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An overview of citric acid production

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ABSTRACT

This article reviews the present state of research on the conversion of low cost substrates to citric acid by fermentation. Fermentation is a powerful incentive for semi-industrialized countries. There is a great demand for citric acid due to its wide industrial applications and less toxicity. Citric acid can be produced using less expensive substrates that are renewable too. Plant biomass is one of the desirable raw materials for fermentation due to its availability in abundance. Using natural sources as substrates we can minimize environmental problems. Always species of *Aspergillus* and *Candida* remain the choice of candidates for the biosynthesis of citric acid. A concise gist of the various natural sources that can be used for the production of citric acid along with the necessary fermentation conditions is presented.

1. Introduction

Biotechnology is an interdisciplinary field which is largely oriented towards the industrial applications of microorganisms for the conversion of waste to useful products. The number of substances excreted by microorganisms is endless. They are classified as simple compounds (eg. lower alcohols, acids etc.) and complex compounds (eg. natural products and cellulosics) or as preliminary products and compounds evolving from secondary metabolism. Several fermentations procedures are used for the large scale production of organic chemicals and high energy fuels from renewable sources. Further, the exploitation of microorganisms for the benefit of mankind was found to be promising and productive. Mixed culture technology as applied to processes, which relies upon microorganisms for proper functioning, is in its infancy. Much is yet to be explored in terms of sequential transformations of substrates acted upon by a mixed culture population of microorganisms. The environmental impetus to move away from traditional chemical techniques towards biologically based production has revealed *Aspergilli and Candida* as very attractive cell factories. So an updated review of citric acid production by *Aspergillus* and *Candida* including aspects of economy of available raw materials as substrates and their utility is considered very relevant.

2. Chemistry of citric acid

Citric acid, a tricarboxylic acid ($C_6H_8O_7 \cdot H_2O$) is a common metabolite of plants and animals and is present in juice of citrus fruits and pineapple. Pure citric acid is colourless, readily soluble in water with a molecular weight of 210.14 g/mol. It is biodegradable, ecofriently, economical, safe and a versatile chemical for sequestering, buffering, wetting, cleaning and dispersing. The acid is mainly used in the preparation of medicinal citrates, confectionary, soft drinks and effervescent salts. Small quantities are employed in silvering and engraving and in dying and calico printing.

3. Fermentation

Microbiological conversion of organics to cellular material and other useful products is fermentation. Despite being an old technology advances in science have kept this process in the forefront.

The fermentation process is advantageous as it is based on renewable sources, it facilitates use of waste for productive purpose, and useful by-products are formed. It involves very mild environment friendly conditions and also consumes less energy. It also faces some drawbacks some of which are:

(i) Use of large quantities of water
(ii) Due to high BOD the waste requires treatment before disposal.
(iii) Infection by foreign microbes can reduce the yield and the technology is sophisticated.

Fermentation is a complex process because several reactions are integrated into one single bio-reaction for which operating conditions have to be precisely tailored and controlled carefully. Variations in results often occur even under apparently similar operating conditions. All the commercial citric acid producers prefer to rely upon their own experience rather than the so called proven facts.

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3.1. Types of fermentation

The development of the processes for citric acid fermentation can be divided into three phases. In the first phase citric acid production was confined to species of *Penicilium* and *Aspergillus* using stationary or surface culture conditions. The beginning of the second phase consisted of the development of submerged fermentation processes for citric acid production using *Aspergillus*. The third stage, which is of recent origin, involves the development of solid state culture, continuous culture and multistage fermentation techniques for citric acid production.

3.2. Biochemistry of citric acid

The products of carbohydrate metabolism form the building blocks of many aerobic and anaerobic microorganisms. The main intermediate of carbohydrate metabolism is citric acid. In certain microorganisms such as *Aspergillus niger*, under specific environmental conditions, citric acid is produced as an over flow product due to the faulty operation of the tricarboxylic acid cycle (TCA). TCA is an intermediate cycle involving the terminal steps in the conversion of carbohydrates, proteins and fats to carbon dioxide and water with concomitant release of energy for the growth, movement, luminescence etc. Studies on the enzyme content of *A. niger* in relation to citric acid accumulation have indicated the vital role played by the TCA cycle in fermentation. Biological formation of citric acid is purely enzymatic. The merit of citric acid fermentation depends on the regulation of the synthesis of each one of the enzymes (involved in the TCA cycle) and its activity is under various control mechanisms such as the cofactors associated with the enzymes. Metal ions form a part of cofactors and in turn controlling trace element concentration can regulate the enzyme activity. Under suitable environmental conditions different species of *Candida* can also produce citric acid.

