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PRODUCTION OF CITRIC ACID: A MICROBIAL PERSPECTIVE

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ABSTRACT: There is high global consumer demand for citric acid due to its low toxicity compared to other acidifiers used mainly in the pharmaceutical and food industries. The pharmaceutical, food, beverage, detergent and cosmetic sectors employ microbial citric acid extensively. It is mainly produced by microbial fermentation using *Aspergillus niger*. Increasing the productivity of citric acid would be of potential interest and therefore there is a clear need to consider all possible ways to achieve optimal citric acid production. With increasing demand and growing markets, the discovery and development of better manufacturing techniques, solutions to improve manufacturing yields and product recovery efficiency are of prior importance. Production of citric acid is affected by various parameters viz., selection of potential citric acid-producing microorganisms, raw materials, fermentation strategies, fermentation conditions and strategies for citric acid recovery. Other prospects for the citric acid manufacturing sector are the improvement of citric acid-producing strains, which have been achieved through mutagenesis and breeding.

Keywords: Citric acid, submerged fermentation, solid-state fermentation, agro-industrial residues, *Aspergillus niger*.

1. INTRODUCTION:

Citric acid is a weak acid that occurs in nature. The term "citric acid" comes from the Latin word "citrus," which means any of group of plants that produce juicy fruits with a slightly sour taste. Citrus plants, such as lemon, Orange, and sweet lime belong to the genus Citrus. purest form of citric acid is water soluble and colourless (Angumeenal & Venkappayya *et.al* 2013). The melting point of citric acid is 153°C and Its molecular weight is 210.14 g/mol. presence of three carboxylic acid functional groups in its framework, it has a different pKa values at pH 3.1, 4.7, and 6.4. Citric acid is a nearly universal intermediate product of metabolism, with traces found in nearly all plants and animals. (Papagianni, 2007).

Citric acid can come from natural sources (eg, lemon, lime, and orange) or synthetic sources (eg, chemical reaction and microbial fermentation). The method of extracting citric acid from lemon juice was developed by a Swedish chemist, Karl Wilhelm Scheele in 1784. This method was adopted in England around 1826 for the commercial production of citric acid from lemons imported from Italy. Until the late nineteenth century, when German botanist Wehmer first discovered the viability of obtaining citric acid through the fermentation of a sugar medium containing inorganic salts with Penicillium glaucum, the method maintained its monopolies as the sole commercial source for citric acid production. However, *Citromyces spp.* based citric acid production has not been widely used in industrial practise because of worries about contamination and the drawn-out fermentation process. *Aspergillus niger* is widely used in the industry for citric acid production. (Bauweleers *et al.*, 2014).

A large number of microorganisms including bacteria and Fungi such as *Arthrobacter paraffin's, Bacillus licheniformis* and *Corynebacterium spp*., *Aspergillus niger*, *A. aculeatus, A. carbonarius, A. awamori, A. foetidus, A. fonsecaeus, C. oleophila, C. guilliermondii, C. citroformans, Hansenula anamola*, and *Yarrowia lipolytica* have been used for citric acid production. Yeast also plays important role in citric acid production such as *Candida, Hansenula, Pichia, Debaryomyces, Torulopsis, Kloekera, Trichosporon, Torula, Rhodotorula, Sporobolomyces, Endomyces, Nocardia, Nematospora, Saccharomyces* (Angumeenal AR and Venkappayya D, 2013).

Based on the market trends, it is evident that there will be an increase in the global demand for citric acid. therefore, crucial to optimize the production of citric acid by looking for alternatives that are cheaper, more respectful of the environment, and with a higher production yield than current methods. In light of this, this review discusses the biochemistry of citric acid production, choice of raw materials, selection of citric acid producing microorganisms, production methods and strategies, effects of various fermentation conditions, and recovery options. The aim is to provide a comprehensive review, compared to other reviews that focus on specific areas (Pazouki and Panda *et al.*,1998).

2. Biochemistry of Citric acid

The krebs cycle, also known as the citric acid cycle or the Tricarboxylic Acid (TCA) cycle. The cycle was first discovered by scientist "Sir Hans Adolf Krebs" (1900 to 1981) In 1953, he shared the Nobel Prize in physiology and medicine with Fritz Albert Lipmann, the father of the ATP cycle. In prokaryotic cells, the citric acid cycle occurs in the cytoplasm; in eukaryotic cells, the citric acid cycle occurs in the mitochondrial matrix. The citric acid cycle serves as the mitochondrial hub for the ultimate steps in carbon skeleton oxidative catabolism for carbohydrates, amino acids, and fatty acids. A coenzyme like nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide is reduced by each oxidative cycle in turn ($FADH_2$). These reduced coenzymes

contribute immediately to the electron transport chain and consequently to the majority of ATP production in the human body (A. Pandey *et al*., 2001, L.P.S. Vandenberghe *et al*., 1999, C.R. Soccol *et al*., 2003).

