Aspergillus citric acid production

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Aspergillus niger produces many primary and secondary metabolites. Citric acid is an organic acid, produced as an intermediate in the tricarboxylic acid cycle (TCA cycle or the citric acid cycle or the Krebs cycle), which is the part of the metabolism of almost all living organisms. This essay would include an introduction about citric acid and its production, the citric acid cycle, industrial production of citric acid, and the biochemistry related to the industrial production.

The natural supply of citric acid limited and thus it has to be produced on an industrial scale. Aspergillus niger produces it by fermentation of glucose or sucrose. This process is one of the major processes in the biotechnological industry.[2] It is used in several industries including the food, beverage, chemical and pharmaceutical industries, and has applications in flavouring, as a preservative, in soaps and detergents, hair drying, explosives, photography, etc. It is required in a considerable amount in large-scale processes.[1][2][3][5] Thus, cheap, economic and newer technologies are being seeked for it’s production.[2] The production of citric acid is about 1. 6 million metric tonnes per annum (as of 2007), which is majorly produced by Aspergillus Niger. [10]

In Aspergillus, the equation of metabolism is:

Carbohydrate ïƒ citric acid ïƒ oxalic acid ïƒ carbon dioxide ïƒ mycelium

As the equation represents, the fermentation of sugar by Aspergillus niger is a three step process and citric acid is produced on the first step from the carbohydrate source. Further in the metabolic pathway, oxalic acid is produced at the second step. All the acids that are produced are finally converted in carbon dioxide, which is required for the growth and maintenance of the mycelium. Any culture of Aspergillus niger would produce much more citric acid than oxalic acid, when grown on a sugar source. [7]

The Citric Acid Cycle

The citric acid cycle, also called the TCA cycle or the Krebs cycle, was discovered by Hans Adolf Krebs, who received a Nobel Prize in Physiology or Medicine in 1953 for this discovery. It is an aerobic pathway, in which glucose is completely oxidized into carbon dioxide. According to the book Biochemistry, by Stryer, " The citric acid cycle is the final common pathway for the oxidation of fuel molecules-amino acids, fatty acids, and carbohydrates." Acetyl coenzyme A is the starting molecule of the cycle. The cycle consists of several oxidation and reduction reactions that would ultimately oxidize an acetyl group and result in two carbon dioxide molecules. The pathway takes place in the mitochondria in eukaryotes. [8]

In the pathway, oxaloacetate, a four-carbon compound and a two-carbon acetyl unit condense together and form a six-carbon compound- citrate, which is a tricarboxylic acid. Citrate is converted into its isomer- isocitrate, which undergoes oxidative decarboxylation and forms a five-carbon compound- Î±-ketoglutarate, which is further oxidatively decarboxylated and forms a four-carbon compound- succinate. In the end, oxaloacetate is regenerated from succinate. Two molecules of carbon dioxide are formed in the cycle, which balances the two carbon acetyl unit that entered the pathway initially.[8] The following equations describe the microbiological production of citric acid:

C6H12O6+1. 5O2â†’C6H8O7+2H2O

C6H12O6+6O2â†’6CO2+6H2O

In the first equation, the oxygen requirement for the oxidation of glucose to citric acid is represented, and the second equation represents the oxygen requirement for the complete oxidation of glucose to carbon dioxide and water, which are required for the maintenance and metabolism in an aerobic organism. Thus, oxygen plays an important role in the production of citric acid. [2]

Citric acid cycle, along with oxidative phosphorylation, produces a large amount of energy used by the organism. Many molecules of ATP, NADH and FADH2 are produced at the various steps of the cycle.

http://www. mpi-magdeburg. mpg. de/research/projects/1088/1114/1118/TCA3. jpgSchematic overview of citric acid cycle.[11]

