Applicant: Catalysta Industries Pvt. Ltd

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Chemical Formula: $C_6H_8O_7$

Chemical Name: Citric Acid

Chemical synthesis routes:

RAW MATERIALS:

Carbon Source: Sucrose,Glucose,Molasses,Starch

Nitrogen Source: Microorganisms require nitrogen for growth and metabolism. Common nitrogen sources include:

Ammonium salts: Ammonium sulphate ($(NH_4)_2SO_4$) or ammonium nitrate (NH_4NO_3) are often used as nitrogen sources.

Organic nitrogen sources: Yeast extract, corn steep liquor, or soybean meal are examples of organic nitrogen sources that can be used.

Mineral Salts: In addition to carbon and nitrogen sources, various mineral salts are required as nutrients for microbial growth and metabolism. These include:

Phosphates: Dipotassium phosphate (K_2HPO_4) or potassium dihydrogen phosphate (KH_2PO_4) provide phosphorus and buffering capacity.

Magnesium sulphate ($MgSO_4$): Provides magnesium ions, which are essential for enzyme activity.

Some of the water,Trace elements etc.

Citric acid (CA) has been widely produced as essential weak organic acid with extensive use in the pharmaceutical and food industries due to being a common metabolite found in animals and plants alike.

Microorganism used in Citric acid production

Aspergillus niger is the highly recommended industrial producer of citric acid. They flourish in sugar medium containing salts at pH 2.5-3.5 and excrete large volumes of CA. An estimated practical yield of CA is 70 % of the theoretical estimate which is 112g per 100g of sucrose.

Many organisms like ***Penicillin sp., Eupenicillin sp., Botrytis sp., Absidia sp., Ustulina vulgaris,*** and more can accumulate citric acid in a sugar and inorganic salt medium. Yeast species belonging to the genera ***Hansenula, Candida, Torula, Saccharomyces, Pichia,*** etc, can produce CA from carbohydrates and n-alkanes. However, producing CA via these organisms may need to be more economical due to the accumulation of unwanted by-products like isocitric acid. A remedial approach could be the development of a mutant strain with lower aconitase activity.

Micro-organisms	References
Fungi	
Aspergillus niger	Hang & Woodams 1984, 1985, 1987, Roukas 1991, Garg & Hang 1995, Lu et al. 1997, Pintado et al. 1998, Vandenberghe et al., 1999b, c
A. aculeatus	El Dein & Emaish, 1979
A. awamori	Grewal & Kalra, 1995
A. carbonarius	El Dein & Emaish, 1979
A. wentii	Karow & Waksman, 1947
A. foetidus	Chen, 1994; Tran et al., 1998
Penicillium janthinelum	Grewal & Kalra, 1995
Yeasts	
Saccahromicopsis lipolytica	Ikeno et al., 1975; Maddox et al., 1985; Kautola et al., 1992 Wojtatowicz et al., 1993; Rane & Sims, 1993
Candida tropicalis	Kapelli et al., 1978
C. oleophila	Ishi et al., 1972
C. guilliermondii	Miall & Parker, 1975; Gutierrez et al., 1993
C. parapsilosis	Omar & Rehm, 1980
C. citroformans	Uchio et al., 1975
Hansenula anamola	Oh et al., 1973
Bacteria	
Bacillus licheniformis	Sardinas, 1972
Arthrobacter paraffinens	Kroya Fermentation Industry, 1970
Corynebacterium sp.	Fukuda et al., 1970

Citric acid production process:

An estimated 99% of citric acid mass production carried out by microbial processes and surface or submerged culture methods. The final product obtained is marketed as a monohydrate acid or an anhydrous salt. About 70% of total CA is used in the beverages and food industries as an acidifier or antioxidant for the preservation and enhancement of

flavours, as well as aromas in ice cream, marmalades, and fruit juices. 20% of total CA produced is utilized by pharmaceutical industries as blood preservatives, vitamin preservants as antioxidants, and effervescent, and iron citrate in cosmetics, tablets, and ointments. In the chemical industry, it is used as a foaming agent for softening textiles and a phosphate substitute for hardening cement.

a. Surface Culture

Surface culture is divided into solids and liquids, though the liquid approach provides higher economic production.

- The culture medium is put on a shallow aluminum tray 5-20 cm deep and maintained at 5-6 pH.
- Uniform air circulation and maintenance of temperature and humidity are ensured in the fermentation chamber.
- Then *A. niger* spores are blown onto the culture medium surface for 5-6 days with uniform passage of dry air.
- The pH of the medium is changed to 1.5 to 2.
- After 24 hours of incubation, the spores germinate, and the white mycelium forms on the medium surface.
- The moulds start to utilize the sugar content of the medium, and after complete utilisation, the residual liquid is extracted from the mycelium mesh.
- This residual liquid contains CA, and *A. niger* produces a small volume of CA as the primary metabolite in this method.

Inoculum Preparation.

A suitable and high-yielding *A.niger* strain is selected from the stock culture.

