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New Perspectives for Citric Acid Production and Application

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Summary

There is a great worldwide demand for citric acid consumption due to its low toxicity when compared with other acidulants used mainly in the pharmaceutical and food industries. Other applications of citric acid can be found in detergents and cleaning products, cosmetics and toiletries, and other. Global production has now reached 1.4 million tonnes and there is annual growth of 3.5–4.0 % in demand/consumption of citric acid. As a result of the adverse market conditions, only big producers have survived. Any increase in citric acid productivity would be of potential interest and hence there is an obvious need to consider all possible ways in which this might be achieved. The production by submerged fermentation is still dominating. However, solid-state processes can create new possibilities for producers. Many by-products and residues of the agro-industry can be used in the production of citric acid. A cost reduction in citric acid production can be achieved by using less expensive substrates. The use of agro-industrial residues as support in solid-state fermentation is economically important and minimizes environmental problems. Other perspectives for citric acid production sector are the improvement of citric acid producing strains, which have been carried out by mutagenesis and selection.

Key words: citric acid, submerged fermentation, solid-state fermentation, agro-industrial residues

Introduction

Citric acid is the most important organic acid produced in tonnage by fermentation. Global production of citric acid in 2004 was about 1.4 million tonnes estimated by Business Communications Co. (BCC) in a recent study of fermentation chemical markets. The report showed that China accounts for 35–40 % of worldwide citric acid production. Leading producers of citric acid for North America and Western European markets include ADM, Cargill, Tate & Lyle, DSM and Junbunzlauer. Israel's Gadot Biochemical Industries and China's Anhui BBCA Biochemical are also major suppliers. Intense competition and relatively low prices caused many smaller citric acid manufacturers in North America and Europe to exit the business in the past decade. Big producers then benefited from the economy of scale (*1*). The citric acid market has been under pressure for more than two years and continues to oscillate with prices falling from $2/kg to $0.70–$0.80/kg. Several producers, including ADM and Tate & Lyle, have cut back on production levels, while two years ago Aktiva closed down a plant in the Czech Republic as a result of the adverse market conditions. Chinese suppliers tend to sell their citric acid at lowest price possible in order to bring in hard currency and this has made it extremely hard for European suppliers to compete (*2*).

Citric acid is widely used to impart a pleasant, tart flavour to foods and beverages. It also finds applications

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as a function of additive detergents, pharmaceuticals, cosmetics and toiletries. About 64 % of U.S. citric acid usage in 2004 was for foods and beverages, 22 % for detergents and cleaning products and 10 % for pharmaceutical and nutritional products. About 2 % went into cosmetics and toiletries. Around 2 % were used in different applications. The actual price of citric acid is about $1 to $1.3 per kilo. Due to the numerous applications and low prices of citric acid, consumption is expected to grow strongly, and considering slight price increases until 2009, the market value for citric acid will exceed $2 billion (*3*).

History of Citric Acid

Citric acid fermentation was first observed as a fungal product by Wehmer in 1893 by a culture of *Penicillium glaucum* on sugar medium. After a few years, he isolated two new fungal strains with the ability to accumulate citric acid, which were designated *Citromyces* (*Penicillium*). However, industrial trials did not succeed due to contamination problems and long duration of fermentation. It was the work of Currie which opened up the way for successful industrial production of citric acid. In 1916, he found that numerous strains of *Aspergillus niger* produced significant amounts of citric acid. The most important finding was that *A. niger* grew well at pH values around 2.5–3.5 and high concentrations of sugars favour citric acid production.

The first citric acid fermentations were carried out in surface cultures. In the 1930s, some units were implanted in England, in Soviet Union, and in Germany for the commercial production. In general, citric acid is commercially produced by submerged microbial fermentation of molasses; the fermentation process using *Aspergillus niger* is still the main source of citric acid worldwide. Although methods were well developed to synthesise citric acid using chemical means, better successes were achieved using microbial fermentations, and over the period of time, this technique has become the method of ultimate choice for its commercial production over chemical synthesis (*4*).

Despite that, the introduction of submerged fermentation presented several problems, including the choice of productive strains with low sensitivity to trace elements. It was necessary to consider raw material much more carefully. Several works were dedicated to the optimization of the conditions for the utilization of cheap material like sugar cane molasses, beet molasses, starch and hydrolysate starch (*5*). Various processes for treating and purifying molasses were developed, especially for the removal of trace metals. Moreover, it was found that a small excess of copper ions was beneficial to achieve high yields of citric acid.

