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PRODUCTION OF CITRIC ACID – A SHORT REVIEW

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ABSTRACT

Citric acid is widely used as a flavouring agent in a long list of preparations. It is one of the few organic acids found in limes and lemons in particularly high concentrations. It can be produced by biotechnological fermentation with the help of fungal species like *Aspergillus niger*. There have been various attempts to produce citric acid efficiently from inexpensive raw materials. This review is an attempt to describe the various organisms and substrates utilized for the production of citric acid over a period of past 10 years.

INTRODUCTION

Citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid) derives its name from the Latin word citrus, a tree whose fruit is like the lemon. Citric acid is a tricarboxylic acid (Fig.1) with molecular weight of 210.14g/mol, which contains three carboxylic functional groups with three different values of p ka (3.1, 4.7 and 6.4). It is a primary metabolic product formed in a tricarboxylic acid (or Krebs) cycle and is found in virtually all plants and animals, being isolated from lemon juice in 1784 (Belen Max, *et al*. 2010). Citric acid was first commercially produced in England around 1826 from imported Italian lemons. Lemon juice remained the commercial source of citric acid until 1919, when the first industrial process started in Belgium. It was synthesized from glycerol by Grimoux and Adams (Grimoux and Adams, 1880) and later from symmetrical dicloroacetone. Wehmer (1893) was the first to demonstrate that citromyces (now penicillum) accumulated citric acid in a medium containing sugar and inorganic salts. Since many organisms have been found to accumulate citric acid: A. niger, A. awamori, A. nidulans, A. fonsecaeus, A. luchensis, A. phoenicus, A. wentii, A.saitoi, A. flavus, Arcemonium species, Botrytis species, Eupenicillium species, Talaromyces species, Trichoderma viride and Ustulina vulgaris (Papagianni, 2007). Curie (1917) found that some strains of A. niger were able to grow in a medium containing sugars and salts at an initial pH of 2.5-3.5. Throughout their growth, these strains excreted large amounts of citric acid, which established the basis of industrial production.

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Biosynthesis of Citric Acid

Sir Hans Krebs postulated the cycle of reactions which describe the oxidation of pyruvate to co_2 and came to be known as citric acid cycle. In 1953, Krebs was awarded Noble prize and the cycle is frequently refered to as Krebs cycle in his honour (David L.Nelson and Michael M.cox, 2004). The first formed substance in this cycle is citric acid, hence it is also known as citric acid cycle. Reaction sequence of krebs cycle is shown in the Fig.2. It can be said that over production of citric acid requires a unique combination of unusual nutritional conditions (excess of carbon source, hydrogen ions and dissolved oxygen and sub-optimal concentrations of certain trace elements- metals and phosphate), which influence the fermentation performance (Kristiansen and Sinclair, 1978). According to Habison *et al*. (1983) and Rohr and Kubicek (1981), a deficiency of manganese or phosphate and nitrogen limitation, inhibits the A. niger anabolism and the resulting degradation of proteins leads to increased ammonium ion concentrations.

