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(Review Article)

Review on production of citric acid by fermentation technology

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Abstract

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. In view of surges in demand and growing markets, there is always a need for the discovery and development of better production techniques and solutions to improve production yields and the efficiency of product recovery. To support the enormous scale of production, it is necessary and important for the production process to be environmentally friendly by utilizing readily available and inexpensive agro-industrial waste products, while maintaining high production yields. This review article for fermentation of citric acid and Microbial production of citric acid, Substrates and strategies of citric acid production for Surface fermentation, Submerged fermentation, Solid-state fermentation and also the effects of various Factors affecting of citric acid fermentation conditions are Carbon source, Nitrogen limitation, Phosphorus source, Lower Alcohols, pH of culture medium, Trace elements, Aeration and Other factors. citric acid recovery options and the numerous applications of citric acid, based on the literature review information of citric acid production by fermentation technology.

Keywords: Review; citric acid production; fermentation technology; Factors effect on fermentation.

1. Introduction

Citric acid, or 2-hydroxy-propane-1,2,3-tricarboxylic acid (C6H8O7.H2O), is a naturally occurring weak organic acid found in all citrus fruits. The name of this organic acid is derived from Latin word citrus, which refers to trees of the genus Citrus, including lemon trees. Citric acid in its pure form is readily soluble in water and colourless [1]. It is solid at room temperature. Citric acid has a melting point of 153°C and it decomposes at higher temperatures. Citric acid has a molecular weight of 210.14 g/mol and possesses three different pKa values, at pH 3.1, 4.7 and 6.4, owing to presence of three functional groups of carboxylic acid [2].

Citric acid is a weak organic tricarboxylic acid found in citrus fruits. Citrus fruits (lemons, oranges, tomatoes, beets etc.) are those fruits which contains sufficient amount of citric acid and they are classified as acid fruits. It is a natural constituent and metabolite of plants and animals, is versatile and widely used organic acid in the field of food and pharmaceuticals industries. Citric acid is a good preservative and acidic in taste. Citric acid can be easily manufacture and easily soluble. It is used in flavoring agent and increases stability of the fruit [3-5]. Citric acid as nonagricultural allowed non-synthetic under 'acids', with annotation that must be produced by microbial fermentation of carbohydrate sources. Olden days citric acid is produced by three method fermentation, chemical synthesis and extraction from citrus fruits [6]. Recently wide range of production of citric acid has been reported in response to different levels of nutrient supplementation produce. Almost 50% citric acid produce worldwide. Global production of citric acid in 2004 was about

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1.4 million tonnes and annual growth is 3.5- 4% demand of citric acid [7-8]. Citrus fruit production was estimated as 120 million tons per year globally. India ranks 6th position for the production of citrus fruits in the world. Worldwide the citrus fruit wastes are also generated in millions of tons after extraction of juice [9].

The method of extracting citric acid from lemon juice was pioneered by a Swedish chemist, Karl Wilhelm Scheele (1742–1786), in 1784. This method was adopted in England around 1826 for the commercial production of citric acid using lemons imported from Italy. The method maintained its monopoly as the only commercial source for citric acid production until the late nineteenth century, when a German botanist, Wehmer, in 1893, first observed the feasibility of obtaining citric acid through the fermentation of a sugar medium containing inorganic salts with Penicillium glaucum [10]. Two years after this discovery, Wehmer successfully isolated two strains which were able to produce citric acid. These strains were later named Citromyces spp. (Penicillium). However, the production of citric acid using Citromyces spp. did not gain much popularity in industrial practice because of contamination problems and the long fermentation process time [11].

In 1916, a study conducted by James Currie made a breakthrough for successful economic industrial production of citric acid from Aspergillus niger. He discovered that significant amounts of citric acid could be obtained from various strains of A. niger. The most important findings were the ability of A. niger to grow at a pH of around 2.5–3.5, which curbed the formation of gluconic and oxalic acid, and the increase in citric acid production with increasing sugar concentration. This single piece of research laid the foundation for present-day industrial citric acid production, which was established in the USA by the pharmaceutical company Pfizer in 1923 [12].

Over the years, a great variety of microorganisms has been studied, ranging from fungi and bacteria to yeasts. However, the microbial conversion of organic materials to citric acid is a complex biochemical reaction which requires careful control and specifically tailored operating conditions. Factors affecting the fermentation process include the concentration and type of carbon source, the pH of the fermentation medium, phosphate and nitrogen limitations, aeration, the morphology of the citric-acid producing microorganism and the concentrations of trace elements. Some of the nutrients, such as trace metals like manganese, phosphate and nitrogen, must be below defined limits to have a positive effect on the fermentation process. However, other elements, such as oxygen and sugar, are required in excess [10,13].

