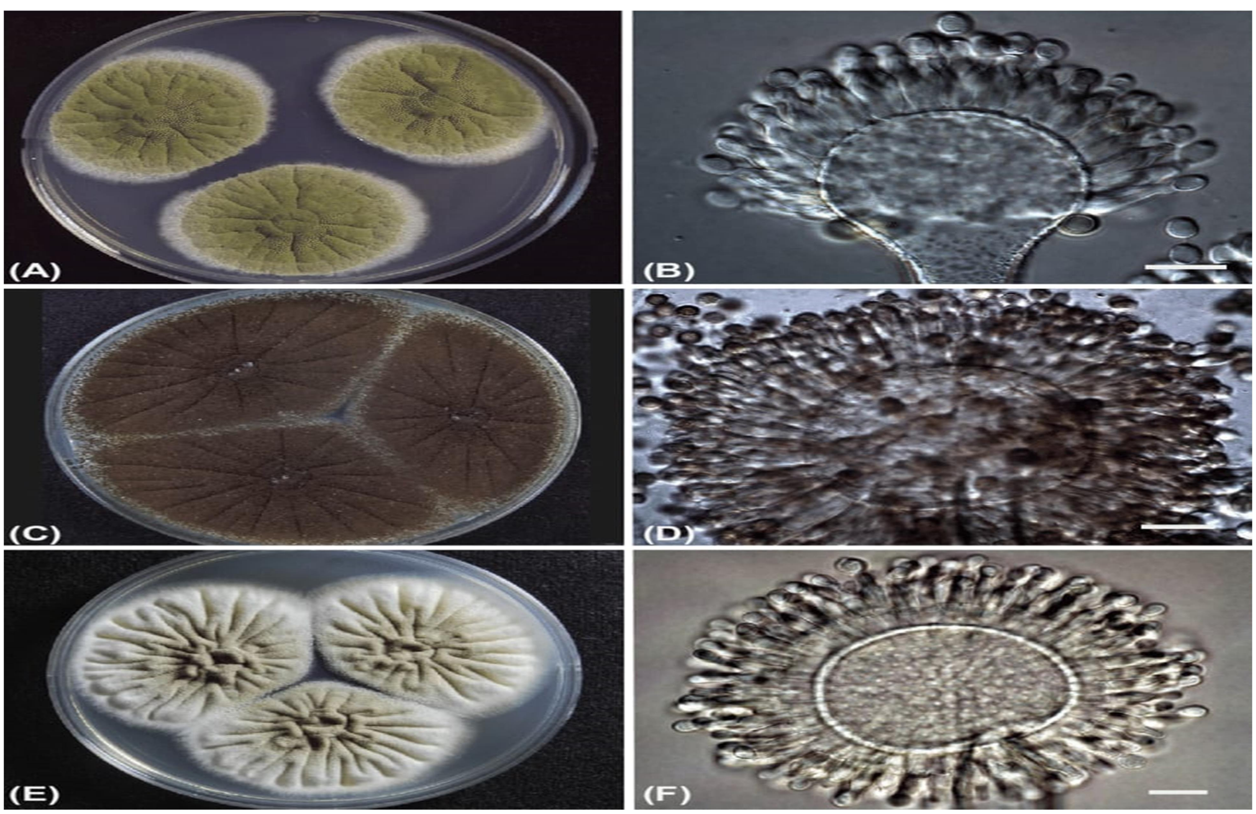
SOLID STATE FERMENTATION

- The solid substrate is soaked with water up to 65-70% of water content. After the removal of excess water, the mass undergoes a steaming process.
- Sterile starch paste is inoculated by spreading Aspergillus niger conidia in the form of an aerosol or as a liquid conidia suspension on the substrate surface
- The pH of the substrate is about 5-5.5 and incubation T 28-30C.growth can be accelerated by adding Alpha-amylase. although the fungus can hydrolyze starch with its own alpha-amylase. During the citric acid production pH dropped to values below 2.
- The solid-state surface process takes 5 to 8 days at the end of which the entire is extracted with hot water. In other cases, mechanical passes are also used to obtain more citric acid from the cells.
- These all steps followed during the solid-state fermentation to produce citric acid

SUBMERGED FERMENTATION

- Beet molasses substrate (12-15%, reducing sugar content) nutritive salts, such as ammonium nitrate or potassium dihydrogen phosphate are added, pH of the substrate is maintained at 5.5 to 5.9.
- The process can usually run in one or two stages, using hydrophilic spores suspensions or germinated conidia from the propagator stage.

- The development of the hyphae and the aggregation generally requires a period from 9 to 25 hours at T 32c
- Mycelia aggregation and spherical pellets, the productive form can be detected after 24 hours of inoculation.
- The change of pH in this phase is from 5.5 to 3.5 for beet molasses substrate and 2.2 for the sucrose substrate.
- Fermentation lasts up to 6-8 days and later citric acid is purified from mycelium.
- These all steps followed during the submerged fermentation to produce citric acid



PRODUCT RECOVERY

- The biomass is separated by filtration. Then the liquid broth is transferred to the recovery process.

PURIFICATION

Purification is a simple way of getting pure citric acid followed by two simple techniques.
1.Precipitation
2.Filtration
- Precipitation is the most commonly used technique.
- It is performed by the addition of calcium oxide hydrate (milk of lime) to form the slightly soluble tri-calcium citrate tetrahydrate. The precipitated tri-calcium citrate is removed by filtration and washed several times with water. It is then treated with sulphuric acid forming calcium sulfate, which is filtered off. Mother liquor containing citric acid is treated with active carbon and passed through cation and anion exchangers. Several anion-exchange resins are commercially available.