2.2.1 Steps of the Citric Acid Cycle:

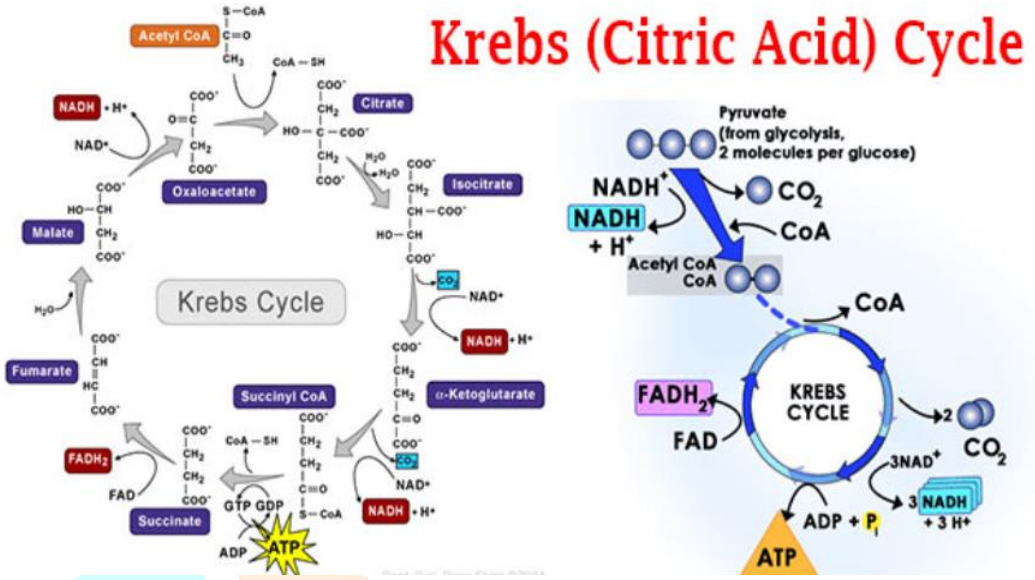

Fig. 1. Schematic representation of the metabolic reactions involved in citric acid production (A. Pandey *et al*., 2001, L.P.S. Vandenberghe *et al*., 1999, C.R. Soccol *et al*., 2003).

The Net Equation

$$\text{Acetyl CoA} + 3\ \text{NAD} + \text{FAD} + \text{ADP} + HPO_4^{-2} \longrightarrow 2\ CO_2 + \text{CoA} + 3\ NADH^+ + FADH^+ + \text{ATP} \quad (1)$$

2.2.1.1 Citrate synthesis

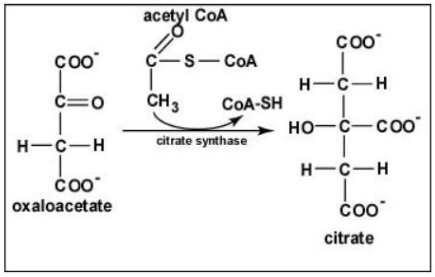

Fig. 2. The conversion of oxaloacetate to citrate (C.R. Soccol *et al*., 2003).

The first reaction of the cycle is the condensation of acetyl-CoA with oxaloacetate to form citrate, which is catalysed by citrate synthase. When oxaloacetate binds to acetyl-CoA, a water molecule attacks the acetyl, allowing coenzyme A to be released from the complex. (A. Pandey *et al*., 2001, L.P.S. Vandenberghe *et al*., 1999, C.R. Soccol *et al*., 2003).

2.2.1.2 Isomerization of citrate

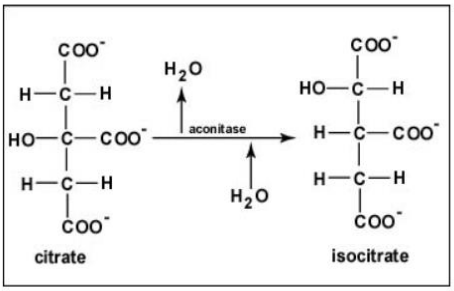

Fig. 3. The conversion of citrate to isocitrate (C.R. Soccol *et al*., 2003).

Aconitase, it's an enzyme with an iron-sulphur that allows the hydroxyl group migration and makes isocitrate out of citrate (A. Pandey *et al*., 2001, L.P.S. Vandenberghe *et al*., 1999, C.R. Soccol *et al*., 2003).

2.2.1.3 Oxidative decarboxylation of isocitrate

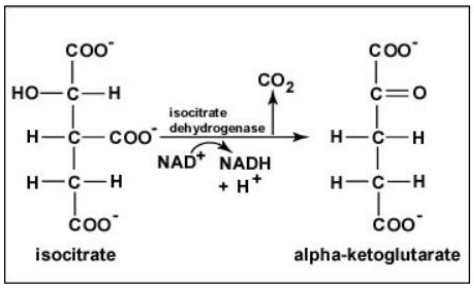

Fig. 4. The conversion of isocitrate to alpha-ketoglutarate (C.R. Soccol *et al*., 2003).

Isocitrate dehydrogenase catalyses the oxidative decarboxylation of isocitrate to form alpha-ketoglutarate during this step. NADH is generated from NAD in the reaction. Isocitrate dehydrogenase catalyses the oxidation of the -OH group at the 4′ position of isocitrate to produce an intermediate, which is then removed of a carbon dioxide molecule to produce alpha-ketoglutarate (A. Pandey *et al*., 2001, L.P.S. Vandenberghe *et al*., 1999, C.R. Soccol *et al*., 2003).

2.2.1.4 Oxidative decarboxylation of alpha-ketoglutarate via the alpha-ketoglutarate dehydrogenase complex

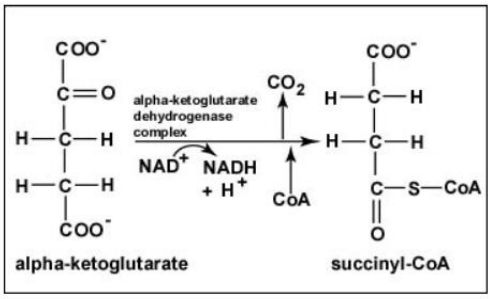

Fig. 5. The conversion of alpha-ketoglutarate to succinyl coenzyme A (C.R. Soccol *et al*., 2003).

