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Production of Citric Acid from an Organic Waste of Sugarcane Molasses by Fermentation using Micro-organisms: A Review

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ABSTRACT

Citric Acid is one of the most important naturally occurring organic acids with various applications in Industry, Pharmaceuticals, Foods & Beverages, Metal industry and many others which are briefly discussed in this paper. So, its economic production becomes very demand of time. This study reviews the current advancements and procedure of biochemical production of Citric acid alongwith the history of its production techniques. The latest production is from organic waste of sugarcane molasses using a fungus. Production techniques and raw materials are briefly discussed with their importance and alternatives. The factors which affect the rate of production are also reviewed. This method can be beneficial in two ways: Cost effective production and cleansing of environment alongwith.

Keywords: - Citric acid, Bioproduction, Molasses, Fermentation

INTRODUCTION

Citric acid is one of the most important commercial products in the world [3].Citric acid is an important organic compound with molecular formula $C_6H_8O_7$ and IUPAC name as (2 – hydroxy–1, 2, 3 –propane tricarboxylic acid). It is a natural constituent of citrus fruits and common metabolite of plants and animals and is the most versatile and widely used organic acid in the field of food (60%) and pharmaceuticals (10%) [1]. It has got several other applications in various other fields. Currently, the global production of citric acid is estimated to be about 7, 36,000 tones/year and the entire production is carried out by fermentation [2]. Molasses is a by-product of the sugar processing industry [4]. Molasses contain lots of sugars and organic acids. In many places this waste has very small usability and often can be problem of environmental pollution because molasses contain calcium oxide which can reduce the oxygen level of the soil [5]. Molasses can be utilized as a substrate in the production of citric acid by using microorganisms, because the molasses have high sugar content.

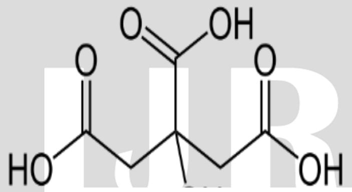

Figure 1 Structure of Citric acid

HISTORY OF DEVELOPMENTS

Karls Scheels from England first isolated Citric acid in 1874 from the lemon juice brought from Italy. Italy had ruled for its manufacturing for almost a decade, and thus it was expensive. This led to immense attempts across the globe to find alternative production methods including chemical and microbial techniques. Initially, Wehmer in 1923 observed that citric acid is a byproduct of calcium oxalate produced by a culture of *Penicillium glaucum*. However, industrial manufacturing did not succeed due to multiple reasons [16]. The commercial production is credited to Currie, in 1917, who found the ability of *Aspergillus niger* to produce considerable amounts of citric acid in sugar based medium. Additively, high concentrations of sugar favored its production. In about 1930, some industrial units were established in England, Soviet Union, and Germany for the commercial production of citric acid. However, the biochemical methods were only developed in about 1950 with the discovery of the glycolytic pathway and the tricarboxylic acid cycle (TCA). Later on, an improvised method employing submerged fermentation was developed in United States [28]. Methods were well developed for chemical synthesis of citric acid, however better yields were achieved using microbial fermentations only. Later on, this technique became the method of choice for commercial production, mainly due to economic advantage of biological production over chemical synthesis [29]. Further research was done to improve the microbial strains, and to maintain their production Capacity. It was also important to consider raw material more carefully. Several works were dedicated to the optimization of the conditions for the utilization of cheap material like sugar cane molasses, beet molasses and starch [30].

APPLICATIONS OF CITRIC ACID

Citric acid is mainly used in food industry because of its acidic taste and its aqueous solubility [6]. It is worldwide accepted as "GRAS" (generally recognized as safe), approved by the Joint FAO/WHO Expert Committee on Food Additives [7]. The pharmaceutical and cosmetic industries retain 10% of its utilization and the remainder is used for various other purposes. Table 1 is concised to present main applications of citric acid.

Industry	Applications
Drinks and Beverages	Provides tartness and complements fruits and berries flavors. Increases the effectiveness of antimicrobial preservatives. Used in pH adjustment to provide uniform acidity.
Candy, Jellies, Jams and Preserves	Provides tartness, pH adjustment, Minimizes sucrose inversion, Produces dark color in hard candies.
Dairy products	As emulsifier in ice creams and processed cheese, acidifying agent in many cheese products and as an antioxidant.
Pharmaceuticals	As effervescent in powders and tablets in combination with bicarbonates, Provides rapid dissolution of active ingredients, Acidulate 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 Industry	Removes metal oxides from surface of ferrous and nonferrous metals, for preparational 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, adhesives, paper, polymers, tobacco, waste treatment, etc.