Further purification
- After purification, It can be produced in two forms

1.Monohydrate
2.Anhydrous.
<u>Mono hydrate</u>

- Contains one water molecule for every citric acid molecule
- Require repeated crystallization until the water content is approx. 7.5- 8.

Anhydrous

- Processed to remove all water from the end product
- Prepared by dehydrating the monohydrate citric acid product at a temperature above 36.6°C.

Factors affecting citric acid fermentation

Carbon source

Studies over several decades have shown that the carbon source affects the citric acid yield directly. Monosaccharides and disaccharides are the preferred carbon source as they are more rapidly metabolized by the fungus than polysaccharides, thus producing higher yield. Polysaccharides are not suitable as the raw material as the decomposition process takes too long to meet the rate of sugar catabolism necessary for the production of citric acid. The slow rate of polysaccharide hydrolysis is due to reduced enzymatic activity, which affects the pH in the fermentation medium. Sucrose is superior to glucose, fructose and lactose, in order of decreasing citric acid yield. The superiority of sucrose has been attributed to the strong extracellular mycelium-bound invertase of *A. niger*, which rapidly hydrolyses sucrose at low pH.

Nitrogen limitation

The concentration of nitrogen has been found to have a strong effect on the production of citric acid, as nitrogen is not only part of a cell's proteins, but also necessary for cellular metabolism. Molasses and other industrial media are usually nitrogen rich, whereas laboratory media require additional ammonium salts as supplements. The type of nitrogen source affects the synthesis of citric acid as well as the fungal growth. Ammonium nitrate promotes reduced vegetative growth, while ammonium sulphate promotes a longer period of vegetative growth. Nitrogen limitation is necessary, because at a concentration greater than 0.25%, oxalic acid accumulates and it will decrease the citric acid yield. A high nitrogen concentration increases the consumption of sugar and fungal growth, while decreasing the amount of citric acid produced (Hang et al. 1977). Ammonium sulphate is the preferred choice of salt as it does not produce the unwanted oxalic acid (Nigam 2009), while reducing the pH of the medium as the salt is consumed.

Phosphorus source

Along with nitrogen and the carbon source, phosphate has also been shown to be a critical factor. For fungal growth, a phosphorus concentration of 0.5–5 g/l is required for citric acid production (Shu & Johnson 1948b). The addition of phosphorus has only a slight effect on the accumulation of citric acid and mycelial growth (Hang et al. 1987). Kubicek and Röhr (1977) deduced that citric acid accumulates with limited phosphate, even when nitrogen is not limited.

pH of culture medium

The pH of the medium changes continuously as a result of microbial metabolic activities, largely because of the secretion of organic acids such as citric acid, and the unwanted gluconic and oxalic acid. The metabolic activities of microbes such as *Aspergillus*, *Rhizopus* and *Penicillium* species are able to reduce the pH quickly to below 3, while other fungi such as *Sporotrichum* and *Pleurotus* species produce a more stable pH between 4 and 5 (del Campo et al. 2006). The pH of the fermentation medium is most important during the sporulation and production phase. In the germination stage, the germinating spores absorb ammonia and release protons, thereby increasing the acidity of the medium and favouring the production of citric acid. At low pH of about less than 2, the formation of unwanted products such as oxalic and gluconic acid is inhibited, and the possibility of contamination by other microorganisms is also reduced, making recovery of citric acid easier (Max et al. 2010).

Trace elements

Studies on divalent metal ions including manganese, zinc, copper, magnesium and iron have shown that they have effects on citric acid production (Käppeli et al. 1978; Dronawat et al. 1995). Tomlinson et al. (1950) concluded that the optimum concentrations of iron and zinc are 1.3 and 0.3 ppm, respectively. These authors further explained the importance of manganese for cell function, sporulation and production of secondary metabolites, and mainly in cell wall synthesis. Manganese deficiency affects the anabolism of *A. niger*, causing a high intracellular ammonium concentration. A decrease in the accumulation of citric acid with iron has been observed, as well as changes in mycelial growth (Mischak et al. 1985). Grewal and Kalra (1995) deduced that at a high zinc concentration, the fungi maintained growth without accumulation of citric acid (Drysdale & McKay 1995). Nickel, molybdenum and cobalt are some other trace metals reported to affect the citric acid accumulation of *A. niger* (Habison et al. 1983). The interdependence of medium constituents has to be taken into account as it is crucial to citric acid production; therefore, robust control of these trace elements is required for the optimum production of citric acid.

USES OF CITRIC ACID

- It is used in the detergent industry as a phosphate substitute.
- Used as a preservative and flavoring agent.
- Emulsifying agent in ice cream.
- Also used as an antioxidant.

Conclusions

This review focuses on the details of citric acid production. The economic potential, biochemistry, choices of microorganism employed, raw materials, types of fermentation technique, factors affecting citric acid production, quantification techniques and recovery techniques are discussed. Currently, citric acid is the most produced organic acid in the world. Global production is expected to increase further with increasing demand. Although citric acid production using *A. niger* provides satisfactory performance at the moment, there is still room for greater improvements in increasing yield and minimizing waste by developing novel fermentation techniques and the optimization of *A. niger* using genetic manipulation. Microbial strains which are capable of fermenting any substrates have been developed to maximize the utilization of agro-industrial waste and provide a more efficient and environmentally friendly production process.

REFFERENCE

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