To form the 4-carbon compound succinyl-CoA, alpha-ketoglutarate is oxidised, carbon dioxide is removed, and coenzyme A is added. NAD+ is reduced to NADH + H+ during this oxidation. Alpha-ketoglutarate dehydrogenase is the enzyme that catalyses this reaction. (L.P.S. Vandenberghe *et al*., 1999, C.R. Soccol *et al*., 2003).

2.2.1.5 Cleavage of succinyl coenzyme A

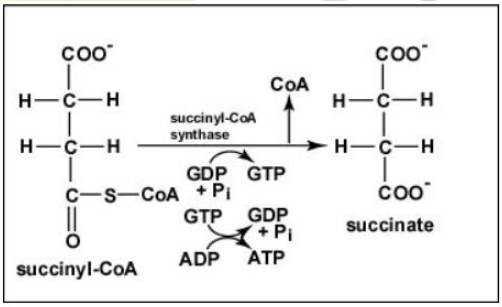

Fig. 6. The conversion of succinyl coenzyme A to succinate (C.R. Soccol *et al*., 2003).

To make succinate, CoA is removed from succinyl-CoA. By substrate-level phosphorylation, the energy released is used to produce guanosine triphosphate (GTP) from guanosine diphosphate (GDP) and Pi. GTP can then be converted into ATP. This citric acid cycle reaction is catalysed by the enzyme succinyl-CoA synthase. (A. Pandey *et al*., 2001, L.P.S. Vandenberghe *et al*., 1999, C.R. Soccol *et al*., 2003).

2.2.1.6 Oxidation of succinate

Succinate is converted to fumarate through oxidation. FAD is reduced to FADH2 during this oxidation. FAD is reduced to FADH2 during this oxidation. Succinate dehydrogenase is an enzyme that catalyses the removal of two hydrogens from succinate. (A. Pandey *et al*., 2001, C.R. Soccol *et al*., 2003).

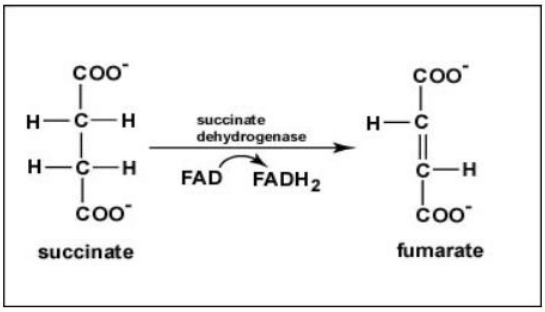

Fig. 7. The conversion of succinate to fumarate (C.R. Soccol *et al*., 2003).

2.2.1.7 Hydration of fumarate

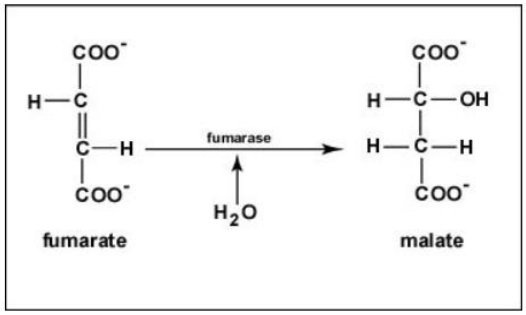

Fig. 8. The conversion of fumarate to malate (C.R. Soccol *et al*., 2003).

Fumarate catalyses the reversible hydration of fumarate to L-malate (fumarate hydratase). Fumarase continues the rearrangement process by reintroducing previously removed Hydrogen and Oxygen into the substrate. (A. Pandey *et al*., 2001, L.P.S. Vandenberghe *et al*., 1999, C.R. Soccol *et al*., 2003).

2.2.1.8 Oxidation of Malate

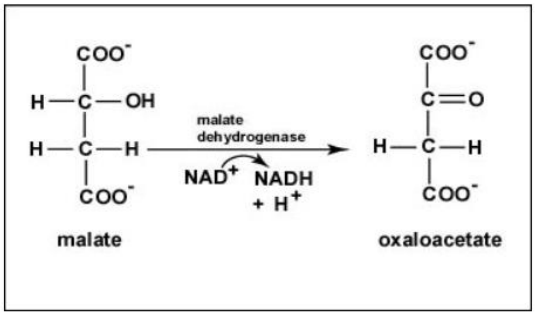

Fig. 9. The conversion of malate to oxaloacetate (C.R. Soccol *et al*., 2003).

Malate dehydrogenase oxidises malate to produce oxaloacetate, the starting compound of the citric acid cycle. NAD+ is reduced to NADH + H+ during this oxidation. (H.S. Grewal *et al.*,1995, A. Pandey *et al*., 2001 C.R. Soccol *et al.*, 2003).

3. Economic importance of Citric Acid:

Citric acid is a versatile and harmless food additive. It is accepted worldwide as GRAS (generally recognized as safe), endorsed by the Joint FAO/WHO Expert Committee on Food Additives (A. Pandey *et al*., 2001, L.P.S. Vandenberghe *et al*., 1999, C.R. Soccol *et al*., 2003). The food and pharmaceutical industries use citric acid widely due to its general recognition of safety, pleasant acidic taste, high water solubility, chelating and buffering properties.