There are annual growths of 3.5–4.0 % in demand/consumption of citric acid. In the last years, a considerable interest has been shown in using agricultural products as alternative sources of carbon and their wastes such as maize, apple and grape pomace, pineapple, mandarin orange and brewery wastes, citrus and kiwi fruit peel for citric acid production by *Aspergillus niger.* The industry is seeking newer cheap and economic process technology.

Microbial Production of Citric Acid

Microorganisms

A large number of microorganisms including fungi and bacteria such as *Arthrobacter paraffinens*, *Bacillus licheniformis* and *Corynebacterium* ssp., *Aspergillus niger*, *A. aculeatus*, *A. carbonarius*, *A. awamori*, *A. foetidus*, *A. fonsecaeus*, *A. phoenicis* and *Penicillium janthinellum;* and yeasts such as *Candida tropicalis*, *C. oleophila*, *C. guilliermondii*, *C. citroformans*, *Hansenula anamola* and *Yarrowia lipolytica* have been employed for citric acid production (*6–11*). Most of them, however, are not able to produce commercially acceptable yields due to the fact that citric acid is a metabolite of energy metabolism and its accumulation rises in appreciable amounts only under conditions of drastic imbalances. Among the mentioned strains, the fungus *A. niger* has remained the organism of choice for commercial production because it produces more citric acid per time unit. The problem in the production of citric acid for yeasts is the simultaneous formation of isocitric acid. The main advantages of using *A. niger* are its ease of handling, its ability to ferment a variety of cheap raw materials, and high yields. Industrial strains which produce commercial citric acid are not freely available and only a few can be obtained from international culture collections.

The improvement of citric acid producing strains has been carried out by mutagenesis and selection. The most employed technique has been by inducing mutations in parental strains using mutagens (*9,10,12*). Mutants of *Aspergillus niger* are used for commercial production (*13*). Among mutagens, γ-radiation, UV radiation and chemical mutagens are often used. To obtain hyper-producer strains, UV treatment can frequently be combined with some chemical mutagens. The »single-spore technique« and the »passage method« are the principal methods of selecting strains. The first one has the disadvantage that mineral acid and organic acids (gluconic and oxalic acids) simulate the presence of citric acid (*8–10,14*).

Different methods of fermentation can lead to different yields of citric acid production by the same strain. Thus, a strain which produces good yields in the solid fermentation or liquid surface is not necessarily good producer in the submerged fermentation. In that way, each method and raw material of industrial interest should be tested with known producer strains (*11*).

In any technique used in citric acid production the inoculation of microorganism is done by means of spores which are added into the fermentation medium (*11*). Spores can be inoculated either mixing them with the air, which is introduced in substrate, or in form of a spore suspension. Spores are produced in glass bottles on solid substrates at optimum temperature (*9*). The type of sporulation medium and time of incubation affect spore viability and citric acid production by mycelia grown from *A. niger*. It was mentioned that potato dextrose agar gives high citric acid yields. Viability increases with time of incubation, but higher production of citric acid was achieved in less than 7 days of spore incubation (*15*). The capacity of germination of the spores tends to reduce with the time but in some cases, short periods of up to

7–8 days do not present significant difference in relation to spores collected after 3 days.

Substrates

Several raw materials such as hydrocarbons, starchy materials and molasses, have been employed as substrates for commercial submerged citric acid production (Table 1) (*6,10,12–25*), although citric acid is mostly produced from starch or sucrose based medium using submerged fermentation. Generally, citric acid is produced by fermentation using inexpensive raw material (*5*), including crude natural products, such as hydrolysate starch, sugar cane broth and by-products like sugar cane and beet molasses (*11*).

