

absence of trace metals. Such conditions provoke an overflow in metabolism that results in an overproduction of citric acid. Oxalic and gluconic acids are also produced if the pH is above 2. Potassium ferrocyanide is added to reduce the concentration of metals and is a growth inhibitor that promotes the production of citric acid. The metabolic imbalance that results in citric acid production also alters the morphology of fungi. The hyphae become short, stubby, and forked in small pellets (0.2–0.5 mm in diameter). Limitation of certain nutrients such as nitrogen and phosphate stimulates citric acid formation. High dissolved-oxygen concentrations must be maintained throughout the culture. Even short interruptions in oxygen provision can result in irreversible decreases in acid production rate.

Citric acid was historically produced by surface fermentation of beet molasses, and this process is still employed by some manufacturers. It is labor intensive, but power requirements are lower than for submerged fermentation. The surface process is realized on surface-aerated trays with liquid depth of 5–20 cm. Sterilized diluted beet molasses, with a sugar concentration of 150 g/l and a pH of 6, is placed on trays in a temperature-controlled (30°C) and aerated clean room. Sterilized additional nutrients and alkali ferrocyanide are added into the medium. Spores of a selected strain of *A. niger* are spread over the liquid on trays. The clean chamber is aerated with filter-sterilized air to provide oxygen to the organisms and to remove fermentation heat. Mycelium forms a layer on the surface of the medium. After 7–10 days of incubation the trays are emptied, the mycelium is removed, and the medium is transferred to the recovery section. The production of undesirable products such as gluconic and oxalic acids can be avoided by strain selection.

After the Second World War submerged fermentation processes utilizing molasses or pure sugar solutions were developed. The submerged process is realized in deep stainless-steel vessels of 100 m³ or larger by batch or fed-batch operation. The fermenters may be mechanically agitated or aerated towers with an internal recycle draft tube. Aeration is provided to the fermenter by air sparging (0.1 to 0.4 vvm), and temperature is controlled with cooling coils. Agitation is usually gentle (50–100 rpm) to avoid shear damage on molds. Diluted molasses supplemented with other nutrients is on-line sterilized and added to the fermenter. Sterilization in the fermenter is also possible. The initial pH is adjusted at 2.5 to 3. Spores of *A. niger* are allowed to germinate in an inoculum medium before being transferred to the main fermenter. In some cases, spores are directly introduced into the fermentation media (5 to 25 × 10⁶ spores/l). Since dissolved-oxygen concentration is critical for citric acid production, oxygen-enriched air may be used in some cases. About 80% of the supplied carbon is converted to citric acid in a typical fermentation. Batch operation usually results in productivities of 0.5–1 kg/m³ h. Fed-batch operation can be performed to avoid substrate inhibition and to prolong the production phase one or two days after growth cessation. Typical volumetric yields of fed-batch processes are around 130 kg/m³. When citric acid production stops, usually after 4–5 days, the fermenters are emptied and the biomass is separated from the broth by filtration. The liquid is transferred to the recovery section.

Precipitation is usually accomplished by addition of calcium hydroxide (lime) to the heated fermentation broth to obtain calcium citrate tetrahydrate. The precipitate is then washed and treated with dilute sulfuric acid, yielding an aqueous solution of citric acid and CaSO₄ (gypsum) precipitate. After bleaching and crystallization, either anhydrous or monohydrate citric acid is obtained. Solvent extraction is another option for recovery of citric acid, although it is not used commercially. Extraction avoids the use of lime and