3.1 Industrial application of citric acid

Due to its pleasant taste, high water solubility, chelating, and buffering properties, citric acid is widely used as a safe acidulant in the food, sugar, confectionery, and beverage industries. In carbonated drinks, it is used to give fruit and berry flavors. As a high-performance water reducer in the confectionery industry (Buchard & Merrit, 1979); in the wine industry to prevent fogging of wine, in candies to add dark color and acidity, as an antioxidant synergistic effect of fats, oils, and fat foods (Buchard & Merrit, 1979); in sherbet as a flavor additive (Buchard & Merrit, 1979); in ice cream as an emulsifier. In addition, Citric acid esters such as triethyl, tributyl, and acetyl tributyl are used as non-toxic plasticizers for plastic films used to protect food (Buchard & Merrit, 1979). The chelating and pH regulation properties of citric acid are used in the food industry to improve the stability of frozen foods by preventing deterioration of the color and flavor of frozen foods. Trisodium citrate is widely used as a blood preservative that prevents coagulation by complexing calcium. Acetic acid is used to prevent kidney stones along with sodium citrate (Gul & Monga, 2014). As a crosslinker, it has multiple uses Before producing biodegradable films such as bioplastics (L.P.S. Vandenberghe *et al.*, 1999).

suitable for environmentally friendly packaging, in ultrafine protein fibers for biomedical applications. Hydroxyapatite for the production of polyols and bioceramic composites for orthopedic tissue engineering. Citric acid is used to soften water and is useful in household cleaners and dishwashing liquids or soaps. Chelating metal ions such as Ca^{2+} and Mg^{2+} ions in hard water, creates bubbles and functions more effectively without softening the water. Due to its excellent metal chelating properties, citric acid can be used as a cleaning agent. Therefore, citric acid solutions are used to clean power plant boilers and similar equipment to remove and prevent scale deposits. Due to its excellent metal chelating properties, citric acid is widely used for cleaning radionuclide-contaminated nuclear (Kantar & Honeyman, 2006) and for bioremediation of heavy metal-contaminated soil. Brazilian researchers first showed in 2005 that citric acid can be used in place of toxic mineral acids to obtain pectin from apple squeezed residue (Canteri-Schemin *et al.*, 2005). The pectin extraction yield by citric acid showed the highest average value (13.75%) (W. Jianlong et al., 2000)

4 Microbial Production of Citric Acid

4.1 Citric acid production from bacteria and fungus

A large number of microorganisms including bacteria and fungi such as *Arthrobacter paraffin's, Bacillus licheniformis* and *Corynebacterium spp*., *Aspergillus niger*, *A. aculeatus, A. carbonarius, A. awamori, A. foetidus, A. fonsecaeus, C. oleophila, C. guilliermondii, C. citroformans, Hansenula anamola*, and *Yarrowia lipolytica* have been used for citric acid production. although Most of them, are unable to produce commercially acceptable yields because citric acid is a metabolite of energy metabolism and its accumulation only grows in appreciable amounts under conditions of drastic imbalances. The *Aspergillus niger* widely used for commercial production of citric acid, because it produces more citric acid per unit of time. The main advantages of using *Aspergillus niger* for citric acid production are its easy to handling, ability to ferment a variety of inexpensive raw materials, and high yields (Angumeenal AR and Venkappayya D, 2013).

Industrial strains that produce commercial citric acid are not freely available and only some can be obtained from international culture collections (Y.Ikeno *et al.*, 1975). Some of the bacterial strain and fungal strain plays important role in citric acid production. There for the Improvement of citric acid-producing strains has been achieved by mutagenesis and selection. The most commonly used technique has been the induction of mutations in the parental strains using mutagens. *Aspergillus niger* mutants are employed in industry for the production of citric acid. Among mutagens, UV radiation and chemical mutagens are often used to obtain hyper productive strains, UV treatment can often be combined with certain chemical mutagens. The "single spore technique" and the "passage method" are the main methods of strain selection. Different fermentation methods can lead to different yields of citric acid production by the same strain. Therefore, a strain that produces good yields in solid or liquid surface fermentation. But it is not necessarily a good producer in submerged fermentation. Thus, each method and raw material of industrial interest should be tested with strains from known producers (F. Yokoya,1992).

If any technique used in the production of citric acid, the inoculation of the microorganism is done using spores which are added to the fermentation medium. The spores can be inoculated either by mixing them with air which is introduced into the substrate or as a spore suspension. The spores are produced in glass vials on solid substrates at optimum temperature. sporulation and incubation time affects spore viability and citric acid production by cultured mycelium of *Aspergillus niger*, It has been mentioned that potato dextrose agar provides high yields of citric acid. Viability increases with incubation time, but increased citric acid production was obtained within 7 days of spore incubation (M.G.F. Vergano *et al.*,1996).

4.2 Citric Acid Production from yeast

The most significant organic acid produced by microbial activities. More than 90% of the citric acid produced worldwide is acquired by fermentation (Singh Dhillon *et al.*, 2011).

Potential manufacturers of citric acid include *Candida, Hansenula, Pichia, Debaryomyces, Torulopsis, Kloekera, Trichosporon, Torula, Rhodotorula, Sporobolomyces, Endomyces, Nocardia, Nematospora, Saccharomyces, and Zygosaccharomyces.* However, some *Candida species, including C. lipolytica, C. tropicalis, C. oleophilic, C. intermedia, C. Guillermodid, C. paratropicalis, C. zeylanoides, C. catenulata, C. parapsilosis, C. citroformans, C. brae, C. subtropicalis.* It was discovered at the end of the 1960s that *Yarrowia lipolitica* produced citric acid with n-alkanes as a substrate with relatively high yields. Due to their metabolic adaptability and development, using yeasts in citric acid production has several benefits, such as the ability to use a variety of carbon sources. studies on the strains, substrates, and fermentation conditions of yeasts used to produce citric acid (Singh Dhillon, 2011).