Molasses is preferably used as the source of sugar for microbial production of citric acid due to its relatively low cost and high sugar content (40–55 %) (*6*). Since it is a by-product of sugar refining, the quality of molasses varies considerably, and not all types are suitable for citric acid production. The molasses composition depends on various factors like the variety of beet and cane, methods of cultivation, conditions of storage and handling (transport, temperature variations), *etc.* Both beet and cane molasses are suitable for citric acid production, however, beet molasses is preferred to sugarcane due to its lower content of trace metals, supplying better production yields than cane molasses, but there are considerable yield variations within each type. In the case of cane molasses, generally it contains some metals (iron, calcium, magnesium, manganese, zinc) which retard citric acid synthesis and it requires some pretreatment for the reduction of them. Palmyra jaggery, a sugar syrup from the palmyra palm is a novel substrate for increasing the yield of citric acid production (*26*). The addition of phytate (an important plant constituent) at the beginning of incubation of beet molasses results in about 3-fold increase in citric acid accumulation (*27*).

A variety of agro-industrial residues and by-products has also been investigated with solid-state fermentation techniques for their potential to be used as substrates for citric acid production such as cassava bagasse, coffee husk, wheat bran, apple pomace, pineapple waste, kiwi fruit peel, grape pomace, citrus waste, *etc.* (Table 2)

Table 1. Raw materials employed in submerged and semi-solid production of citric acid

Raw material	Strain	γ(citric acid) kg/m^3	Yield %
Beet molasses	*A. niger* ATTC 9142	109	–
	Yarrowia lipolytica A101	54	68.7[a]
Black strap molasses	*A. niger* GCM 7	86	–
Brewery wastes	*A. niger* ATTC 9142	19	78.5
Cane molasses	*A. niger* T 55	–	65
	A. niger GCMC-7	113.6	100
Carob pod extract	*A. niger*	86	
Coconut oil	*C. lipolytica* N-5704	–	99.6[b]
Corn starch	*A. niger* IM-155	–	62
Date syrup	*A. niger* ATTC 9142	–	50
Glycerol	*C. lipolytica* N–5704	–	58.8[b]
Hydrolysate starch	*Y. lipolytica* DS-1	–	–
	Y. lipolytica A-101	–	75
	A. niger UE-1	74	49
n-paraffin	*C. lipolytica* N-5704	–	161[b]
Olive oil	*C. lipolytica* N-5704	–	119[b]
Palm oil	*C. lipolytica* N-5704	–	155[b]
Rapeseed oil	*Y. lipolytica* A-101	–	57
	A. niger	–	115[b]
Soybean oil	*Y. lipolytica* A-101	–	63
Soybean oil	*C. lipolytica* N-5704	–	115[b]
Wood hemicellulose	*A. niger* IMI-41874	27	45[a]
	S. lipolytica IFO 1658	9	41
Xylan hydrolisate	*A. niger* YANG No. 2	72	–
Yam bean starch	*A. niger* YW-112	–	74[a]

[a]based on sugar consumed, [b]based on oils and fatty acids

Table 2. Raw materials employed in solid-state production of citric acid

Raw material	Strain	w(citric acid) g/kg	Yield %
Apple pomace	*A. niger* NRRL 2001	766[a]	–
	A. niger NRRL 2270	816[a]	–
	A. niger NRRL 599	771[a]	–
	A. niger NRRL 328	798[a]	–
	A. niger NRRL 567	883[a]	–
	A. niger BC1	124	80
Carob pod	*A. niger* ATCC 9142	264	60
Carrot waste	*A. niger* NRRL 2270	29[a]	36
Cassava bagasse			
Flasks	*A. niger* LPB-21	347[b]	67
Semi-pilot scale	*A. niger* LPB-21	260[b]	–
	A. niger CFTRI 30	234	
Cellulose hydrolysate and sugar cane	*A. niger*	29	44
Coffee husk	*A. niger* CFTRI 30	150[b]	–
Corncob	*A. niger* NRRL 2001	250	50
	A. niger NRRL 2270	603.5	
Deoiled rice bran	*A. niger* CFTRI 30	92	–
Grape pomace	*A. niger* NRRL 2001	413[a]	88
	A. niger NRRL 2270	511[a]	–
	A. niger NRRL 599	498[a]	–
	A. niger NRRL 328	523[a]	–
	A. niger NRRL 567	600[a]	–
Kiwifruit peel	*A. niger* NRRL 567	100[a]	–
Kumara (starch containing)	*A. niger* YANG No. 2	103[b]	–
Molasses (sugarcane bagasse)	*A. niger* DS1		
	Clarified	198	64.5
	Non-clarified molasses	179	62.5
Mussel processing Wastes (polyurethane foams)	*A. niger*	300	–
Okara (soy residue)	*A. niger*	51[a]	53
Orange waste	*A. niger*	46	–
Pineapple waste	*A. niger* ATCC 1015	132[b]	–
	A. niger ACM 4942	194[b]	74
Rice bran	*A. niger* CFTRI 30	127	–
Sucrose (sugar cane bagasse)	*A. niger* CFTRI 30	174[b]	–
Sugarcane-pressmud and wheat bran (4:1)	*A. niger* CFTRI 30	116	
Wheat bran	*A. niger* CFTRI 30	85	–