MICRO-ORGANISM USED FOR CITRIC ACID PRODUCTION

Citric acid can be produced either through surface fermentation culture or submerged culture by utilizing the activity of microorganisms [8]. Fermentation is the process of energy production in cells in anaerobic and aerobic conditions. Fermentation can be interpreted as a gradual change by enzymes of some bacteria, yeasts and fungi. Aspergillus Terreus is a fungus of the Ascomycota phyla. Its morphological features are same as the most of the species in this phylum that are filament, hyphae secured or have septa, and can be found abundantly in nature [9]. Another similar fungi species is Aspergillus Niger which can grow optimum at 35-37 °C [10]. In addition, the process of growth of these fungi is as aerobic microorganisms. Aspergillus Terreus also produces several enzymes like proteases, amylases and α -glactosidases that can be used as biocatalysts in the production of citric acid by fermentation [11]. Citric acid is the primary metabolite product formed from TCA Cycle (Tricarboxylic Acid Cycle) [12]. A large number of other micro-organisms including bacteria, fungi and yeasts have been employed to produce citric acid. Most of them, however, are not able to produce commercially acceptable yields. This fact could be explained by the fact that citric acid is a metabolite of energy metabolism and its accumulation rises in appreciable amounts only under conditions of drastic imbalances [13]. Kubicek and Rohr [14] (1986) reviewed the strains reported to produce citric acid. Many micro-organisms can be used to produce citric acid. Among these, only *A. niger* and certain yeasts such as *Saccharomycopsis* sp. are employed for commercial production [15]. However, the fungus *A. niger* has remained the organism of choice for commercial production.
The main advantages of using this fungus are:
(a) Its ease of handling,
(b) Its ability to ferment a variety of cheap raw
(c) High yields.

1. PRODUCTION TECHNIQUES AND RAW MATERIALS

Mainly fermentation process for formation of citric acid is initiated by starch or sucrose based materials however other raw materials such as molasses, several starchy materials and hydrocarbons have also been employed [15]. Rohr et al. [16] (1983) classified raw materials used for citric acid production into two groups: (i) with a low ash content from which the cations could be removed by standard procedures (e.g. cane or beet sugar, dextrose syrups and crystallized dextrose); (ii) raw materials with a high ash content and high amounts of other non sugar substances (e.g. cane and beet molasses, crude unfiltered starch hydro-lysates). Several attempts have been made to produce citric acid using molasses, which is preferred due its low cost and high sugar content (40-55%). The composition of molasses depends on various factors, e.g. the kind of beet and cane, methods of cultivation of crops and fertilizers and pesticides applied during cultivation, conditions of storage and handling (e.g. transport, temperature variations), production procedures, etc. Generally, cane molasses contains calcium, magnesium, manganese, iron and zinc, which have a retarding effect on the synthesis of citric acid. However, both cane and beet molasses are suitable for citric acid production but, beet molasses is preferred due to its lower content of trace metals. Consequently, some pre-treatment is required for the removal/reduction of trace metals in sugarcane molasses. Still, cane molasses creates difficulties in achieving good fermentation yields [1].

FACTORS AFFECTING KINETICS OF PRODUCTION

Medium and Components

Carbon source**:** Citric acid accumulation is strongly affected by the nature of the carbon source [17]. The presence of easily metabolized carbohydrates has been found essential for good production of citric acid. Hossain et al. [18] (1984) showed that sucrose was the most favorable carbon source followed by glucose, fructose and galactose. Galactose showed very less growth of fungi and did not favor citric acid production. Other sources of carbon such as sorbose, ethanol, cellulose, manitol, lactic, malic and α-acetoglutaric acid, allow a limited growth and low production. Starch, pentoses (xyloses and arabinoses), sorbitol and pyruvic acid slow down growth, though the production is minimal [18].

Nitrogen source**:** Citric acid production is directly influenced by the nitrogen source [3]. Usually, ammonium salts like urea, ammonium sulfate, ammonium chloride, peptone, malt extract, etc are preferred. Nitrogen consumption leads to pH decrease, which is very important point in citric acid fermentation. A high concentration of nitrogen increases the fungal growth and the consumption of sugars, but decreases the amount of citric acid produced [19].

Phosphorous source: Concentration of phosphate in the medium also plays important role in the yields of citric acid production [8]. The most suitable source found is Potassium dihydrogen phosphate. Shu and Johnson [20] (1948) reported that for maximum production of citric acid, phosphorous at of 0.5 to 5.0 g/L concentration was required by the fungus. Phosphate is known to be essential for the growth and metabolism but low levels of phosphate favors the yields. However, excess of phosphate leads to the formation of certain sugar acids, a decrease in the fixation of CO_2, and the stimulation of growth [21].