Citric acid is commercially produced from molasses, sucrose, or glucose using *Aspergillus niger*. Additionally, there is a lot of interest in various yeasts that can produce citric acid from a variety of carbon sources. Yeasts are preferred in the production process because they may utilize a range of carbon sources and they tolerate some conditions like high substrate concentrations, metal ions, and low oxygen levels, and pose fewer health risks. Other significant factors for their selection include the use of less reactive substrates, the reduction in substrate and waste treatment, the cost of product recovery, and simpler genetic modifications using molecular techniques (Liu X-Y *et al.*, 2010).

5. Substrates

Citric acid is mostly produced from starch or sucrose based medium utilizing submerged fermentation, several raw materials such as hydrocarbons, starchy materials, and molasses have been used as substrates for industrial submerged citric acid production (L.P.S. Vandenberghe *et al.*,1999)

In most cases, citric acid is produced through fermentation using low-cost raw materials (Pazouki M *et al.*, 1998), such as hydrolysate starch, sugar cane broth, and by-products such as sugar cane and beet molasses (F. Yokoya, 1992).

Molasses is preferred as a sugar source for microbial citric acid production due to its relatively low cost and high sugar content (40–55%) (H.S. Grewal *et al.*1995). As it is a by-product of sugar refining, the quality of molasses varies widely and not all types are suitable for citric acid production. The composition of molasses depends on various factors such as the variety of beet and cane, cultivation methods, storage and handling conditions (transportation, temperature changes), etc. Both beet and cane molasses are suitable for the production of citric acid, however, beet molasses is preferred over sugar cane due to its low trace metal content,

providing better production yields than cane molasses, but there are considerable variations in yield within each type. In the case of cane molasses, it generally contains certain metals (iron, calcium, magnesium, manganese, zinc) which delay the synthesis of citric acid and require pre-treatment to reduce them. Palmyra jaggery, a sugar syrup from palmyra palm is a new substrate to increase the yield of citric acid production (P. Ambati *et al*., 2001).

Along with cassava bagasse, coffee husk, wheat bran, apple pomace, pineapple waste, kiwi fruit peel, grape pomace, citrus waste, etc., a type of agribusiness residues and by products have also been examined for usage as substrates for citric acid production.

6. Method for citric acid production

6.1 Submerged fermentation

For the synthesis of citric acid, the submerged approach is frequently utilized. Submerged fermentation is thought to be responsible for around 80% of global production. This fermentation technique, which is used on a big scale, necessitates more sophisticated equipment and strict monitoring. On the other hand, it offers various benefits, including increased productivity and yields, lower labourer costs, less contamination risk, and reduced labour consumption. There are two types of fermenters used: conventional stirred fermenters and tower fermenters, with the latter being preferred due to price, size, and operation advantages (Rohr et al., 1983). Fermenters should be made of high-quality steel and equipped with an aeration system capable of maintaining a high dissolved oxygen level. Fermenters for citric acid production do not need to be pressure vessels because sterilization is accomplished simply by steaming without applying pressure. In this fermentation process, an external water film can be used to cool the fermenters.

Submerged fermentation uses a variety of media, including sugar and starch-based media. Molasses and other raw materials necessitate for pre-treatment, nutrient addition, and sterilization. Inoculation is accomplished by either adding a spore suspension or pre-cultivated mycelia. When using spores, a surfactant is added to disperse them in the medium. In general, an inoculum size of 10% fresh medium is required for pre-cultivated mycelia. Submerged fermentation typically takes 5 to 10 days to complete, depending on the process conditions. It can be done in batch, continuous, or fed batch systems, with batch being the most common. (L.P.S. Vandenberghe *et al.*, 1999).

6.2 Solid-state fermentation

Solid-state fermentation, commonly known as the Koji technique, it was first developed in Japan, where there are many raw materials such as fruit wastes and rice bran. It is the simplest method for producing citric acid and has been used as a substitute for agro-industrial waste (M.B. Kolicheski *et al.* 1995, L.P.S. Vandenberghe *et al*.2000, J. Pallares *et al.*1996, D.B. Xu *et al*.1989, M. Hossain *etal.*1984). Solid-state culture is defined by the growth of microorganisms on an insoluble material that serves as both physical support and a source of nutrients in a low-water activity environment (J.M. González *et al.*,1997). Because the fungus grows on the surface of the substance, there are some parallels with the surface process. The substrate is solid and soaked to around 70% moisture, depending on the absorption capability of the substrate. The original pH of the substance is usually adjusted to 4.5–6.0, and the temperature of the material is usually maintained at 28-30°C, depending on the microorganism used. The solid culture process is completed in 96 hours under optimal conditions(L.P.S. Vandenberghe *et al.*, 1999, M.B. Kolicheski *et al.*, 1995, L.P.S. Vandenberghe *et al.*, 2000, J. Pallares *et al.*, 1996, D.B. Xu *et al.*, 1989, M. Hossain *et al.*, 1984).

The organism most commonly used in solid-state fermentation is *Aspergillus niger*. Strains with high nitrogen and phosphorus requirements are not ideal microorganisms for solid culture due to the slower diffusion rate of nutrients and metabolites that occur at lower water activity in the solid state process. the presence of trace elements may not affect citric acid production as adversely as occurs in submerged fermentation; therefore no pre-treatment of the substrate is necessary. This is one of the important benefits of solid-state fermentation (M. Hossain *et al.*,1984).