[a]based on sugar consumed, [b]based on dry matter

(*10,28–42*). It has been an increasing trend towards efficient utilization of and value-addition to these residues, besides being a form of reducing environmental concerns. These residues are very well adapted to solid-state cultures due to their cellulosic and starchy nature. A cost reduction in citric acid production can be achieved by using less expensive substrates, such as industrial waste products mentioned.

Citric acid production techniques

Citric acid production synthesis by fermentation is the most economical and widely used way of obtaining this product. More than 90 % of the citric acid produced in the world is obtained by fermentation, which has its own advantages: operations are simple and stable, the plant is generally less complicated and needs less sophisticated control systems, technical skills required are lower, energy consumption is lower and frequent power failures do not critically affect the functioning of the plant.

Citric acid production by fermentation can be divided in three phases, which include preparation and inoculation of the raw material, fermentation, and recovery of the product. The industrial citric acid production can be carried in three different ways: by submerged fermentation, surface fermentation and solid-state fermentation or »Koji« process (*11,43-48)*. All of these methods require raw material and inoculum preparation. In industrial citric fermentation, the large-scale spore production is made by using appropriate means and conditions such as direct inoculation in the production fermentor. Sometimes it is necessary to remove the remainder mineral of the raw material and add other nutrients such as phosphorous, magnesium and nitrogen for development of the mycelium and a good production of the citric acid.

Several types of fermentors have been used for citric acid production in solid-state fermentation such as Erlenmeyer conical flasks, glass incubators, trays, rotating and horizontal drum bioreactors (Fig. 1), packed-bed column bioreactor, single-layer packed-bed, multi-layer packed-bed, *etc.* (*6,9,10,37,43–45*). Classically, the solid-state process has been carried out in trays, which facilitates aeration. Higher yields (347 g/kg dry cassava bagasse) were obtained in flasks without any aeration, and very little sporulation was observed (*45*). Equivalent yields (309 g/kg of dry cassava bagasse) were obtained in column reactors only with variable aeration. This fact showed great perspective in using solid culture process for citric acid production in simple tray type fermentors. In fact, the scale-up study of production of citric acid showed that in tray bioreactors with 4-cm bed thickness 263 g/kg of dry cassava bagasse were attained (*45*).

Submerged fermentation

The submerged technique is widely used for citric acid production. It is estimated that about 80 % of world production is obtained by submerged fermentation (*10, 14,46*). This fermentation process employed in large scale requires more sophisticated installations and rigorous control. On the other hand, it presents several advantages such as higher productivity and yields, lower labour costs, lower contamination risk and labour consumption.

Submerged fermentation can be carried out in batch, fed batch or continuous systems, although the batch mode is more frequently used. Normally, citric fermentation is concluded in 5 to 12 days, depending on the process conditions.

Surface fermentation

Liquid surface culture is the classic citric production process and was the first industrial manufacture; submerged fermentation was developed only after that (*11,47,48*). Surface fermentation is still used in industries of small and medium scale because it requires less effort in operation, installation and energy cost.

The process is carried out in fermentation chambers where a great number of trays is arranged in shelves. The culture solution is held in shallow trays with capacity of 0.4 to 1.2 m^3 and the fungus develops as a mycelial mat on the surface of the medium. The trays are made of high purity aluminium, special grade steel or polyethylene, however steel trays supply better yields of citric acid (*11,47,48*). The fermentation chambers are provided with an effective air circulation, which passes over the surface in order to control humidity and temperature by evaporative cooling. This air is filtered through