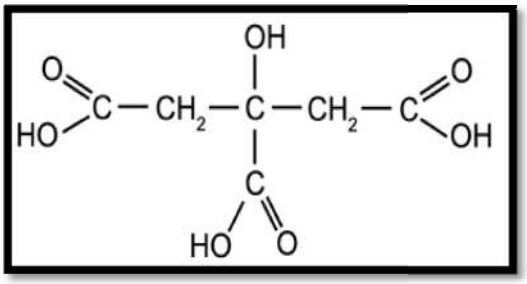

Fig.1. Chemical structure of citric acid

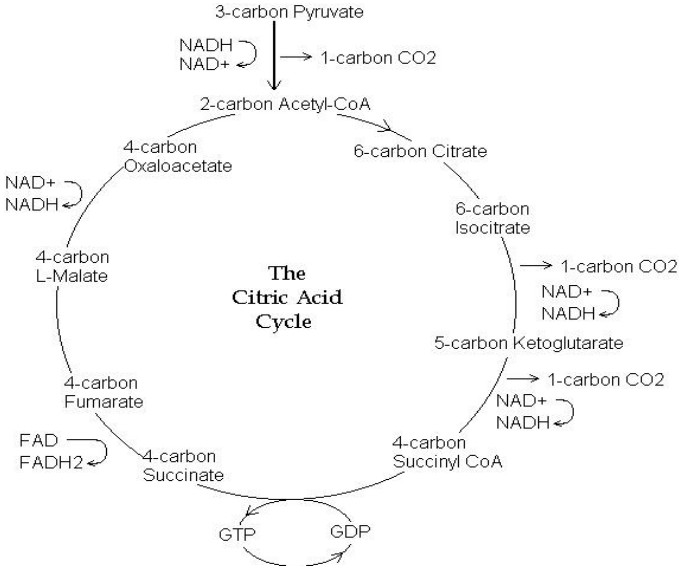

Fig.2. Biochemical reactions of Krebs cycle

This increase is able to counter balance the inhibition exerted by citric acid on phosphate-fructokinase (Habison *et al*., 1983), being a positive end-effector (Arts *et al*., 1987 and Habison *et al*., 1979). High concentrations of NH_4+ and glucose also repress the synthesis of α-ketoglutarate dehydrogenase, inhibiting the citric acid catabolism via the Krebs cycle, leading to its accumulation (Röhr and Kubicek, 1981). Citric acid is the result of high speed flow of income and reduction in outflow velocity (Papagianni *et al*., 2005). Other smaller issues are of interest to other enzymes: invertase (Boddy *et al*., 1993 and Rubio and Maldonado, 1995), hexokinase (SchreferlKunar *et al*., 1989 and Steinböck *et al*., 1994), glucose oxidase (Dronawat *et al*., 1995; Hayashi and Nakamura, 1981; Mischak *et al*., 1985 and Rogalski *et al*., 1988), phosphofructokinase (Habison *et al*., 1983; Jernejc *et al*., 1992; KubicekPranz *et al*., 1990 and Legiša and Benèina, 1994) and other enzymes of pentose phosphate pathway (Legiša and Mattey, 1986), pyruvate kinase (De Graaff *et al*., 1988; De Graaff *et al*., 1992; MeixnerMonori *et al*., 1984; Ruijter *et al*., 1996; Torres, 1994 and Torres, 1994) and citrate synthase (Kubicek and Röhr, 1980 and Lowenstein, 1969)

Citric Acid producing Micro organisms

The micro organisms selected for biotechnological production of citric acid are listed in the Table 1 (36-41, Ikram-Ul-Haq, *et al*., 2002, Nwoba Emeka G., *et al*., 2012, Savas Anastassiadis, *et al*., 2006, Ashish Kumar and Jain, V.K., 2008, Murad A. El-Holi and Khalaf S.Al-Delaimy, 2003, Aftab Nadeem, *et al*., 2010). Few micro organisms such as Bacillus licheniformis, B.subtilis, Corynebacterium spp., A. niger, A. awamori, A. foetidus, Penicillum restrictum, Candida lipolytica, C. intermedia and Sacchromyces cervisiae have been used for the biotechnological production of citric acid. However, A. niger a filamentous fungus remained the best choice for citric acid production due to ease of handling, its ability to ferment a variety of cheap raw materials and high yields (Raja Rao,p. and Kruthi Reddy M., 2013).