6.3 Surface fermentation

Surface fermentation, also known as liquid surface culture, it was the original industrial production technology of citric acid. Even if liquid fermentation is performed in recent years. It was popular, but there are still small and medium-sized ones. Industries using this method (Bauweleers *et al.*, 2014). Surface fermentation offers the following benefits: Installation and energy cost reduction because it is unnecessary (Energy for aeration and agitation) and also no bubbles. But it's labor-intensive (Drysdale & McKay, 1995) and Sensitive to changes in media composition (Bengasietal, 2014). This method consists of two phases. Both are characterized by the rapid absorption of carbohydrates. The first stage is a fungal outbreak as a mat of mycelium on the surface of the medium. In the second stage, carbohydrates are converted and used to citric acid (Kiel *et al.*, 1981). The procedure is the same as before run in a fermentation chamber using a tray of high purity made up of materials such as stainless steel, aluminium or polyethylene. However, stainless steel tray is preferred as it is resistant to deformation in long-term use (Bauweleers *et al.*, 2014). Because the chamber needs to be effectively ventilated aseptic air is transferred aseptically and continuously medium surface through a bacterial filter. Air provides the required oxygen demand for microorganisms due to the highly aerobic nature of the process; also controls humidity and temperature (Evaporative cooling). Air also helps remove carbon dioxide, an inhibitor of citric acid production concentrations above 10% (Soccoletal, 2006). After completing the second phase citric acid is obtained by washing the mycelial mat (M.Y. Lu *et al.*, 1998).

7. Factors affecting citric acid production

7.1 Phosphorous source

In the first works, it was verified that the presence of phosphate in the medium had a great influence on the yield of citric acid. Low phosphate levels have a positive effect on citric acid production. This effect acts at the level of enzymatic activity and not at the level of gene expression. On the other hand, the presence of an excess of phosphate leads to a decrease in the fixation of carbon dioxide, which in turn increases the formation of certain sugar acids, and the stimulation of growth (H.S. Grewal *et al*.1995, C.P. Kubicek *et al.*, 1986, L.P.S. Vandenberghe *et al.*, 1999).

7.2 Nitrogen source

Physiologically preferred ammonium salts are such as urea, ammonium nitrate and sulfate, peptone, malt extract, etc. Ammonium acids are preferred because their consumption leads to a decrease in pH, which is essential for citric fermentation. However, it is necessary to maintain the pH values on the first day of fermentation before some biomass production. The concentration of nitrogen source required for citric acid fermentation is 0.1 to 0.4 g/L. High concentrations of nitrogen increase fungal growth and sugar consumption but reduce the amount of citric acid produced (L.P.S. Vandenberghe *et al.*,1999).

7.3 Carbon source

Citric acid accumulation is strongly influenced by the carbon source. carbohydrates are essential for good Production of citric acid. Sucrose is the most advantageous carbon source, followed by glucose, fructose, and galactose. Their some carbohydrates which have negative effect on citric acid production. One of the example is galactose. Galactose contributed to very low fungal growth and citric acid Accumulation. Other carbon sources Sorbose, ethanol, cellulose, mannitol, lactic acid, malic acid, and α-acetoglutaric acid allow limited growth and low production (Y. Ikeno *et al.,* 1987).

7.4 Trace elements

The nutrition of trace elements is probably the main factor affecting the yield of citric acid. Many divalent metals like zinc, manganese, iron, copper, and magnesium are known to affect the production of citric acid From *Aspergillus niger*. Benuzzi and Segovia (1996) reported the existence of different copper concentrations in pellet medium. It was very important to improve what was right Structure of citric acid related to cell physiology and Acid production. The Optimal concentration of $CuSO_4.5H_2O$ was 78 mg / l. Magnesium also plays important role in citric acid production. Optimal Magnesium sulfate concentration was found in It ranges from 0.02 to 0.025% (Kapoor *et al.,* 1983). Lower alcohols: The addition of lower alcohols enhances acid production from commercial glucose and other crude carbohydrates. Methanol, ethanol, isopropanol, or methyl acetate are acceptable alcohols. The optimal amount of methanol/ethanol depends upon the strain and the composition of the medium, generally the optimum range being 1-3%. (Hamissa, 1978; Mannomani and Sreekantiah, 1988; Georgieva *et al.,* 1992; Dasgupta *et al.,* 1994). Mannomani and Sreekantiah (1987) reported that the addition of ethanol resulted in a two-fold increase in citrate synthetase activity and A 75% decrease in aconitase activity. They also found that vegetable oil influenced acid production. Alcohols have been demonstrated to primarily alter the phospholipid content of the cytoplasmatic membrane in microorganisms, which in turn affects membrane permeability (Orthofer et al., 1979). However, Meixner *et al.* (1985) argued against a job of membrane permeability in acid accumulation. Ingram and Buttke (1984) found that alcohols stimulate acid production by affecting growth and sporulation through the action not only on the cell permeability but also on the spatial organization of the membrane, or changes in lipid composition of the semipermeable membrane. Miscellaneous: Some compounds which are inhibitors of metabolism like calcium fluoride, salt, and potassium fluoride have been found to accelerate acid production, while, yellow prussiate of potash has been found to decrease the yield. There are many compounds, which act in some ways to favour citric acid accumulation. a number of them are capable to impair the action of metal ions and other toxic compounds that influence growth during the initial phase. a number of these are 4-Methylumbelliferone, 3-hydroxy-2-naphthol, benzoic acid, 2-naphthenic acid, iron cyanide, quaternary ammonium compounds, amine oximes, starch, EDTA, vermiculite, etc (Singh Dhillon *et al.,* 2011).