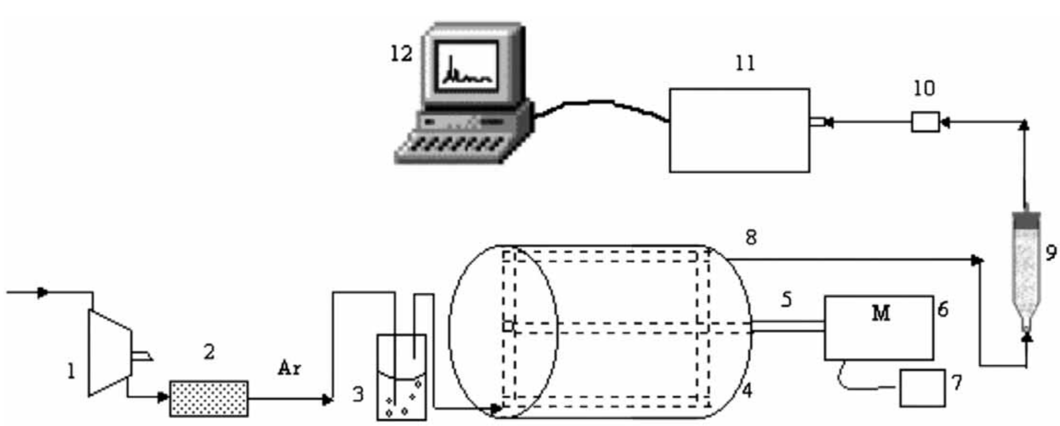

Fig. 1. Outline of the horizontal drum bioreactor and auxiliary equipments:
(1) compressor, (2) air filter, (3) humidifier, (4) horizontal drum bioreactor, (5) axis, (6) motor, (7) speed controller, (8) air discharge, (9) silica gel column, (10) automatic injector, (11) gaseous chromatograph, (12) computer

a bacteriological filter and the chambers should always be in aseptic conditions and must be conserved principally during the first two days when spores germinate. The most common contaminations are mainly caused by penicillia, other aspergilli, yeasts and lactic bacteria.

During fermentation, which is completed in 8 to 12 days (*8,11*), high amount of heat is generated, so high aeration rates are needed in order to control the temperature and to supply air to the microorganism. After fermentation, the tray contents are separated into crude fermentation fluid and mycelial mats which are washed to remove the impregnated citric acid (*11*).

Solid-state fermentation

Solid-state fermentation, also known by »Koji« process, was first developed in Japan where abundant raw materials such as fruit wastes and mainly rice bran are available. It is the simplest method for citric acid production and it has been an alternative method for using agro-industrial residues (*29,30,46,49,50*). Solid-state culture is characterized by the development of microorganisms in a low-water activity environment on an insoluble material that acts both as physical support and source of nutrients (*28*). Some similarities are observed with the surface process since the fungus also develops on material surface. The substrate is solid and it is moistened to about 70 % moisture, depending on the substrate absorption capacity. The initial pH of the material is normally adjusted to 4.5–6.0 and the temperature of incubation is about 28–30 °C, depending on the microorganism used (*10,29,30,49,50*). The solid culture process is completed within 96 hours under optimal conditions (*8*).

The most common organism used in solid-state fermentation is *A. niger*. However, there have also been reports with yeasts. The strains with large requirements of nitrogen and phosphorus are not ideal microorganisms for solid culture due to lower diffusion rate of nutrients and metabolites occurring at lower water activity in solid-state process. The presence of trace elements may not affect citric acid production so harmfully as it does in submerged fermentation, thus, substrate pretreatment is not required. This is one of the important advantages of the solid culture (*50*).

Chemical factors affecting citric acid production

Citric acid accumulation is strongly influenced by the type and concentration of carbon source (*48,51*). The presence of carbohydrates which are rapidly taken up by microorganisms has been found essential for a good production of citric acid (*11*). Among the easily metabolized carbohydrates, sucrose is the most favourable carbon source followed by glucose, fructose and galactose (*10,11,52*). As presented previously, several raw materials can be employed successfully for citric acid production. However, there are some critical factors that should be taken into account such as costs or need of pretreatment for choosing the type of substrate. Molasses has trace elements which must be precipitated by potassium ferrocyanide. Table 3 shows the main factors that affect citric acid production.