Raw Materials for the Biotechnological Production of Citric Acid

For the production of citric acid to be feasible, cheap raw materials are necessary. Raw materials for the production of citric acid should have the following characteristics: cheap, low levels of contaminants, rapid production rate, high yield, little or no byproduct formation, ability to be fermented with little or no pretreatment and year-round availability. The different raw materials and the parameters which are used for the production of citric acid are listed in Table 2. A comparative study was carried out between the various raw materials that are used for the production of citric acid from the years 2002-2014. It was found that Glucose produced maximum amount of citric acid (167g/l) by using the micro organism Candida oleophila ATCC20177 (Savas Anastassiadis, *et al*., 2006), Followed by the Ferro cyanide treated molasses which produced 106.65g/l of citric acid using the Hyper mutant strain of *A. niger* GCMC-7 (Ikram-Ul-Haq, *et al*., 2002), then comes Whey with sugars and additives which produced 106.5 g/lof citric acid by using the micro organism *A. niger* ATCC9642 (Murad A.El-Holi and Khalaf S.Al-Delaimy, 2003).

Citric acid can also be produced by using Artabotrys Adoratissimus (tail grape) as the raw material, In this case, it was found that the reaction involved for the conversion is pseudo first order reaction (Anuradha,k., *et al*., 2013). According to Svetlana V. Kamzolova1, Alina R. Fatykhova1 *et al*., Emiliya G. Dedyukhina1 *et al*., Savas G. Anastassiadis *et al*., Nikolay P. Golovchenko1 and Igor G. Morgunov1*et al*., few yeast species such as Yarrowia lipolytica N15 produced 98 g/l by using waste from biodiesel industries as the raw material by maintaining certain parameters such as the temperature, pH, aeration and agitation (Kamzolova *et al*., 2011). The minimum amount of citric acid was produced from corncobs. The micro organism used was *A. niger* ATCC10549 and it produced 4.8 g/1 kg cry corncobs (Ahmed Ashour, *et al*., 2014).

Fermentation Process

There are various fermentation processes used in the manufacture of citric acid, surface culture process, sub merged culture process using *A. niger* and sub merged culture process using a yeast (Arvind H. Patel, 2012). Batch, fed-batch, repeated batch and continuous fermentation are most frequently used. The advantage of continuous culture compared to batch culture is the possibility to continue the process for a longer period of time.

Table 1. Micro organisms used for biotechnological production of citric acid

S.no	Name of the organism	Yield	Reference	Year
1.	Hyper mutant strain of A. niger GCMC-7	106.65 g/l	(36)	2002
2.	A. niger ATCC9642	106.5 g/l	(40)	2003
3.	Candida oleophila ATCC20177	167 g/l	(38)	2006
4.	A. niger DS1	0.087/100 gDS.h	(39)	2008
5.	A. niger M-101	34.2% - ethanol,	(41)	2010
6.		47.36%-methanol		
	Yarriwia lipolytica N15	98 g/l – maximum 71 g/l - minimum	(42)	2011
7.	A. niger EGN006	23.261±1.447 g/l	(37)	2012
8.	A. niger cultivated on DPJ	3.17 g/100 ml	(43)	2011
9.	A. niger ATCC9142	2.7 g/dm3	(44)	2012
10.	A. niger ATCC9142	Maximum with respect to temperature	(45)	2013
11.	A. niger spp.	36% - maximum, 6% - minimum	(46)	2013
12.	A. niger	Maximum with respect to temperature and other parameters	(49)	2013
13.	A. niger ATCC10549, A. flavipes ATCC11013, A. alliancens UI315	4.8 g/1 kg dry corncobs	(47)	2014
14.	A. niger MTCC281	16.47+-0.73 g/l	(48)	2014

Table 2. Different raw materials and the parameters used for the production of citric acid