3.3. Microbial strains

Starting from fungi to yeast, a few bacteria are also suitable for citric acid production. The fermentation capabilities of the selected strains are sometimes improved using mutagens (Pandey, Soccol, Rodriguez-Leon, & Nigem, 2001: pp. 113–126; Soccol, Vandenberghe, Rodrigues, & Pandey, 2006; Vendenberghe et al., 1999). Mutation is a process by which the characteristics of a strain are improved physiologically and morphologically which results in improving the process kinetics. Under such conditions membrane permeability of the microbe is also altered. Studies on branch formation in filamentous fungi have shown that mutations with various chemicals cause alterations in the cell wall composition and changes in physical conditions which cause a marked effect on the frequency of branching.

A. niger is a common black mold which can act as a citric acid producing cell factory (Haq, Khurshid, Ashraf, Qadeer & Rajoka, 2001). Other species of *Aspergillus* namely, *Aspergillus aculeatus, Aspergillus awamori, Aspergillus carbonaries, Aspergillus wenti, Aspergillus foetidus* (Papagianni, 2007) were also found to produce citric acid in appreciable amounts. Among the yeast *Saccaromicopsis lipolytica* (Rane & Sims, 1993; Wojtatowics, Marchin & Ericksen, 1993), *Candida tropicalis (*Kapelli, Muller, & Fiechter, 1978*), Candida oleophils, Candida guilliermondi* (Angumeenal, Kamalakannan, Prabhu & Venkappayya, 2003a, 2003b), *Candida parapsilosis* (Omar & Rehm, 1980), *C. citroformans* (Uchio, Maeyashiki, Kikuchi, & Hirose, 1975) were good citric acid producing candidates. Bacterial species were also used earlier for citric acid fermentation. However, among all *A.niger* remains the best choice for citric acid production due to its ease of handling, its ability to ferment a variety of raw materials and yield high yield (Vandenberghe, Soccol, Pandey, & Lebeault, 1999). The production of citric acid also depends on an appropriate strain, along with aeration, carbon source, nitrogen and phosphate, pH and trace elements. The morphology of the producer microorganism also plays a role in controlling and augmenting citric acid formation (Belen Max, Salgado, Rodriguez & Dominguez, 2010).

An undisturbed metabolic flow of carbohydrates through glycolysis is required to get a high citric acid accumulation by *A. niger*. Initial intracellular pH in spores should be as high as possible if a high rate of metabolic flow through hydrolysis is desired for high yields of citric acid. But the pH of the culture may change depending upon microbial metabolic activities. Nature of substrate and production technique has influence on pH. A pH of 2.0 was optimum for molds to produce citric acid (Srivatsava, & De, 1980), whereas a pH in the range of 5–6 is favoured with molasses. When working with yeasts, initial pH values of above 5 are required. It is reported that below pH 5, citric acid production decreases and accumulation of other products like erythritol, arabitol and mannitol is favoured. Transport of citrate across cell membrane is also affected (Anastasisdis & Rehm, 2005; Karasu, Yalcin, Bozdemir & Ozhas, 2010).

The optimum temperature for citric acid formation will differ based on strain and medium composition (Shuler, Kargi, 2002). Hence it is necessary to optimize and determine the appropriate favourable temperature before scaling up the process. However, the optimum temperature reported for yeast lies between 22 and 35 °C. *A.niger* and other fungi require an optimum temperature of 25–30 °C (Corolla & Kennedy, 2001). An increase in temperature beyond 30 °C has been found to increase oxalic acid accumulation irrespective of the fermentation conditions. Therefore, maintenance of favoured temperature is very much essential for effective citric acid biosynthesis.

3.4. Medium composition

Fermentation of a substrate to citric acid is directly related to the quality and quantity of the sugar source. The nature of the substrate which in turn depends on the carbon source will also have a marked influence on the metabolic activity of the microbial strains. Among the synthetic substrates tried so far, sucrose was the best candidate followed by glucose, fructose and galactose (Vandenberghe et al., 1999). To make citric acid production cost-effective nowadays a lot of plant and fruit sources rich in sugar content are used as substrates. They happen to be renewable sources making the fermentation process more economical.

Molasses is the effluent got from sugar industry and is the non crystallisable residue remaining after sucrose isolation. It is reported to be in sugar and hence a good substrate for citric acid fermentation (Adham, 2002; Cevrimli, Kariptas, & Ciftci, 2009; El Aasar, 2006). However, while using such substrates suitable treatment procedures are needed to make them suitable for fermentation.

Some of the natural sources available may contain nitrogen and phosphate and so their supplementation may not be required. But their presence is required at optimum levels. Otherwise citrate formation will be affected. Nitrogen can be supplied through KNO_3 or NH_4NO_3 and phosphate by adding K_2HPO_4 or KH_2PO_4 in limited quantities.

3.5. Metal ions

Fungi and yeast require trace quantities of metal ions (hence called as trace elements) for their growth. Presence of magnesium and zinc are essential for the microbial growth (Angumeenal, Kamlakanna, Prabhu & Venkappayya, 2002). The metal ions are expected to cause a mutational change in the species and also