Table 3. Chemical factors affecting citric acid production

Factor	Positive effect	Level	Negative effect
Carbon source	Sucrose Glucose Fructose Galactose	14–22 %	Starch Xylose Arabinose Sorbitol Pyruvic acid
Phosphorus source	Potassium dehydrogen phosphate	low (0.5 to 5.0 g/L)	
Nitrogen source	Ammonium nitrate Ammonium sulfate Peptone Malt extract Urea	under 25 % 0.1 to 0.4 gN/L	High concentrations (biomass production)
Trace elements	Zinc Copper Magnesium sulfate	low levels (0.02–0.025 %)	Manganese (1 ppm)
Lower alcohols	Methanol Ethanol *n*-propanol Iso-propanol Methylacetate	1–4 % (volume per mass)	
Oils and fats		0.05–0.3 %	
Other compounds	Calcium fluoride Sodium fluoride Potassium 3-hydroxy-2-naphtoic acid 4-methyl-umbelliferone Benzoic acid 2-naphtoic acid Iron cyanide EDTA Vermiculite H_2O_2		Potassium ferrocyanide Quaternary ammonium compounds Amine oxides

Phosphorous source

In the first works it was verified that the presence of phosphate in the medium had a great effect on the yield of citric acid. Low levels of phosphate have positive effect on citric acid production. This effect acts at the level of enzyme activity and not at the level of gene expression. On the other hand, the presence of 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 (*6,8,10*).

Nitrogen source

Citric acid production is directly influenced by the concentration and nature of the nitrogen source. Physiologically, ammonium salts are preferred, such as urea, ammonium nitrate and sulphate, peptone, malt extract, *etc*. (*6,10*). Acid ammonium compounds are preferred because their consumption leads to pH decrease, which is essential for the citric fermentation. However, it is necessary to maintain pH values in the first day of fermentation prior to a certain quantity biomass production. The concentration of nitrogen source required for citric acid fermentation is 0.1 to 0.4 g/L (*8,11*). High nitrogen concentrations increase fungal growth and sugar consumption but decrease the amount of citric acid produced (*10*).

Trace elements

Trace metal ions have a significant impact on citric acid accumulation by *A. niger* (*53*). Divalent metal ions such as zinc, manganese, iron, copper and magnesium have been found to affect citric acid production. It is crucial to take into account the interdependence of medium constituents. There is elevated production of citric acid only if a rigorous control of the trace elements availability is accomplished, mainly in the submerged process.

Lower alcohols

Lower alcohols added in pure material inhibit citric acid production but if added into crude carbohydrates these alcohols enhance the production. Methanol, ethanol, *n*-propanol, isopropanol or methylacetate neutralize the negative effect of the metals in citric acid production generally in amounts about 1 to 5 % (*8*). Even so, optimal amount of methanol and ethanol depends upon the strain and the composition of the medium. Alcohols have been shown to act principally on membrane permeability in microorganisms by affecting phospholipid composition. Other studies showed that alcohols stimulate citric acid production by affecting growth and sporulation on space organization of the membrane or changes in lipid composition of the cell wall (*54*).

Other compounds

Oils and fats are used in citric acid production to control the foam formation. In addition, some oils stimulate productivity and maintenance of fermentation processes (*8,11*). This lipids act as carbon sources and they are consumed during the fermentation, which is necessary to maintain its level above 0.05–0.3 % (*6,8,11*).

Physical factors affecting citric acid production

pH

The pH of a culture may 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 decrease. Changes in pH kinetics also depend highly on the microorganism. With *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp., pH can drop very quickly to less than 3.0. For other groups of fungi such as *Trichoderma*, *Sporotrichum*, *Pleurotus* sp., pH is more stable between 4.0 and 5.0. The nature of the substrate and production technique also influence pH kinetics (*11*). In this way initial pH must be very well defined and optimized depending on the microorganism, substrate and production technique.

Aeration

Since citric acid production is an aerobic process, oxygen supply has a determinant effect on its production. Increased aeration rates lead to enhanced yields and reduced process time. An interruption of aeration during batch fermentation is quite harmful (*11*). Dissolved oxygen concentration influences the citric acid formation directly. It is important to maintain the oxygen concentration above 25 % saturation (*10*). Critical dissolved oxygen tension is 9–12 % of air saturation for growth phase and 12–13 % of air saturation for the production phase (*6,11*). The high demand of oxygen is reached by constructing appropriate aeration devices, which is also dependent on the viscosity of the fermentation broth. This is an additional reason why small compact pellets are the preferred mycelial forms of *A. niger* during the production. When the organism turns into development of filaments, the dissolved oxygen tension rapidly falls to less than 50 % of its previous value, even if the dry mass has not increased by more than 5 %.