7.5 pH

The pH of the culture can change in response to microbial metabolic activities. The most obvious reason is the secretion of organic acids, such as citric acid, which will cause the pH to drop. Changes in pH kinetics are also highly dependent on microorganisms. With *Aspergillus sp.*, *Penicillium sp*., and *Rhizopus sp*., the pH can drop very quickly to less than 3. For other groups of fungi such as *Trichoderma*, *Sporotrichum*, and *Pleurotus* sp., the pH is more stable between 4.0 and 5. The nature of the substrate and the production technique also influence the pH kinetics. In this way, the initial pH must be very well defined and optimized according to the microorganism, the substrate, and the production technique (F. Yokoya, 1992).

7.6 Aeration

The amount of oxygen supplied is crucial since the biosynthesis of citric acid is extremely aerobic. Therefore, altering aeration rates might negatively impact the efficiency and yield of fermentation. The partial pressure of the dissolved carbon dioxide in the medium decreases at high aeration rates. As carbon dioxide replaces the oxaloacetate supply for citrate synthase, it serves as a substrate for pyruvate carboxylase. Pyruvate decarboxylase catalyses a reaction that results in carbon dioxide, however excessive aeration can result in some losses. The rise in carbon dioxide levels is harmful to final biomass and citrate levels (Angumeenal & Venkappayya, 2013). However, carbon dioxide is a positive effect on citric acid synthesis (Vandenberghe *et al.,* 1999). The high partial pressure of carbon dioxide Prevents the release of filamentous fungal spores, Improves the accumulation of citric acid. No matter how expensive Aeration rate mainly leads to foaming of the medium during the growth stage requiring the use of antifoaming agents Medium and/or mechanical defoaming agents. economically is better to gradually increase the ventilation volume. This is consistent with the study by Kubicek *et al.*,(1980) Who revealed Destructive processes (eg interruption of ventilation) were irreversibly destroyed for up to 20 minutes during the idiopathic period. The ability to live organisms to synthesize and store citric acid, Although the viability of the organism was maintained. The role of hypoventilation is to hold breathing activity *Aspergillus niger* to shift metabolism from biomass production to citric acid synthesis. It was also concluded that more ventilation results would be obtained Increased sporulation and decreased citric acid accumulation (Soccol *et al.,* 2006).

7.7 Inhibitor

The addition of 0.1 to 1 mm sodium malonate to the fermentation medium inhibited the mycelial boom to some extent and stimulated citric acid manufacturing markedly (up to 39%) besides affecting the complete titratable acidity. It was exactly at those ranges that malonate has been proven to inhibit succinate dehydrogenase specifically in *Aspergillus niger*. Because of this interruption in the tricarboxylic acid cycle, in addition, the metabolism of citric acid already formed was likely reduced, thereby main to accelerated citric acid accumulation in the medium. Challenger and Landberk have confirmed that *Aspergillus niger* and

other species of *Aspergillus* have the capability to possess the metabolite malonate. It is hence possible that low concentrations of added malonate are completely metabolized in the course of the early length of the fungal boom without adversely affecting citric acid production. However, Barton and Ghiretti have mentioned 73% inhibition of citric acid by using yeasts upon addition of high concentrations of l7 mm of malonate. In general, stimulation of some metabolic approaches upon addition of low concentrations of materials which inhibit the equal methods at greater concentrations is often observed, although the motives for this stimulation are not clear. The addition of the sodium salt of ethylene diamine tetra acetic acid (EDTA) inhibited fungal growth, total titratable acidity, and citric acid production, although the results were more stated at higher concentrations. Since extra of the glycolytic and tricarboxylic acid cycle enzymes are based on a metallic ion, particularly Mg^{2+}, for their activity, it is probably that a higher concentration of EDTA is utilized in the formation of a metal-ion chelate with certain metals critical for the exercise of enzymes (directly or indirectly) associated to the synthesis and accumulation of citric acid in the medium. The addition of 0.2 mm iodoacetate strongly inhibited fungal growth, whole titratable acidity, and citric acid production by 60, 75, and 95%. Respectively, the presence of 10 mm iodoacetate inhibited fungal growth and citric acid production. At lower concentrations, however, it markedly stimulated citric acid production (by 38 and 10.9%, respectively) without materially affecting fungal growth. Total titratable acidity was decreased by using 20%, suggesting a suppression of the production of organic acids other than citric acid. Compared with the control, the percentage of citric acid to total titratable acids was elevated from 35% to 60% and 50% upon the addition of 0.01 and 0. l mm iodoacetate, respectively. Iodoacetate has been reported to be a substitute-specific inhibitor of glyceraldehyde-3-phosphate dehydrogenase, especially at a concentration of l mm. At higher concentrations, other enzymes with sulfhydryl corporations at the active site are additionally affected. it is probable that interruption of the glycolytic cycle due to iodoacetate inhibition might be accountable for the inhibition of fungal boom and, consequently, of citric acid production. The reason for the enhancement of citric acid manufacturing at lower concentrations of iodoacetate is no longer clear. The presence of 1 mm sodium azide in the medium completely inhibited fungal growth, complete titratable acidity, and citric acid production. Lower stages triggered slight stimulation of citric acid production but inhibited whole titratable acidity significantly. The inhibitory effects of sodium azide on oxidative phosphorylation might explain the inhibition observed at 1 mg concentration, which has been reported by numerous workers (Singh Dhillon *et al.*, 2011).

8. Recovery of citric acid

The recovery of citric acid from a fermented broth is usually done by two procedures: precipitation and solvent extraction. The first method is the most used and is applicable in all types of processes. The second requires a fermented broth with few impurities. In both methods, it is necessary to remove fermented broth, mushroom micelles, and suspended solids by filtration (F. Yokoya, 1992).