Aspergillus niger is superior to other microorganisms for the commercial synthesis of citric acid because of its better production yield. It is easy to handle, can ferment various cheap raw materials and delivers high yields. As such, strains of this microorganism can be improved to create industrial strains for use in commercial production, and mutagenesis and strain selection have been carried out for such improvement. Different mutagens, including radiation, such as ultraviolet, X-rays and gamma-rays, and chemicals, such as ethyl methane sulphonate and diethyl sulphonate, have been used to induce the mutation of A. niger.[14].

2. Method Used in Production of Citric Acid

2.1. Extraction from Citrus Fruits

In this process, the citrus fruits especially lemons are washed the peeled and crushed in between squeezer to obtain juice. The filter juice containing 3-4% citric acid is limed to get calcium citrate. The calcium citrate with sulphuric acid to form a solution from which citric acid is crystallized.

2.2. Chemical Synthesis

In this process, twofold excess of hydrogen cyanide added rapidly to a slurry of 15g of symmetrically dichloroacetone in 10 ml. Ethanol at 0 0 C about 100 mg of sodium cyanide added and the mixture is stirred at 0-10 0 C for 2 hours and then 60 0 C for 2 hours. After cooling 250 ml of cold concentrated hydrochloric acid is continuously added with stirring. The solution is kept at 0 0 C overnight and finally boil under reflux for 24 hours. The hydrochloric acid evaporated and water is added. The formed mixture is extracted with ether. For the above quantity of chemicals approximately 117gm of product is reported.

2.3. Fermentation

Fermentation process is generally regarded as a biological method for citric acid production. Citric acid production using Aspergillus niger in submerged fermentation using as substrate. Literature regression equations used to model the fermentation for determining the optimum fermentation conditions. For fermentation process the medium is taken as 250 ml in flask with 50 ml medium and 1 ml of spore inoculum, incubated in a rotary shaker at 150 rev./min. The

Maximum citric acid is reported in the literature. Many researchers working on the fermentation process and finding out optimized parameters. By using optimized parameters such as pH, temperature, sugar concentration, ammonium nitrate concentration and potassium ferrocyanide concentration got the maximum citric acid [15].

2.4. Accumulation of citric acid

In general, the build-up of citric acid involves deactivation of aconitase and/or isocitrate dehydrogenase. Activity in the Krebs cycle produces intermediates necessary for biomass formation during the formation of citric acid [16,17]. The Krebs cycle is a series of eight reactions that take place in the mitochondrion. These reactions take a two-carbon molecule (acetate) and completely oxidize it to carbon dioxide. The cycle is summarized in the following chemical equation:

2.5. Acetyl CoA + 3NAD + FAD + ADP + HPO−2 4 → 2CO2 + CoA + 3NADH+ + FADH+ + ATP

Therefore, the accumulation of citric acid probably results from enhanced (deregulated) biosynthesis instead of inhibited degradation [18].

2.6. Microbial production of citric acid by fermentation

Species of Aspergillus such as A. wenti, A. foetidus, A. aculeatus, A. awamori, A. fonsecaeus, A. phoenicis and A. carbonaries, as well as Trichoderma viride and Mucor pyriformis, have been found to produce significant amounts of citric acid. Besides fungi and bacteria, yeast species such as Candida tropiclaus, Candida oleophilis, Candida guilermondi, Yarrowia lipolytica, Torulopsis, Hansenula, Debaromyces, Torula, Pichia, Kloekera, Saccharomyces and Zygosaccharomyces are capable of producing citric acid from n-alkanes and carbohydrates [19-22]. The drawback of using yeast is that it produces large quantities of isocitric acid, which is an undesirable by-product; therefore, mutant strains that have low aconitase activity are required. In addition, the increasing cost of oil makes it less feasible economically as oils are now used as the principal carbon source, in a manner analogous to the previous use of alkanes [23].

Aspergillus niger has so far maintained its place in citric acid production as it has advantages over other bacterial microorganisms such as Arthrobacter paraffinens, Bacillus licheniformis, Bacillus subtilis, Brevibacterium flavum, Corynebacterium spp. and Penicillium janthinellum. It is easy to handle, can ferment a broad range of low-cost raw materials and provides high yields [24-25]. Mutagenesis has been used in recent years to improve the citric-acid producing strains so that they can be used in industrial applications. The most common methods include the use of mutagens to induce mutations on the parental strains. The mutagens utilized for improvements are gamma radiation, ultraviolet radiation and often chemical mutagens. Microorganisms for citric production have to be inoculated by spores, which are transferred to the fermentation medium. The various transfer media include air, and can be in the form of a suspension which is then introduced into bottles containing the substrate. Ideally, for high yields, an incubation time of 7 days is required for A. niger. However, after the 7 days of incubation, the capacity for germination tends to reduce with time [26].