Trace elements: Concentration of trace elements is the main factor which influences 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 in different directions. Zinc favored the production of citric acid if added with KH_2PO_4. On the other hand, the presence of manganese ions and iron and zinc (in high concentrations) could cause the reduction of citric acid yields only in phosphate free medium. Copper was found to complement the ability of iron at optimum level, to enhance the biosynthesis of citric acid. A low level of manganese was capable to reduce the yield of citric. Citric acid accumulation decreased by the addition of iron, which also had some effect on mycelial growth [22]. Lower alcohols: Addition of lower alcohols enhances citric acid production from commercial glucose and other crude carbohydrate. Appropriate alcohols are methanol, ethanol, isopropanol 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% [23, 24].

Miscellaneous**:** Some compounds which act as inhibitors such as calcium fluoride, sodium fluoride and potassium fluoride have been found to accelerate the production of citric acid, while, potassium ferrocyanide has been found to decrease the yield [1].

OTHER PROCESS PARAMETERS

pH: The pH of a culture may change in response to microbial metabolic activities. The reason being the secretion of organic acids such as citric, acetic or lactic acids, which will cause the pH to decrease [25]. Changes in pH kinetics depend highly also on the micro-organism. Besides, the nature of the substrate also influences pH kinetics. Generally, a pH below 2.0 is required for optimum production of citric acid. A low initial pH also checks contamination. A pH of 2.2 was reported to be optimum for the growth of the mould as well as for the production of citric acid [26].

Aeration**:** Aeration has been shown to have an impulsive effect on fermentation of citric acid. Enhanced aeration leads to increased yields and less time consumption for fermentation. It is important to maintain the oxygen concentration above 25% saturation. The high oxygen demand can be fulfilled by establishing proper aeration devices. This is the reason why small compact pellets are the preferred mycelial forms of fungus during fermentation. When the organism turns into filamentous developments, e.g. due to metal contamination, the dissolved oxygen tension rapidly falls to less than 50% of its previous value, even if the dry weight has not increased by more than 5% [1]. High aeration rates lead to high amounts of foam, especially during the growth phase. Therefore, the addition of antifoaming agents and the construction of mechanical "defoamers" are required to tackle this problem [27].

CONCLUSION

Since the beginning of this century, citric acid production has been intensively studied and multiple alternatives to this process have been found to follow its great demand as it is industrially very important. Care has also been taken to produce economic production of the compound. This study is a review on the detailed process and conditions employed in the fermentation of sugarcane molasses using a fungus *Aspergillus niger.* All the inhibitors and promoters are discussed alongwith the process parameters which affects the process of production.

REFERENCES

1. Vandenberghe, L. P., Soccol, C. R., Pandey, A., & Lebeault, J. M. (1999). Microbial production of citric acid. *Brazilian Archives of Biology and Technology*, *42*, 263-276.
2. Vandenberghe, L. P., Soccol, C. R., Pandey, A., & Lebeault, J. M. (2000). Solid-state fermentation for the synthesis of citric acid by Aspergillus niger. *Bioresource Technology*, *74*(2), 175-178.
3. Soccol, C. R., Vandenberghe, L. P., Rodrigues, C., & Pandey, A. (2006). New perspectives for citric acid production and application. *Food Technology and Biotechnology*, *44*(2), 141-149.
4. Zhang, S., Wang, J., & Jiang, H. (2021). Microbial production of value-added bioproducts and enzymes from molasses, a by-product of sugar industry. *Food chemistry*, *346*, 128860.
5. Sansoucy, R., Aarts, G., & Leng, R. A. (1992). Molasses/urea blocks. *Tropical Feeds.(GOHL, B., Ed.) FAO, Rome*.
6. Nangare, S., Vispute, Y., Tade, R., Dugam, S., & Patil, P. (2021). Pharmaceutical applications of citric acid. *Future Journal of Pharmaceutical Sciences*, *7*(1), 1-23.
7. Joint FAO/WHO Expert Committee on Food Additives. Meeting, & World Health Organization. (2009). *Evaluation of Certain Food Additives: Sixty-ninth Report of the Joint FAO/WHO Expert Committee on Food Additives* (Vol. 69). World Health Organization.
8. Max, B., Salgado, J. M., Rodríguez, N., Cortés, S., Converti, A., & Domínguez, J. M. (2010). Biotechnological production of citric acid. *Brazilian journal of Microbiology*, *41*, 862-875.
9. Campbell, C. K., & Johnson, E. M. (2013). *Identification of pathogenic fungi*. John Wiley & Sons.
10. Kozakiewicz, Z., & Smith, D. (1994). Physiology of aspergillus. In *Aspergillus* (pp. 23-40). Springer, Boston, MA.
11. Ward, O. P., Qin, W. M., Dhanjoon, J., Ye, J., & Singh, A. J. A. I. A. M. (2005). Physiology and biotechnology of Aspergillus. *Advances in applied microbiology*, *58*, 1-75.
12. Akram, M. (2014). Citric acid cycle and role of its intermediates in metabolism. *Cell biochemistry and biophysics*, *68*(3), 475-478.