S.no	Raw materials	Parameters	Reference	Year
1.	Ferro cyanide treated molasses	Concentration of sugar-150 g/l Temperature-30°C pH-6.0 Aeration required Agitation-200 rpm Time-6 days	(36)	2002
2.	Whey with sugars and additives	Concentration – whey with 15% (w/v) sucrose with or without 1% methanol Temperature-105°C pH-3.0 (initial) Time-20 days Aeration and agitation required.	(40)	2003
3.	Glucose	Concentration of glucose-33.3 g/l Temperature-30°C Time-4.85 days Aeration required Agitaion-200 rpm	(38)	2006
4.	Treated sugarcane bagasse supplemented with sucrose	Concentration of sucrose-310 g/l Temperature-30°C pH-4.0 Varied aeration rates of 0.25, 0.75, 1.25 and 1.75 l/min	(39)	2008
5.	Lower alcohols (ethanol-10%, methanol-15%)	Ethanol- 1% Methanol-1.5% sugar added- 150 g/l Temperature - 30°C , pH- 4.0 ±0.2 Time – 8 days, shaking period – 200 rpm	(41)	2010
6.	Waste from biodiesel industries(pure glycerol and waste glycerol)	Temperature- 28±0.5 °C pH- 4.5±0.1 20% NAOH Aeration required Concentration- 60% Agitation- 800 rpm Concentration of starch- 60 g/l	(42)	2011
7.	Cassava starch	Temperature - 30°C pH- 5.5 Time- 6 days Aeration and agitation required.	(37)	2012
8.	Deprotienised leaf juice (DPJ) Lucerne	2% w/v solution of DPJ, 25 ml of 2% DPJ + 0.75g glucose. Temperature-30°C to 32°C Time- 7 days	(43)	2011
9.	Corn starch hydrolysate	pH - 5.5, Temperature: 25-30°C Initial sugar concentration- 40 w/v, time- 4 days.	(44)	2012
10.	Sugar cane bagasse	pH- 4-4.5, Temperature: 25- 30°C Time- 4 – 5 days, Initial sugar- 14-18%.	(45)	2013
11.	Oat bran	Concentration-0.25(1% w/v) NH4NO3 Temperature: 28°C pH- 4.5 Time- 6 days. Temperature - 37°C	(46)	2013
12.	Artabotrys Adoratissimus (tail grape)	Time – 5 days Aeration and agitation required	(49)	2013
13.	Corncobs	Concentration- 30 w/w corncobs Temperature- 30°C pH- 5 or 5.5 to 7.4 Time- 10 days.	(47)	2014
14.	Jackfruit and its waste	Concentration- 15%-20% Temperature: 30°C Agitation- 160-200 rpm Time- 6 days. (144 hours)	(48)	2014

Recovery

There are three different separation methods that can be employed for recovering citric acid from fermentation broth. These are precipitation, ion exchange and solvent extraction. Precipitation is the most commonly used method. It is more economical to remove citric acid as calcium citrate by lime precipitation than by ion-exchange treatment. The ion exchange method may be used for treating lime juice rather than filtered acid juice. Solvent extraction is possible alternative to the classical method but because the available solvents tend to extract some of the impurities too, it is easier to apply to products from glucose or alkane base substrates. The advantage of this process is that it avoids the use of lime and sulfuric acid and the concomitant problem of gypsum (calcium sulphate) disposal. Gypsum is produced as a waste product during the purification process of citric acid and its disposal is a completed issue.

Industrial uses and Applications of Citric Acid

Citric acid is used as a flavoring agent in a long list of preparations. It is one of the few organic acids found in limes and lemons in particularly high concentrations. It's anhydrous states is produced in powders, appears to be white in color and has a strongly acidic taste. When it comes to industrial applications, citric acid is used in variety of preparations. The largest industrial application of citric acid is for making detergents. In liquid detergents, sodium citrate is used as a builder, to increase the effectiveness of the surfactants, due to its high solubility and biodegradability. While in the form of powder detergents, sodium citrate is used as a co-builder and processing acid. Sodium citrate also contributes alkalinity to enhance surfactant performance. The pharmaceutical industries uses citric acid again mainly as a flavoring and stabilizing agent in multiple pharmaceutical preparations. The largest use of citric acid in the pharmaceutical industry is for the effervescent effect it produces, when combined with bicarbonates or carbonates in antacids and dentrifices (http://www.slideshare.net/ShangThomas/gte-dec2011-ar2the-uses-of-citric-acid-in-the-industry).

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