2.7. Substrates

A wide range of substrates is utilized in the fermentation process of the microorganisms. Materials such as hydrocarbons, molasses and starchy materials are commonly used. The review by mentions examples such as beet molasses, black strap molasses, cane molasses, carob pod extract, n-paraffin, glycerol, corn starch, hydrolysate starch, yam bean starch, wood hemicellulose, olive oil, rapeseed oil, palm oil and soya bean oil. Owing to the need to use less expensive substrates with the aim of reducing the production costs of citric acid and making it more environmentally sustainable, the noncrystallizable effluents (molasses) after sucrose isolation from sugar refineries may be used. Molasses offers reduced cost and a high sugar content of 40–55% in the form of fructose, glucose and sucrose. The quality of molasses varies according to its source. Therefore, it requires pretreatment [e.g., mixing with K4Fe (CN)6 at pH 4.5, 90°C for 15 min and then removal of the precipitate by filtration] to make it suitable for fermentation [7,27]

2.8. Fermentation strategies in citric acid production

Citric acid production by fermentation has become established as the most widely used and economical process to obtain citric acid. Over 90% of the citric acid used around the globe today is produced from fermentation. This method offers advantages such as having simple, stable and less complicated operations; requiring less complex control systems and lower technical skill; consuming less energy; and not being critically affected by frequent plant power failure. In general, all fermentation processes, irrespective of the type of fermentation, have three phases: preparation and inoculation, fermentation, and recovery of the citric acid. Over the years, citric acid fermentation has undergone a series of developments. In the 1910s, production was limited to species of Penicillium and Aspergillus utilizing surface or stationary culture conditions. In the 1940s, Shu and Johnson (1948a) developed submerged fermentation from Aspergillus. This research provided the basis for submerged fermentation [7].

2.9. Surface fermentation

Surface fermentation, also known as liquid surface culture, was the original citric acid industrial production technique. Even though in recent years submerged fermentation has gained popularity, there are still small- and medium-scale industries that make use of this method [11]. Surface fermentation offers advantages such as lower installation and energy costs (as it does not require energy for aeration and agitation), and is also foam free. However, it is labour intensive and sensitive to changes in composition of the media. This method consists of two phases, both of which are characterized by a rapid uptake of carbohydrates. The first phase is the development of the fungus as mycelial mat on the surface of the medium and the second phase utilizes carbohydrates by converting them to citric acid [28-30]. The process is conventionally performed in fermentation chambers, using trays made from materials such as special-grade steel, high purity aluminum or polyethylene. However, stainless steel trays are preferred, as they are resistant to deformation with prolonged use [11].

2.10. Submerged fermentation

This is the most widely used fermentation technique in the world today. Eighty per cent of the world's production is estimated to be from the submerged method [31]. Submerged fermentation was developed after surface fermentation. It requires more sophisticated installation, higher energy cost and rigorous control, and there is formation of foam (which can be resolved using antifoaming agents), but it provides higher productivity and yields, has reduced capital, maintenance and labour costs, and carries lower contamination risks. In addition, it is less sensitive to change in the medium composition, providing a wider range of substrates and better control of substrates; this advantage makes molasses usable as a medium for citric acid production [13]. Submerged fermentation is mostly operated as a batch system [32]. However, continuous systems are possible and are used in practice. Submerged fermentation also includes the shake flask technique, which is usually used for the optimization of fermentation conditions [24]. This is basically an Erlenmeyer flask which is placed on a shaker and stirred continuously throughout the fermentation process [33]. Performed a comparative study between surface culture and submerged culture techniques. The outcome from this study was that surface fermentation is superior to submerged fermentation in terms of yield and productivity of citric acid.

2.11. Solid-state fermentation

The solid-state process, or 'Koji' fermentation, originates from Japan, which has an abundance of agro-industrial residues/wastes [34]. This process involves the cultivation of microorganisms in the absence of free liquid on moist solid materials [21]. The solid materials act as a physical support and source of nutrients for the microorganism. Under optimal conditions, the process should be completed in 4 days [28]. The main advantage of solid-state fermentation is its superior yield and the ability to utilize inexpensive and widely available agro-industrial residues as substrates for bio-production, making it more environmentally friendly than submerged fermentation [35]. It requires less water and has lower operating costs, and does not require complex equipment. There is no need for pretreatment as the system is less sensitive to the presence of trace elements compared to submerged fermentation [19].