13. Sauer, M., Porro, D., Mattanovich, D., & Branduardi, P. (2008). Microbial production of organic acids: expanding the markets. *Trends in biotechnology*, *26*(2), 100-108.
14. Meixner-Monori, B., Kubicek, C. P., Harrer, W., Schreferl, G., & Rohr, M. (1986). NADP-specific isocitrate dehydrogenase from the citric acid-accumulating fungus Aspergillus niger. *Biochemical Journal*, *236*(2), 549-557.
15. Vandenberghe, L. P. S., Rodrigues, C., De Carvalho, J. C., Medeiros, A. B. P., & Soccol, C. R. (2017). Production and application of citric acid. In *Current developments in biotechnology and bioengineering* (pp. 557-575). Elsevier.
16. Rohr, M., Kubicek, C. P. and Kominek, J. (1983) Citric acid. In- Biotechnology, Vol. 3, Reed, G. and Rehm, H. J. (Eds.), Verlag-Chemie, Weinheim, pp. 419-454
17. Yalcin, S. K., Bozdemir, M. T., & Ozbas, Z. Y. (2010). Citric acid production by yeasts: fermentation conditions, process optimization and strain improvement. *Current research, technology and education topics in applied microbiology and microbial biotechnology*, *9*, 1374-1382.
18. Hossain, M., Brooks, J. D., Maddox, I. S. (1984) The effect of the sugar source on citric acid production by Aspergillus nig
19. Kareem, S. O., Akpan, I., & Alebiowu, O. O. (2010). Production of citric acid by Aspergillus niger using pineapple waste. *Malaysian Journal of Microbiology*, *6*(2), 161-165.
20. Shu, P. and Johnson, M. J. (1948) Citric acid production by submerged fermentation with Aspergillus niger. Ind. Eng. Chem., 40, 1202
21. Stitt, M., & Krapp, A. (1999). The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment*, *22*(6), 583-621.
22. Kapoor, K. K., Chaudary, K. and Tauro, P. (1983), Citric acid. In-Industrial Microbiology, Reed, G. (Ed.), 4th Edn. AVI Publishing Co. Inc. Westport, Conn., 709-747
23. Hamissa, F. A. (1978) Effect of alcohol and related compounds on citric acid production from beet molasses by Aspergillus niger. Chem. Microbiol. Technol., 5, 157-160.
24. Dasgupta, J., Nasim, S., Khan, A. W. and Vora, V.C. (1994) Production of citric acid in molasses medium: effect of addition of lower alcohols during fermentation. J. Microbiol. Biotechnol., 9, 123-125
25. Soccol, C. R., Vandenberghe, L. P., Rodrigues, C., Medeiros, A. B. P., Larroche, C., & Pandey, A. (2008). Production of organic acids by solid-state fermentation. In *Current developments in solid-state fermentation* (pp. 205-229). Springer, New York, NY.
26. Kim, S. K., Park, P. J., & Byun, H. G. (2002). Continuous production of citric acid from dairy wastewater using immobilizedAspergillus niger ATCC 9142. *Biotechnology and Bioprocess Engineering*, *7*(2), 89-94.
27. Jiang, J., Zu, Y., Li, X., Meng, Q., & Long, X. (2020). Recent progress towards industrial rhamnolipids fermentation: process optimization and foam control. *Bioresource technology*, *298*, 122394.
28. Aboud-Zeid, A., and Ashy, M. A. (1984) Production of citric acid: A review. Agric. Wastes, 9, 51-76
29. Mattey, M. (1992) The production of organic acids. Crit. Rev. Biotechnol., 12, 87-132
30. S. Sarangbin, Y. Watanapokasin, Yam bean starch: A novel substrate for citric acid production by the protease-negative mutant strain of Aspergillus niger, Carbohydr. Polym. 38 (1999) 219–224.