Organic acids – Citric acid and Lactic acid

The organic acids find their wide application in the food, pharmaceutical and chemical industries. Some organic acids like citric acid, lactic acid, fumaric acid, propionic acid, malic acid, α-ketoglutaric acid, 5- ketoglutaric acid, 2- ketoglutaric acid, gluconic acid, acetic acid, kojic acid, itaconic acid are produced through fermentation. Advances in the fermentation technology have helped to manufacture organic acid at industrial scale. The chemical synthesis of these organic acids requires very harsh conditions and involves many steps which make their large scale production impractical. The microbial fermentation is a very simple method to synthesize these organic acids in very pure form. The fermentation methods require less energy input and are cost effective due to simple media formulations in many cases. In the proceeding section citric acid and lactic acid production will be discussed.

Citric acid :

Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) is one of the world's major fermentation products with the annual production of over 550,000 tonnes, and its demand is increasing at the rate of 2-3% every year. It was first isolated in 1784 from lemon juice and crystallized by Scheele. Until the 1920s, citric acid was extracted from the lemon juice and referred as 'natural citric acid'. Whehmer first time in 1923 described that citric acid is a metabolic product of *Penicillium* and *Mucor*. In 1923, Pfizer became the first industry to produce citric acid through fermentation based process in USA, by culturing *Aspergillus niger* in surface culture in a medium containing sucrose and mineral salts. As on today most of the citric acid is produced by fermentation, and the major producers are located in Western Europe, USA and China. Citric acid has GRAS (generally regarded as safe) status and its major applications are summarized in Table 6.

Table 6: Uses of citric acid in different industries

Industry	Application
Food and beverage products	Acidulant, pH regulator, flavour enhancer, preservative and antioxidant synergist
Bakery	Leavening agent
Chemical industry	Antifoam, softener, electroplating, pickling agent, alkyd resins and plastics
Detergents	To replace polyphosphate
Steam boiler	Removal of scales formed due to salt deposition
Oil wells	Removing the iron clogged in the pores of the sand
Medical and cosmetics	Effervescent type denture cleansers, and in shampoos and cosmetics

Microorganisms:

Citric acid is a primary metabolite and excreted in traces by *Aspergillus wentii, A. clavatus, Penicillium luteum, P. citrium, Mucor piriformis, Paecilomyces divaricatum, Citromyces pfefferianus, Candida guilliermondii, Saccharomycopsis lipolytica, Trichoderma viride, Arthrobacter paraffineus,* and *corynebacterium* sp. Many microorganisms including filamentus fungi, yeasts and bacteria could be used to produce citric acid, however, the mutants of A. niger are generally used for commercial production.

Citric acid production media :

The commonly used carbon sources in the citric acid production media include starch from potatoes, starch hydrolysates, glucose syrup from saccharified starch, sucrose, sugarcane syrup, sugarcane molasses, sugar beet molasses. Starch used during fermentation is hydrolysed either by amylases secreted by the growing fungus or by extraneous amylases. Trace elements Cu, Mg, Mn, Fe, Zn, and Mo are necessary in ppm range for optimal growth of the fungus. These may exert inhibitory effects if the optimal concentration exceeds.

Iron is one of the very important trace element required for the production of citric acid by *A. niger*, because the optimal growth of this fungus requires high iron concentration (since iron is co-factor of aconitase), but only 0.05-0.5 ppm is needed to lower aconitase activity which leads to maximizing citric acid production. The pH of medium also influences citric acid production. The fermentation starts at pH 5 and in first 48 h during trophophase, the pH falls below 3.0 as a result of utilization of ammonium ions. The pH below 3.0 during idiophase prevents the formation of oxalic acid and gluconic acid this also reduces the risk of contamination. (p 25).

germinate at 32 ºC in medium containing 15% sugar to form pellets of mycelium (0.2-0.5 mm diameter). These pellets are used for inoculating the production medium. Aspergilli are genetically unstable, so minimum number of preliminary steps is performed to produce the final inoculum and in some cases spores are also directly used to inoculate the production medium.

Surface and solid-substrate fermentations:

The liquid surface methods are the oldest production method and about 20 % of the world's supply of citric acid is met by these processes. The bioreactors used in these processes are shallow aluminum or stainless steel trays (5-20 cm deep). It is a labour intensive process than the deep vat fermentations, due to manual cleaning of pipes, trays, and walls of the system. Media containing beet molasses (320-400 g/L), NH_4NO_3 (1.6-3.2 g/L), CaH_2PO_4 (0.3-1.0 g/L), $MrSO_4.7H_2O$ (0.2-0.5 g/L), $ZnSO_4$ (0.01-0.1 g/L) and calcium hexacyanoferrarte (0.4-2.0 g/L) is sterilized continuously and pumped onto the trays. The fermentation is initiated by inoculating the medium by spraying dry spores or spore suspension. Sterile air is blown over the surface to control the incubation temperature (30 ºC) and to lower the CO_2 level produced during the fermentation. The spores germinate within 24 h and as the growth proceeds, the pH of the medium declines to 1.5-2.0 due to the uptake of the ammonium ions, and citric acid production begins. The fermentation is continued for 8-12 days and a production of about 1.0 kg of citric acid monohydrate /m^3 per day is achieved.