Aeration should be performed through the medium during the whole process with the same intensity, even though, due to economic reasons, it is usually preferred to start with low aeration rates. The incorporation of the oxygen together with air in submerged process results in increment of citric acid production, but it is economically unviable (*11*). However, it is possible to circulate the oxygen in the fermentor since the carbonic gas is removed from the process. High aeration rates lead to high amounts of foam, especially during the growth phase, so the addition of antifoaming agents and the construction of mechanical »defoamers« are required to tackle this problem (*10*).

It has been reported that forced aeration at the beginning of the process in solid-state fermentation packed-bed reactor affects metabolic rates, and thus, citric acid productivity. The study of citric acid production by *A. niger* in SSF revealed the importance of a CO_2 rich atmosphere. Vandenberghe (*30*) showed that an environment with high concentrations of CO_2 has a positive effect on citric acid synthesis. The high partial pressure of CO_2 probably retards spore liberation of the filamentous fungi and favours citric acid synthesis with cassava bagasse as support/substrate. In fact, low oxygen environment is directly involved in the growth limitation, which is crucial for citric acid production. Low aeration rates

(0.18 m^3/kg dry CB/h) are supposed to limit the respiration activity of *A. niger* and, consequently, to turn the metabolism to citric acid synthesis and not to biomass production. It was also observed that strongly aerated cultures (0.3 m^3/kg dry CB/h) increased sporulation. As the sporulation increased, the accumulation of citric acid decreased (*30*,*45*).

Product Recovery

The recovery of citric acid from fermented broth is generally performed through three procedures: precipitation, extraction and adsorption (mainly using ion exchange resins). The first method is the most frequently used and it is applicable in all types of processes. The second one requires a fermented broth with little impurities. In both of the methods there is the need to remove the fermented broth, micelles of the fungus, and materials in suspension by filtration (*11*).

Precipitation method is the classical method and it is performed by the addition of calcium oxide hydrate (milk of lime). The acid is transformed into tri-calcium citrate tetrahydrate, which is lightly soluble. The precipitate is recovered by filtration, treated with sulphuric acid forming calcium sulphate (gypsum), which is filtered off. Mother liquor of citric acid solution is treated with active carbon and passed through cation and anion exchangers. Finally, the liquor is concentrated in vacuum crystallizers at 20–25 °C, forming citric acid monohydrate (*6*,*9–11*). Anhydrous citric acid is obtained at crystallization temperature higher than 36.5 °C. The crystals are separated by centrifugation and the dry stage is conducted at a temperature bellow 36.5 °C for monohydrate product and above this for anhydrous product (*8*,*11*). Generally, a bed flowing dryer is used. Two kinds of wastes are generated through precipitation technique: the microorganism residue contains proteins, amino acids, inorganic matter, sugar, colloid, pigment, biotin, *etc.*, and the other is calcium sulphate. The first one can be dried and used as forage or supplied to forage factory and the second can be supplied to cement factories (*11*).

The solvent extraction is another alternative to purification and crystallization of citric acid. The mother liquor contains small amount of impurities captured by the solvents. This method has the advantage of avoiding the use of calcium hydroxide and sulphuric acid, which are employed in great amounts, and the production of gypsum. In this case a mixture of *n*-octyl alcohol, tridodecylamine and isoalkane is used. Other solvents such as acetone, methanol and ethanol were tested in order to extract citric acid from solid particles in solid-state processes. Better results using extraction technique at normal temperature (20–25 °C) were achieved with acetone, followed by water, ethanol and methanol. Liquid-liquid extraction of citric acid has been found to be a promising alternative to the conventional process. Suitable extractants as phosphorous-based oxygen-containing and amine-based extractants, with functional groups effective for reversible complexation with acids, should be used (*13*).

The main problems in citric acid production are still the separation and purification steps. Several stages of filtration, precipitation, crystallization and drying make the process expensive and complicated. In recent years some methods were developed to decrease the cost of the recovery, trying to overcome the drawback of precipitation scheme, which is responsible for the formation and disposal of enormous amounts of calcium sulphate, leading to pollution problems.

