STUDY MATERIALS

PHARMACEUTICAL CHEMISTRY PART-3

SEC-I, SEMESTER I

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Factors affecting citric acid fermentation

Carbon source: Studies over several decades have shown that the carbon source affects the citric acid yield directly. Monosaccharides and disaccharides are the preferred carbon source as they are more rapidly metabolized by the fungus than polysaccharides, thus producing higher yield. Polysaccharides are not suitable as the raw material as the decomposition process takes too long to meet Carbon source Studies over several decades have shown that the carbon source affects the citric acid yield directly. Monosaccharides and disaccharides are the preferred carbon source as they are more rapidly metabolized by the fungus than polysaccharides, thus producing higher yield. Polysaccharides are not suitable as the raw material as the decomposition process takes too long to meet the rate of sugar catabolism necessary for the production of citric acid. The slow rate of polysaccharide hydrolysis is due to reduced enzymatic activity, which affects the pH in the fermentation medium. Sucrose is superior to glucose, fructose and lactose, in order of decreasing citric acid yield. The superiority of sucrose has been attributed to the strong extracellular mycelium-bound invertase of *A. niger*, which rapidly hydrolyses sucrose at low pH.

Nitrogen limitation: The concentration of nitrogen has been found to have a strong effect on the production of citric acid, as nitrogen is not only part of a cell's proteins, but also necessary for cellular metabolism. Molasses and other industrial media are usually nitrogen rich, whereas laboratory media require additional ammonium salts as supplements. The type of nitrogen source affects the synthesis of citric acid as well as the fungal growth. Ammonium nitrate promotes reduced vegetative growth, while ammonium sulphate promotes a longer period of vegetative growth. Nitrogen limitation is necessary, because at a concentration greater than 0.25%, oxalic acid accumulates and it will decrease the citric acid yield. A high nitrogen concentration increases the consumption of sugar and fungal growth, while decreasing the amount of citric acid produced.

Phosphorus source: Along with nitrogen and the carbon source, phosphate has also been shown to be a critical factor. For fungal growth, a phosphorus concentration of 0.5–5 g/l is required for citric acid production. The addition of phosphorus has only a slight effect on the

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accumulation of citric acid and mycelial growth deduced that citric acid accumulates with limited phosphate, even when nitrogen is not limited.

Lower Alcohols: Lower alcohols added in pure material inhibit citric acid production but added into crude carbohydrate these alcohols enhance production. Methanol, ethanol, n-propanol, Isopropanol.

pH of culture medium: The pH of the medium changes continuously as a result of microbial metabolic activities, largely because of the secretion of organic acids such as citric acid, and the unwanted gluconic and oxalic acid. The metabolic activities of microbes such as Aspergillus, Rhizopus and Penicillium species are able to reduce the pH quickly to below 3, while other fungi such as *Sporotrichum* and *Pleurotus species* produce a more stable pH between 4 and 5. The pH of the fermentation medium is most important during the sporulation and production phase. In the germination stage, the germinating spores absorb ammonia and release protons, thereby increasing the acidity of the medium and favoring the production of citric acid. At low pH of about less than 2, the formation of unwanted products such as oxalic and gluconic acid is inhibited, and the possibility of contamination by other microorganisms is also reduced, making recovery of citric acid easier.

Trace elements: Studies on divalent metal ions including manganese, zinc, copper, magnesium and iron have shown that they have effects on citric acid production. Concluded that the optimum concentrations of iron and zinc are 1.3 and 0.3 ppm, respectively. These authors further explained the importance of manganese for cell function, sporulation and production of secondary metabolites, and mainly in cell wall synthesis. Manganese deficiency affects the anabolism of *A. niger*, causing a high intracellular ammonium concentration. A decrease in the accumulation of citric acid with iron has been observed, as well as changes in mycelial growth deduced that at a high zinc concentration, the fungimaintained growth without accumulation of citric acid. Nickel, molybdenum and cobalt are some other trace metals reported to affect the citric acid accumulation of *A. niger*. The interdependence of medium constituents has to be taken into account as it is crucial to citric acid production; therefore, robust control of these trace elements is required for the optimum production of citric acid.