Solid-state fermentation processes are performed at small scale using steam sterilized wheat bran or sweet potato waste. The pH of the medium is adjusted to 4.5 and inoculated with spores of *A. niger*, and spread over trays to a depth of 3-5 cm. Sterile air is circulated and temperature is maintained around 28 ºC. The fermentation is allowed to proceed for 5- days after which the citric acid from the medium is extacted using hot water.

Submerged fermentation processes:

The submerged fermentation is carried out in stirred tanks of 40-200 m^3 capacity or in larger airlift fermenters of 200-900 m^3 size, about 80% of the citric acid is produced through these processes. It is very necessary to use stainless steel fermenter vessel or alternatively special glass or plastic lining is done to prevent the leaching of heavy metals because of the lowering of the pH during fermentation.

The structure of the mycelium in the trophophase influences the productivity of citric acid during fermentation. Small compact pellets (1 mm) with fluffy centers and smooth surface consisting of short forked bulbous hyphae characteristically have optimal citric acid production rates. High manganese level in the medium is responsible for development of long unbranched hyphae with loose texture which produce lesser citric acid. This necessitates the pretreatment of raw materials to reduce manganese concentration to below 0.02 mM. The low manganese concentration also limits the pentose phosphate pathway and shifts the glucose flux to glycolysis and citric acid production. Addition of copper ions to the medium prevents the uptake of magnesium by the fungus which also diminishes the aconitase activity involved in further metabolism of citrate. The level of iron in the medium is also low in order to inhibit the activity of aconitase enzyme.

The sugar concentration of 140 g/L in the production medium is necessary for promoting activity of both glycolytic enzymes and pyruvate carboxylase. Ammonium salts at 0.1-0.4 g/L restrict the increase in biomass and also activates citric acid production by counteracting the inhibitory effect of citrate on phosphofructokinase. The fermentation is carried out at 30 ºC with a high aeration rate of 0.2-1 vvm during the idiophase. The initial pH of medium is 5-7 which decreases during the idiophase to 2 or below inhibiting the glucose oxidase activity, responsible for gluconic acid production. The fermentation is carried for 8 days and yield of 0.7-0.9 g citrate per gram glucose is achieved. The overall yield of citrate is around 18.0 kg/m^3 per day.

Citric acid recovery:

The citric acid is recovered by separation of fungal mycelium from the fermentation broth by rotary filtration or centrifugation. The filtrate is treated with lime ($Ca(OH)_2$) to precipitate out oxalic acid formed in sub-optimal production conditions in the form of calcium oxalate. The precipitates of calcium oxalate is separated by filtration and the pH of the filtrate is raised to 7.2 ± 0.2 and heated to 70-90 ºC with lime forming calcium citrate precipitates. These precipitates are separated by filtration by means of rotatory filters and treated with sulphuric acid to release citric acid. This is again filtered to separate citric acid in solution from the calcium sulphate precipitates. The citric acid solution thus obtained is diluted and passed through activated carbon for decolourising. Finally the solution is evaporated to form citric acid crystals. To avoid the use of lime

and sulphuric acid, other methods of purification like solvent extraction, ion-pair extraction and electrodialysis are used.

Lactic acid:

Lactic acid (2-hydroxypropanoic acid) was discovered and isolated in 1780 by the Swedish chemist Scheele from sour milk and the first organic acid produced microbiologically in 1881 by Charles E. Avery at Littleton, Massachusetts, USA. It is classified as GRAS (generally regarded as safe) by Food and Drug Authority (FDA) in the USA and its annual consumption is estimated to be 30 000 tonnes. Lactic acid is used in various industries for different applications (Table7).