Electrodialysis is an electrochemical separation process in which electrically charged membranes and electrical potential difference are used to separate ionic species from aqueous solutions. This technique was tested in citric acid recovery and shows that the separation of ionic species from clarified fermentation broths is more economical (*6*). The problem is that electrodialysis technique costs were found to be about 50 % greater than current industrial-scale citric acid recovery process. The great specific electromembrane and electric energy costs appear to limit the electrodialysis applications only to high value-added products. The use of electrodialysis would need the development of new integrated fermentation processes to minimize waste formation and enhance productivity (*55*).

Applications of Citric Acid

Citric acid is a versatile and innocuous alimentary additive. It is accepted worldwide as GRAS (generally recognized as safe), approved by the Joint FAO/WHO Expert Committee on Food Additives (*9*,*10*,*48*). The food and pharmaceutical industries utilize citric acid extensively because of its general recognition of safety, pleasant acid taste, high water solubility and chelating and buffering properties.

Citric acid is used in cosmetics and toiletries as buffer, and in a wide variety of industrial applications as a buffering and chelating agent. Citric acid is also a reactive intermediate in chemical synthesis. In addition, its carboxyl and hydroxyl groups permit the formation of a variety of complex molecules and reactive products of commercial interest. Table 4 presents the main applications of citric acid (*6*,*9*,*10*,*48*).

Table 4. Citric acid applications

Applications	Industry	Functions
Beverages	Wines and ciders	Prevents browning in some white wines. Prevents turbidity of wines and ciders. Used in pH adjustment.
	Soft drinks and syrups	Provides tartness. Stimulates natural fruit flavour. As acidulant in carbonated and sucrose based beverages.
Food	Jellies, jams and preservatives	Used in pH adjustment. Acts as acidulant. Provides the desired degree of tartness, tang and flavour. Increases the effectiveness of antimicrobial preservatives.
	Dairy products	As emulsifier in ice creams and processed cheese. Acidifying agent and antioxidant in many cheese products.
	Candies	Acts as acidulant. Provides tartness. Minimizes sucrose inversion. Produces dark colour in hard candies. Prevents crystallization of sucrose.

Table 4. – continued

Applications	Industry	Functions
	Frozen fruit	Protects ascorbic acid by inactivating trace metals. Lowers pH to inactivate oxidative enzymes.
	Fats and oils	Synergist for other antioxidants, as sequestrant. Stabilizing action.
	Animal feed	Feed complementation
Agriculture		Micronutrient evaluation in fertilizers. Enhances P availability in plants.
Pharmaceutics	Pharma-ceuticals	As effervescent in powders and tablets in combination with bicarbonates. Anticoagulant. Provides rapid dissolution of active ingredients. Acidulant in mildly astringent formulation.
	Cosmetics and toiletries	Buffering agent. pH adjustment. Antioxidant as a metallic–ion chelator.
Other	Industrial applications	Acts as buffer agent. Sequestring metal ions. Neutralizes bases. Used in nontoxic, noncorrosive and biodegradable processes that meet current ecological and safety standards.
	Metal cleaning	Removes metal oxides from the surface of ferrous and nonferrous metals, for operational cleaning of iron and copper oxides.
		In electroplating, copper plating, metal cleaning, leather tanning, printing inks, bottle washing compounds, floor cement, textiles, photographic reagents, concrete, plaster, refractories and moulds, adhesives, paper, polymers, tobacco, waste treatment, chemical conditioner on teeth surface, ion complexation in ceramic manufacture.

Conclusions

Citric acid is the most produced organic acid measured in tonnage. Its production has now reached 1.4 million tonnes per year and continues to increase more each year. The main reason for constant increase is the large number of applications that can be found for citric acid, mainly in the food and pharmaceutical industries. Traditional processes, such as the submerged fermentation using the fungus *Aspergillus niger*, dominate the global production. However, different techniques of production are continuously being studied showing new perspectives for the production of citric acid. In this context, solid-state fermentation appears where agro-industrial residues can be used as substrate-supports to the filamentous fungi *Aspergillus niger*. Significant optimization of all citric acid processes can be observed with genetic amelioration of producer strains, which nowadays is the powerful tool of the citric acid market.

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