Aeration: The highly aerobic nature of the bio-production of citric acid makes the amount of oxygen supplied a critical factor. Therefore, varying aeration rates can have adverse effects on

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the fermentation performance and yield. At high aeration rates, there is reduced partial pressure of the dissolved carbon dioxide in the medium. Carbon dioxide is a substrate for pyruvate carboxylase as it replaces the supply of oxaloacetate for citrate synthase. The reaction catalyzed by pyruvate decarboxylase produces carbon dioxide, but extreme aeration can incur some losses. Increased levels of carbon dioxide are damaging to the final biomass and concentrations of citrate.

Other factors: Other factors that have effects on citric acid production include lipids, such as groundnut oil, and sodium monofluoroacetate and also showed that lipid will improve the yield of citric acid with no effect of the dry weight of mycelium. showed the effects of calcium fluoride, sodium fluoride and potassium fluoride on the industrial production of citric acid. The main factors that affect citric acid production.

Applications of Citric Acid

- Citric acid mainly used in food industry, pharmaceuticals, chemical industry, cosmetics, printings, food preservative, electro pickling, copper plating, beverage & others. Some specific applications are given below.

- Citric acid monohydrate is widely used as organic acid & pH control agent, flavouring and preservative in food production like as candy, cookies, biscuits, jams, jellies, snacks, instant foods and sauces.

- It is used as acidity regulator and antioxidant in beverage such as alcoholic beverage, carbonated soft drink, syrups, juice drinks, tea & coffee, ice-cream, sports &energy drink.

- It can be used in thrombin inhibitor and fungicide in pharmaceutical.

- It can be used as antioxidant & pH regulator in agriculture/ animal food/ poultry food such as chicken feed, boiler feed.

- It is widely used in cleaning agent, surfactant in various industries such as cleaning agent & anti-crease agent.

- Remove metal oxide from surface of ferrous & nonferrous for operational cleaning of iron & copper oxides.

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- In electroplating, copper plating, metal cleaning, leather tanning, printings inks, bottle washing compounds, floor cement, textiles, photographic reagent, plaster.
- It can be use in tartness and complements fruits & barriers flavours in beverage.
- It is used as an acidulent in creams, gels & liquid of all kinds.
- It is used as acidifying agent in many cheese products & as an antioxidant in dairy products.
- It can be used as an alternative to nitric acid in passivation of stainless steel.
- It is used as an odorless alternative to white vinegar for home dyeing with acid dyes.
- It is used as one of the active ingredients in the production of antiviral tissues.
- It is an alpha hydroxy acid and used as an active ingredient in chemical peels.
- It can be used in food colouring to balance the pH level of a normally basic dye.
- It can be added to ice cream as an emulsifying agent to keep fats from separating, to caramel to prevent sucrose crystallization, or in recipes in place of fresh lemon Juice.

Production Of Penicillin

There is only one choice for the antibiotic production process: the synthesis of *benzylpenicillin* (*penicillin G*, originally known as ‘penicillin’). This, the most renowned antibiotic and the first one has been manufactured in bulk, is still universally prescribed. Although originally made by surface liquid culture, *penicillin G* is now produced by air-lift fermentation under aerated conditions. *Penicillin G* is not a typical fermentative antibiotic. It is made by a fungus, *Penicillium chrysogenum*. The number of antibiotics from fungal sources is few, though they do include *penicillin G* and *V* and *cephalosporin C*. These three antibiotics are the major starting materials for the semi-synthetic -lactam antibiotics. The systemic antifungal antibiotic *griseofulvine* is also of fungal origin. Most antibiotics are produced by fermentation using bacteria, including streptomycin and the tetracycline family among numerous others. The manufacture of streptomycin from *Streptomyces griseus* will not be considered in this experiment. *Streptomycin* is the next important antibiotic after *penicillin G* to be made available to the clinician. It has played an important role in fighting tuberculosis.

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All of the above processes are operated as batch fermentations, in which a volume of sterile medium in a vessel is inoculated. The broth is fermented for a defined period. The tank is then emptied and the products are separated to obtain the antibiotic. The vessel is then recharged for batch operation with medium and the sequence repeated, as often as required. Continuous fermentation is not common practice in the antibiotics industry. The antibiotic concentration will rarely exceed 20 gl 1^{-1} and may be as low as 0.5 gl 1^{-1}.

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