Table 7: Applications of lactic acid in different industries

Industry	Application
Food	Preservative, acidulant, buffering agent, pickling agent and dough conditioner
Meat	Prolongs the shelf life of poultry and fish during packaging
Textile	Finishing, antimony lactate as a mordant during dyeing
Metal	Electroplating bath, plasticizer and corrosion inhibitor
Leather	Acidulant
Pharmaceutical	Ointments, lotions, anti acne solutions, humectants, and parenteral solutions, calcium lactate (anti caries agent) and biodegradable polymers for sutures in medicine
Medical	Orthopedic implants, controlled drug release
Chemical	Ethyl/butyl lactate

Lactic acid bacteria:

There are two groups of lactic acid bacteria, one is heterofermentative and other is homofermentative. The heterofermentative (e.g. *Luconostoc mesenteroides*) lactic acid bacteria produce many byproducts other than lactic acid and are not suitable for commercial processes. In case of homofermentative bacteria (*Lactobacillus* sp.), very little substrate is used for producing cell mass and other metabolites and majority of the carbon source is converted to lactic acid, and here the percent conversion of sugars to lactic acid is virtually equivalent to the theoretical yield of two moles of lactic acid per mole of hexose sugar utilized. In Table 8 some *Lactobacillus* species and their preferred carbon sources are listed.

Table 8: Some homofermentative lactic acid bacteria and their preferred carbon sources:

Organism	Carbon source
Lactobacillus delbrueckii	Glucose
Lactobacillus leichmannnii	Glucose
Lactobacillus bulgaricus	Lactose
Lactobacillus helviticus	Lactose and galactose
Lactobacillus amylophyllus	Starch
Lactobacillus amylovirus	Starch
Lactobacillus lactis	Glucose, sucrose and galactose
Lactobacillus pentosus	pentoses of sulfite waste liquor

The homofermentative lactic acid bacteria are facultative anaerobes which can be grown in low oxygen concentration and are used for industrial production of lactic acid.

Lactic acid biosynthesis :

The lactic acid is an anaerobic fermentation end product of glycolysis i.e. pyruvate is reduced to lactic acid by lactate dehydrogenase (EC 1.1.1.27) generating NAD for glycolysis (Fig 11). The lactic acid bacteria are able to produce either D(-)-lactic acid, L(+)-lactic acid or the racemic mixture of both D and L isomers (Fig 12). Two stereospecific lactate dehydrogenases (LDH) are present in organisms synthesizing the L(+) or D(-)-lactic acid. In some *Lactobacilli*, accumulation of L(+)-lactic acid induces

racemase which converts it into D(-)-lactic acid until equilibrium is obtained. D-Lactic acid is levorotatory and L-form is dextrorotary whereas the salts of D- and L- lactic acid have reversed optical rotations.

Fermentation process :

Semirefined corn sugar (dextrose), molasses, or whey are some commonly used carbon sources for the industrial production of lactic acid. The typical medium employed in the fermentation process consists of 10-15 % dextrose, 10 % calcium carbonate, and small amounts of nitrogenous(p 29).

conventional batch reactor yielding 0.83 g/l of lactic acid and of 0.31 g/l/h productivity . High yield of lactic acid is obtained either by continuous cell recycle fermentation process or by fed batch fermentation. Inhibitory effect of lactic acid has also been overcome by electrodialysis fermentation method which continuously remove lactic acid from the fermentation broth resulting in continuation of fermentation activity and 82.2 g/liter lactic acid which is about 5.5 times greater than that produced in non-pH controlled fermentation . Using membrane cell recycle bioreactor higher volumetric productivity of 117 g/l/h has been obtained but high product concentration does not occur. Membrane Cell Recycle Bioreactors (MCRB) in series having lactic acid productivity of 5.7 g/l/h, and 92 g/l lactic acid concentration has also been reported . Continuous fermentation processes using immobilized cells for the production of lactic acid have also been developed.

Recovery of lactic acid :

A number of methods can be applied for the separation of lactate salt from fermented medium which involve extraction by solvents or separation by ion-exchange, adsorption, vacuum distillation and membrane filtration (Eyal et al. 2001). The medium after completion of fermentation consists of either pure lactic acid or its salt or the mixture of the two. Earlier method used addition of excess of calcium carbonate to the medium at the end of the fermentation and pH adjusted to 10, heated and filtered. In this procedure all the lactic acid is converted in to calcium lactate, the bacteria are killed and protein in the medium gets coagulated. A number of other methods have been developed to recover and purify lactic acid. In one such method, the filtrate is concentrated to crystallize calcium lactate following which sulphuric acid is added to precipitate calcium as calcium sulphate and removed by filtration. Lactic acid is again recrystallized as calcium lactate and passed through activated carbon to remove coloured impurities. Lactic acid is also extracted with isopropyl ether directly from the heated and filtered fermentation broth, by counter-current continuous extraction method, and lactic acid is further recovered from the solvent by counter-current washing with water. A preferred process for the lactic acid recovery from the mixture containing free lactic acid and the dissolved lactate salt involves first lowering down of the pH of fermented broth to 3.0-4.2 and then hydrophilic membrane and the volatile amine weak base (VAWB) are used to separate lactic acid from the fermented broth.

The lactic acid is finally regenerated from salts of weak amine base by selectively vaporizing the volatile amine base .