Industrial Production

James Currie discovered in 1917 that Aspergillus Niger could produce citric acid, and the industrial level production of citric acid was started by Pfizer in 1919 (cited in [9]). This method is still the main production technique used in industries. Sucrose or glucose containing medium are used by Aspergillus niger to produce citric acid. The source of sucrose and glucose include corn steep liquor, molasses, hydrolyzed corn starch, and other sugary solutions. The mould is first filtered out of the sugary solution, and then citric acid is precipitated with calcium hydroxide (lime), to form calcium citrate salt. Citric acid is regenerated from this salt by treatment with sulphuric acid. In some modern technologies, the fungus is separated from the sugary liquid by using a rotatory biological contactor (RBC), on which it grows and is rotated to allow exposure to the liquid and air alternately, for about 10 times per minute. Since there is no need to filter the fungus from the sugary solution, the fungus can be used again and again and this makes the process more efficient.[4]

http://upload. wikimedia. org/wikipedia/commons/3/3c/Rotating_Biological_Contactor. png

The various processes by which citric acid is produced industrially include:

- Surface process on liquid substrate
- Solid state fermentation
- Submerged fermentation
- Using A. niger
- Using yeast

- Using immobilization of microorganisms
- Solid-state fermentation [10]

Biochemistry

In order to produce citric acid on an industrial scale by Aspergillus niger, the medium should be optimized such that it is suitable for the overproduction of citric acid by the organism. The following factors should be optimized:

Sugar Source- If the concentration of the sugar source is increased, the yield of citric acid also increases.

Nitrogen and Phosphate- Some research undertaken by Shu and Johnson in 1948 (cited in [9]) has reported that if phosphate is provided when trace elements aren’t present in limiting amounts, there is an additional biomass growth along with side reactions. Another research conducted by Kubicek and Röhr in 1977 (cited in [9]) deducted said that citric acid accumulated when phospohate was limited and nitrogen wasn’t. On the other hand, a research conducted by Kritiansen and Sinclair in 1979 (cited in [9]) showed that nitrogen should be limited.

pH- The pH of the broth should be more than 5 for the germination of the spores. The pH should be less than 2 for the production of citric acid, as this would prevent the contamination by other microorganisms, as well as the production of other organic acids like oxalic acid. This low pH also facilitates the recovery of citric acid easier. Many enzymes are also pH sensitive in the citric acid cycle, and thus the culture pH has a huge impact. [9]

Aeration- The difference in the aeration rate can have a serious effect on the yield. According to Maria Papagianni's paper, " If the aeration rate is too high (a condition that occurs only at laboratory scales), the partial pressure of dissolved CO2 in the medium can become too low. Carbon dioxide is important as a substrate for pyruvate carboxylase which replenishes the supply of oxaloacetate for citrate synthase. Sufficient CO2 is produced by the pyruvate decarboxylase reaction to satisfy the stoichiometric demand of the pyruvate carboxylase reaction, but excessive aeration results in some loss. McIntyre and McNeil (1997) showed that elevated levels of CO2 in the sparged gas actually had a detrimental effect upon the final citrate concentration and final biomass concentration." [9]

Trace Elements- Some trace elements are required for the growth of Aspergillus niger, and the limitation in the concentration of others, like Zn, Mn, fe, Cu and heavy metals, is required for the production of citric acid. [1] [9]

Fungal Morphology- When submerged culture is observed, it is found that the morphology of filamentous fungi can be in the form of pellets and free filaments, depending on culture conditions and the genotype of the strain, and it is not known which of these forms is better for the production of citric acid. [9]

Thus, the sugar or carbon source should be present in high concentration with high acidity, oxygen must be high, hydrogen ions must be present, and the optimum concentrations of trace metals, phosphate and nitrogen should be present in the medium along with the proper aeration, in order to overproduce citric acid. [9]

To conclude, citric acid is produced as an intermediate of the TCA or citric acid cycle in most living organisms, but is majorly produced by Aspergillus niger in large-scale industries, as the demand for citric acid is huge for different purposes. Various studies have been undertaken to find out how the medium in which the organism grows should be optimized, in order for it to overproduce citric acid.