The main problems in citric acid production remain the separation and purification steps. In recent years, some methods have been developed to reduce the cost of recovery, trying to overcome the drawbacks of the precipitation model, which is responsible for the formation and elimination of huge amounts of calcium sulfate, with significant pollution problems. Electrodialysis is an electrochemical separation process in which electrically charged membranes and an electrical potential difference are used to separate ionic species from aqueous solutions. This technique has been tested in the recovery of citric acid. The problem is that the costs of the electrodialysis technique are around 50% higher than the current one's process for recovering citric acid on an industrial scale. The use of electrodialysis would require the development of new integrated fermentation processes to minimize waste formation and increase productivity (D.T. Friesen *et al.*, 1991).

8.1 Precipitation

One of the conventional Citric Acid fermentation broth downstream processing technologies used in the industry by precipitation of calcium salt with the usage of calcium carbonate and sulfuric acid (Pazouki and Panda 1998). Despite the heating which suffers power requirements, the precipitation process is only 90-95% complete, which must also be investigated. (Annadurai et al., 1996). Karkl in 1984 separated Citric Acid from the fermentation broth by adjusting the pH between 6.1 and 7.5. The clarification was once carried out using a cure activated carbon and mixed with $CaCl_2$, calcium acetate, or $Ca(OH)_2$ to precipitate Citric Acid. Calcium citrate was then separated using filtration under vacuum, washed with hot water, and dried at 90–105 °C. The optimum pH and temperature were 7–7.2 and 80 °C, respectively. This classical multistep, calcium carbonate/H_2SO_4 precipitation process suffers from the use of large amounts of chemicals, which will increase the manufacturing fee and generates a considerable amount of environmentally harmful waste (for 1× 103 kg of Citric Acid production, approximately 30 m^3 CO_2, 40× 103 kg of wastewater, and 2×103 kg of gypsum is generated) (Jinglan et al., 2009). The modern approach of Citric Acid production has many drawbacks as mentioned before, the principal being the formation of derivative salts generating secondary pollution. there is a need to shorten the technique and at the same time enhance Citric Acid recovery with the use of some superior techniques. To eliminate the generation of CO_2 and gypsum as by products, many separation techniques are used, i.e., solvent extraction (Pazouki and Panda, 1998), and supercritical CO_2 extraction (S. Mourya and K.S. Jauhri, 2000).

8.2 Solvent Extraction

Although the classical precipitation technique is the most used in large-scale industrial processes, solvent extraction has additionally been developed for the separation of Citric Acid. Amine extraction has been located to be a promising approach to the separation of carboxylic or hydroxycarboxylic acid from an aqueous solution. Citric Acid is readily extracted into several natural solvents, such as high-molecular-weight aliphatic amines (Pazouki and Panda, 1998). Baniel and Gonen (1990) attained 90% healing of Citric Acid with the solvent extraction method. Wenneresten (1980)studied the opportunity of using solvent extraction technique for the recuperation of citric acid from an aqueous solution. Wenneresten (1980, 1983) found that tertiary amines have been positive extractants for Citric Acid. The amine extraction approach takes advantage of the low pH (below the lowest pKa) of the fermentation medium in the place of Citric Acid is present in its protonated form. Due to excessive reactivity in this form, it forms a complex with weakly fundamental tertiary amines. Thiamine extraction has the gain of eating negligible amounts of mineral acids and bases and manufacturing salt by products over the conventional method. It helps relieve product inhibition and additionally

eliminates the need to evaporate massive quantities of water. The eco-friendly solvent extraction method with tertiary amines can be further refined for the technically feasible extraction of citric acid at a pilot scale. Resins have been used as adsorbents, and the eluent is either a water acetone mixture or dilutes H_2SO_4. (Jinglan *et al*., (2009) have proposed a modern Citric Acid refining process primarily based on Simulated Moving Bed technology. This specific resin has a high selectivity to Citric Acid, while the different factors (impurities) present in the fermentation broth are frequently retained. Moreover, the bonding strength between Citric Acid and the resin is no longer as strong as that found in the existing commercial resins (Pazouki and Panda, 1998).

9. Future perspective

Significant interest is an increase in citric acid productivity at a lower cost. There is a need for new technology and innovations not only for bioreactor designs that can overcome scaling issues in fermentation processes but also for online monitoring and control of several parameters. To reduce costs and environmental problems, less expensive substrates such as agro-industry by products and residues can be used for citric acid production. High citric acid yielding strains must be explored through strain improvement, which can be accomplished using a variety of high throughput technologies. The recovery and purification of end products are major challenges that have pushed researchers to work tirelessly to find solutions. The integrated fermentation and product recovery method, which can be applied to large-scale citric acid production, The above-mentioned innovative strategies can not only lower production and recovery costs, but also protect the environment from harmful chemicals used in conventional citric acid production.

10. Conclusions

Its production now reaches 1.4 million tons per year and continues to increase every year. The main reason for the steady increase is the large number of applications that can be found for citric acid, primarily in the food and pharmaceutical industries. Traditional processes, such as submerged fermentation with the fungus *Aspergillus niger*, dominate world production. However, different production techniques are continually being investigated opening new perspectives for the production of citric acid. In this context, solid state fermentation appears where agro-industrial residues can be used as support substrates for the filamentous fungi *Aspergillus niger*. Significant optimization of all citric acid processes can be seen with genetic improvement of producing strains, which is a powerful tool of the citric acid market today.

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