3. Factors affecting citric acid fermentation

3.1. 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 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 [36]. 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 [37-40].

3.2. 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 [41-43].

3.3. 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. The addition of phosphorus has only a slight effect on the accumulation of citric acid and mycelial growth deduced that citric acid accumulates with limited phosphate, even when nitrogen is not limited [44].

3.4. Lower Alcohols

Lower alcohols added in pure material inhibit citric acid production but added into crude carbohydrate these alcohols enhance production. Methanol, ethanol, n-propanol, Isopropanol [45].

3.5. 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. 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 favoring 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 [13,46].

3.6. Trace elements

Studies on divalent metal ions including manganese, zinc, copper, magnesium and iron have shown that they have effects on citric acid production [21,27]. 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 [47-49] deduced that at a high zinc concentration, the fungi-maintained growth without accumulation of citric acid [28]. Nickel, molybdenum and cobalt are some other trace metals reported to affect the citric acid accumulation of A. niger [50]. 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.

3.7. Aeration

The highly aerobic nature of the bio-production of citric acid makes the amount of oxygen supplied a critical factor. Therefore, varying aeration rates can have adverse effects on the fermentation performance and yield. At high aeration rates, there is reduced partial pressure of the dissolved carbon dioxide in the medium. Carbon dioxide is a substrate for pyruvate carboxylase as it replaces the supply of oxaloacetate for citrate synthase. The reaction catalyzed by pyruvate decarboxylase produces carbon dioxide, but extreme aeration can incur some losses. Increased levels of carbon dioxide are damaging to the final biomass and concentrations of citrate [1, 49].

3.8. Other factors

Other factors that have effects on citric acid production include lipids, such as groundnut oil, and sodium monofluoroacetate. [51] showed that lipid will improve the yield of citric acid with no effect of the dry weight of mycelium. [34] showed the effects of calcium fluoride, sodium fluoride and potassium fluoride on the industrial production of citric acid. The main factors that affect citric acid production [52-54].

3.9. Applications of Citric Acid

- Citric acid mainly used in food industry, pharmaceuticals, chemical industry, cosmetics, printings, food preservative, electro pickling, copper plating, beverage & others. Some specific applications are given below.
- Citric acid monohydrate is widely used as organic acid & pH control agent, flavouring and preservative in food production like as candy, cookies, biscuits, jams, jellies, snacks, instant foods and sauces.
- It is used as acidity regulator and antioxidant in beverage such as alcoholic beverage, carbonated soft drink, syrups, juice drinks, tea & coffee, ice-cream, sports &energy drink.
- It can be used in thrombin inhibitor and fungicide in pharmaceutical.
- It can be used as antioxidant & pH regulator in agriculture/ animal food/ poultry food such as chicken feed, boiler feed.
- It is widely used in cleaning agent, surfactant in various industries such as cleaning agent & anticrease agent.
- Remove metal oxide from surface of ferrous & nonferrous for operational cleaning of iron & copper oxides.
- In electroplating, copper plating, metal cleaning, leather tanning, printings inks, bottle washing compounds, floor cement, textiles, photographic reagent, plaster.
- It can be use in tartness and complements fruits & barriers flavours in beverage.
- It is used as an acidulent in creams, gels & liquid of all kinds.
- It is used as acidifying agent in many cheese products & as an antioxidant in dairy products.
- It can be used as an alternative to nitric acid in passivation of stainless steel.
- It is used as an odorless alternative to white vinegar for home dyeing with acid dyes.
- It is used as one of the active ingredients in the production of antiviral tissues.
- It is an alpha hydroxy acid and used as an active ingredient in chemical peels.
- It can be used in food colouring to balance the pH level of a normally basic dye.
- It can be added to ice cream as an emulsifying agent to keep fats from separating, to caramel to prevent sucrose crystallization, or in recipes in place of fresh lemon Juice.

4. Conclusion

Citric acid production has been studied during last decade and great alternatives to this process have been found to follow its great demand. Reviewing broad research achievements and market trends, this study provides a critical overview on citric acid. This review article for fermentation of citric acid and Microbial production of citric acid, Substrates and strategies of citric acid production for Surface fermentation, Submerged fermentation, Solid-state fermentation and also the effects of various Factors affecting of citric acid fermentation conditions are Carbon source, Nitrogen limitation, Phosphorus source, Lower Alcohols, pH of culture medium, Trace elements, Aeration and Other factors. citric acid recovery options and the numerous applications of citric acid, based on the literature review information of citric acid production by fermentation technology and 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. The production of citric acid now more than 1.4 million tonnes per year and day by day that rate is increasing. The important reason for increases the large numbers of applications that can be found citric acid mainly in food and pharmaceutical industry.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

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