STUDY MATERIALS

PHARMACEUTICAL CHEMISTRY PART-2

SEC-I, SEMESTER I

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Micro-Organisms Used for Citric Acid Production:

A large number of micro-organisms including bacteria, fungi and yeasts have been employed to produce citric acid. Most of them, however, are not able to produce commercially acceptable yields. This fact could be explained by the fact that citric acid is a metabolite of energy metabolism and its accumulation rises in appreciable amounts only under conditions of drastic imbalances. The strains reported to produce citric acid. Table 2 shows the micro-organisms used to produce citric acid. Among these, only *A. niger* and certain yeasts such as *Saccharomycopsis sp*. are employed for commercial production. However, the fungus *A. niger* has remained the organism of choice for commercial production. The main advantages of using this microorganism are: (a) its ease of handling, (b) its ability to ferment a variety of cheap raw materials, and (c) high yields.

Extraction from Citrus Fruits

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

Chemical Synthesis

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

Fermentation

Fermentation process is generally regarded as a biological method for citric acid production. Citric acid production using *Aspergillus niger* in submerged fermentation using as substrate.

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Literature regression equations used to model the fermentation for determining the optimum fermentation conditions. For fermentation process the medium is taken as 250 ml in flask with 50 ml medium and 1 ml of spore inoculum, incubated in a rotary shaker at 150 rev./min. The Maximum citric acid is reported in the literature. Many researchers working on the fermentation process and finding out optimized parameters. By using optimized parameters such as pH, temperature, sugar concentration, ammonium nitrate concentration and potassium ferrocyanide concentration got the maximum citric acid.

Accumulation of citric acid

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

Acetyl CoA + 3NAD + FAD + ADP + HPO^{-2} 4 → 2CO2 + CoA + 3NADH+ + FADH+ + ATP

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

Microbial production of citric acid by fermentation

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

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alkanes. *Aspergillus niger* has so far maintained its place in citric acid production as it has advantages over other bacterial microorganisms such as *Arthrobacter paraffinens*, *Bacillus licheniformis*, *Bacillus subtilis*, *Brevibacterium flavum*, *Corynebacterium spp*. and *Penicillium janthinellum*. It is easy to handle, can ferment a broad range of low-cost raw materials and provides high yields. Mutagenesis has been used in recent years to improve the citric-acid producing strains so that they can be used in industrial applications. The most common methods include the use of mutagens to induce mutations on the parental strains. The mutagens utilized for improvements are gamma radiation, ultraviolet radiation and often chemical mutagens. Microorganisms for citric production have to be inoculated by spores, which are transferred to the fermentation medium. The various transfer media include air, and can be in the form of a suspension which is then introduced into bottles containing the substrate. Ideally, for high yields, an incubation time of 7 days is required for *A. niger*. However, after the 7 days of incubation, the capacity for germination tends to reduce with time. Substrates A wide range of substrates is utilized in the fermentation process of the microorganisms. Materials such as hydrocarbons, molasses and starchy materials are commonly used. The review by mentions examples such as beet molasses, black strap molasses, cane molasses, carob pod extract, n-paraffin, glycerol, corn starch, hydrolysate starch, yam bean starch, wood hemicellulose, olive oil, rapeseed oil, palm oil and soya bean oil. Owing to the need to use less expensive substrates with the aim of reducing the production costs of citric acid and making it more environmentally sustainable, the noncrystallizable effluents (molasses) after sucrose isolation from sugar refineries may be used. Molasses offers reduced cost and a high sugar content of 40–55% in the form of fructose, glucose and sucrose. The quality of molasses varies according to its source. Therefore, it requires pretreatment [e.g., mixing with $K_4Fe(CN)_6$ at pH 4.5, 90°C for 15 min and then removal of the precipitate by filtration] to make it suitable for fermentation.

Fermentation strategies in citric acid production

Citric acid production by fermentation has become established as the most widely used and economical process to obtain citric acid. Over 90% of the citric acid used around the globe today is produced from fermentation. This method offers advantages such as having simple, stable and less complicated operations; requiring less complex control systems and lower technical skill; consuming less energy; and not being critically affected by frequent plant power failure. In general, all fermentation processes, irrespective of the type of fermentation,

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have three phases: preparation and inoculation, fermentation, and recovery of the citric acid. Over the years, citric acid fermentation has undergone a series of developments. In the 1910s, production was limited to species of Penicillium and Aspergillus utilizing surface or stationary culture conditions. In the 1940s, Shu and Johnson (1948a) developed submerged fermentation from Aspergillus. This research provided the basis for submerged fermentation.

Surface fermentation

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

Submerged fermentation

This is the most widely used fermentation technique in the world today. Eighty per cent of the world's production is estimated to be from the submerged method. Submerged fermentation was developed after surface fermentation. It requires more sophisticated installation, higher energy cost and rigorous control, and there is formation of foam (which can be resolved using antifoaming agents), but it provides higher productivity and yields, has reduced capital, maintenance and labour costs, and carries lower contamination risks. In addition, it is less sensitive to change in the medium composition, providing a wider range of substrates and better control of substrates; this advantage makes molasses usable as a medium for citric acid production. Submerged fermentation is mostly operated as a batch system. However, continuous systems are possible and are used in practice. Submerged fermentation also includes the shake flask technique, which is usually used for the optimization of fermentation

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conditions. This is basically an Erlenmeyer flask which is placed on a shaker and stirred continuously throughout the fermentation process. Performed a comparative study between surface culture and submerged culture techniques. The outcome from this study was that surface fermentation is superior to submerged fermentation in terms of yield and productivity of citric acid.

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

References:

1. Introduction to Medicinal Chemistry, G. L. Patrick; Oxford University Press, UK, 2013.
2. Medicinal and Pharmaceutical Chemistry, H. Singh, & V. K. Kapoor; Vallabh Prakashan, Pitampura, New Delhi, 2012.
3. Principles of Medicinal Chemistry, 4th ed., W.O. Foye, T. L. Lemke, & D.A William.; B.I. Waverly Pvt. Ltd. New Delhi.
4. Pharmaceutical chemistry, Chandan Saha, B Chakraborty, S. Chakraborty, K Basu; Techno World Publishers.