- Glass vials incubated for 10-14 days and then sporulating media are infected with the selected stock culture.
- Trace elements like zinc, iron salts, and manganese should be maintained in the sporulating media.
- To suspend the mature spores, the appropriate diluent is used like water with the wetting chemical **sodium lauryl phosphate.**

Media Preparation

- The medium must contain a carbon source such as sucrose and inorganic salts for CA production.
- **Sucrose and beet molasses** are preferred carbon sources for CA fermentation media.
- A culture medium with less than 15% sucrose is beneficial to achieve a high CA yield.
- Using fructose or glucose instead of sucrose leads to lower citric acid output.
- On a commercial scale, beet molasses are the preferred carbon source for A. niger.
- Beet molasses are rich in inorganic salts hence they are pre-treated with ferricyanide or ferrocyanide to diminish excess inorganic salt.
- Other elements added to the medium for optimum growth of microorganisms and CA production are nitrogen, phosphorus, potassium, and magnesium.

- Salts like ammonium nitrate, magnesium sulphate, potassium mono hydrogen phosphate, etc., are also added in small amounts.
- pH is adjusted by adding HCl to 3.4 – 3.5 as lower pH facilitates sterilization, less contamination, suppression of oxalic acid formation, and good yield of citric acid synthesis.

Fermentation

- The prepared 1 to 2.5 cm thick media is placed in a shallow pan.
- The inoculum spores are spread onto the medium surface with uniform aeration to allow even distribution of spores in the media.
- Incubation is done at 30-40 degrees Celsius temperature.
- The temperature is constant during fermentation with air current ventilation to allow efficient gaseous exchange.
- After 24 hours of inoculation, the germinating spores produce a thin layer of mycelium on the medium surface.
- The pH of the medium drops to 1.5 – 2.0 as ammonium ions are formed.
- The presence of oxalic acid and yellow pigment after 30 hours of fermentation is an indication of high iron concentration and halted citric acid production.
- Fermentation ceases after 8-14 days with thick, mature white mycelium floating atop liquid media.
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- The surface area to medium volume ratio is proportional to the sugar conversion rate to CA.
- In the shallow pan method, the ratio is reduced, and the yield of CA increases.
- Under appropriate conditions, sugar is exponentially converted to citric acid.
- This method yields between 1.2 and 1.5 kilograms of citric acid monohydrate per square meter of fermentation surface per hour.

Recovery

- Mycelium is separated from the mycelium broth.
- Mycelium is pressed to extract any intracellular citric acid present.
- The filtered broth is treated with Calcium hydroxide and then treated with sulphuric acid in equivalent volume to obtain CA and calcium sulfate is generated as a precipitate.
- Filtration is employed to isolate precipitate, and impure citric acid is generated by demineralizing and decolorizing it with activated carbon.
- After evaporation, pure CA crystals are obtained.

Application of Citric acid:

Citric acid is mainly used in food industry because of its pleasant acid taste an its high solubility in water. It is worldwide accepted as "GRAS" (generally recognized as safe), approved by the Joint FAO/WHO Expert Committee on Food Additives. The pharmaceutical and cosmetic industries retain 10% of its utilization and the remainder is used for various other purposes. Table 1 presents main 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.

Alternative approach: A chemical route for the synthesis of citric acid from orange and grape juices:

Citric acid, a key tricarboxylic acid found in citrus fruits, is a valuable additive in various industries. This process aimed to extract citric acid from orange and grape juices through a chemical route involving three steps: neutralisation, calcium chloride addition, and acidification. The fruits were processed by peeling, crushing, filtering and then subjected to these steps to extract citric acid. By varying the final pH and concentrations of calcium chloride and sulfuric acid, the study achieved maximum yields of citric acid (91.1% from orange and 79.8% from grape) at optimal conditions (pH 10, 40.7% calcium chloride, and 1.9 M sulfuric acid). The purified citric acid crystals were characterized using FTIR and XRD. The findings suggest that oranges are a more promising source for citric acid production than grapes.

Experimental method on lab scale:

1.10 mL of fruit juice was titrated with 0.1 M NaOH using a phenolphthalein indicator to calculate citric acid mass.

2. pH of fresh orange juice was 3.3 and grape juice was 3.7.

3. 2.8 M NaOH (10% w/w) was added dropwise to juice to reach pH 10 for neutralization.

4. Mixture filtered to remove insoluble; filtrate containing sodium citrate was filtered thrice.

5. A known volume of 40.7% $CaCl_2$ was added to sodium citrate solution, heated, and filtered to obtain calcium citrate.

6. Calcium citrate washed with hot water, dried, and weighed.

7. Dried calcium citrate acidified with 1.9 M H_2SO_4, heated, and mixed to obtain a citric acid solution.
8. Mixture filtered to remove calcium sulfate; citric acid crystallized from solution.

If we go with the same process on a large scale, the Chemical route involves multiple steps and precise control, making it more complex than fermentation. Chemicals required for the process can be expensive, increasing production costs.

Achieving high yields requires precise control, which can be challenging on a large scale.

References: Provide references for a research paper or an actual patent.

1.https://www.researchgate.net/publication/352777819_Chemical_route_for_synthesis_of_citric_acid_from_orange_and_grape_juices#:~:text=The%20chemical%20route%20for%20synthesis,M)%20to%20produce%20citric%20acid.

2.https://microbenotes.com/citric-acid-production/

List the contributions of each author:

- Authors 1 and 2 carried out the literature search and found the reaction that would be best to scale up.
- Authors 3 and 4 discovered the different reaction parameters, like yield, Efficiency, and related things.
- Authors 4 and 5 give a proof reading and also mention other minute problems and details for the reaction to carry on.

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