APPLIED MICROBIOLOGY: INDUSTRIAL

Contents

Acetic Acid Production

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Glossary

aerobic process A process that requires the presence of oxygen to proceed.
anaerobic process A fermentation that requires the complete absence of oxygen.
downstream processing A series of unit operations or processes to recover, isolate, and purify the desired component from a fermentation mixture.
fermenter The vessel in which fermentation is conducted.
productivity The amount of the product produced per unit volume per unit time.

Abbreviations

CoA coenzyme A
CODH carbon monoxide dehydrogenase
FDH formate dehydrogenase

Introduction

Acetic acid (CH_3COOH) is the principal constituent of vinegar. The first vinegar was probably a result of spoiled wine, considering that the Latin word *acetum* means sour or sharp wine. Thus, it has been produced as long as wine making has been practiced and therefore dates back to at least 10 000 BC.

Historical Background

Acetic acid was used as a medicinal agent and was probably the first known antibiotic. For most of human history, acetic acid was produced by fermentation of sugar to ethyl alcohol and its subsequent oxidation to acetic acid by microorganisms.

This process was supplemented in the nineteenth century by wood distillation. In 1916, the first dedicated plant for the production of acetic acid by chemical rather than biological means became commercial. This method was based on acetylene-derived acetaldehyde, and it marked the advent of inexpensive, industrial-grade acetic acid and the birth of a viable industry based on its use. The advantages of chemical synthetic routes include high acetate concentrations (35–45%, by wt), high production rates, and acetic acid generated in the free acid form. The major disadvantages are the need for high temperatures, high pressures, and good agitation, the threat of explosion, the high cost of catalysts, and the dependence on nonrenewable, uncertain sources of raw materials (crude oil). In 1995, annual production of acetic acid by the petrochemical route in the United States was 4.68 billion pounds, ranking 35th among all chemicals produced. Production increased at an annual rate of 18% from 1993 to 1995. Vinyl acetate ranked 41st, averaging 3 billion pounds in 1993–95.

Fermentation production routes have traditionally been aimed at the food market. Vinegar production usually requires lower capital investment, has shorter startup times, and can generate different types and flavors of vinegar when different carbohydrate sources are used. Furthermore, the raw material (e.g., corn, sugarcane, and sugar beet) is a renewable resource. The cost of acetic acid from chemical synthesis ranges from 15 to 35¢ lb^{-1} on a 100% basis, whereas it is 35–45¢ lb^{-1} from aerobic fermentation.

Acetic acid has a wide variety of uses, as shown in Figure 1. There is a large market for vinyl acetate due to the demand for synthetic fibers. Calcium magnesium acetate and potassium acetate are relatively new applications used primarily as noncorrosive, environmental friendly alternatives to chloride salts for de-icing roads and for airport runways and as heat exchange fluids.

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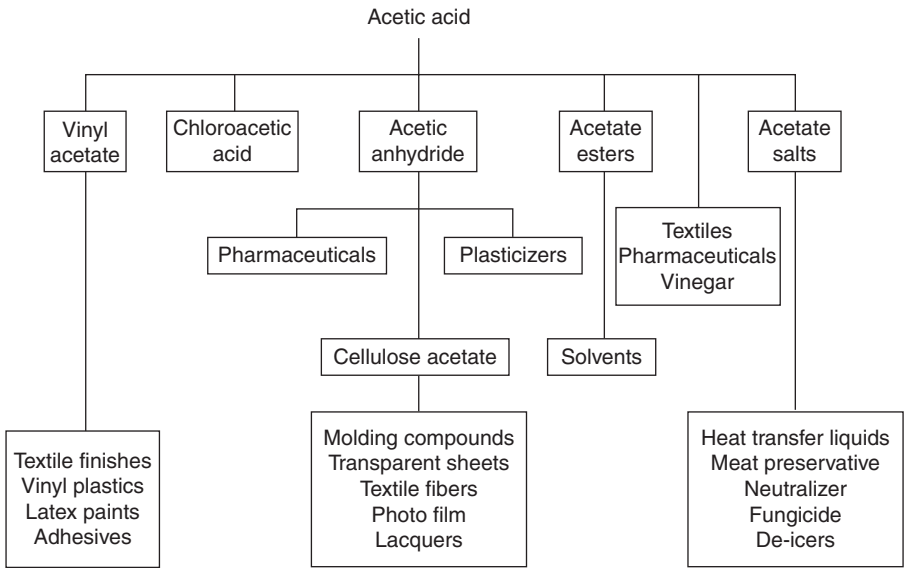

Figure 1 Uses of acetic acid. Reproduced from Cheryan M, Parekh S, Shah MM, and Witjitra K (1997) In: Neidleman SL and Laskin AI (eds.) *Advances in Applied Microbiology*, vol. 3, pp.1–33. New York: Academic Press, with permission.

Acetic Acid Production

Acetic acid as an industrial chemical is currently produced from fossil fuels and chemicals by three processes: acetaldehyde oxidation, hydrocarbon oxidation, and methanol carbonylation. It can also be produced by biological routes using either an aerobic or an anaerobic route.

Aerobic Process

Food-grade acetic acid is produced by the two-step vinegar process. The first step is the production of ethanol from a carbohydrate source such as glucose. This is carried out at 30–32 °C using the anaerobic yeast *Saccharomyces cerevisiae*:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2CH_3CH_2OH$$

The second step is the oxidation of ethanol to acetic acid. Although a variety of bacteria can produce acetic acid, only members of *Acetobacter* are used commercially, typically the aerobic bacterium *Acetobacter aceti* at 27–37 °C. This fermentation is an incomplete oxidation because the reducing equivalents generated are transferred to oxygen and not to carbon dioxide:

$$2CH_3CH_2OH + O_2 \rightarrow 2CH_3COOH + 2H_2O$$

The overall theoretical yield is 0.67 g acetic acid per gram glucose. At the more realistic yield of 76% (of 0.67 g; i.e., 0.51 g per gram glucose), this process requires 2.0 lb of sugar or 0.9 lb of ethyl alcohol per pound of acetic acid produced. Complete aeration and strict control of the oxygen concentration during fermentation are important to maximize yields and keep the bacteria viable. Submerged fermentation has almost completely replaced surface fermentation methods. The draw-and-fill mode of operation can produce acetic acid at concentrations up to 10% (w/w) in continuous culture at pH 4.5 in ~35 h.

Anaerobic Process

In the 1980s, another process for production of acetic acid emerged based on anaerobic fermentation using *Clostridia*. These organisms can convert glucose, xylose, and some other hexoses and pentoses almost quantitatively into acetate according to the following reaction:

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH$$

Clostridium thermoaceticum is also able to utilize five-carbon sugars:

$$2C_5H_{10}O_5 \rightarrow 5CH_3COOH$$

A variety of substrates, including fructose, xylose, lactate, formate, and pyruvate, have been used as carbon sources in an effort to lower substrate costs. This factor is also important if cellulosic renewable resources are to be used as raw materials.

Typical acidogenic bacteria are *Clostridium aceticum, C. thermoaceticum, Clostridium formicoaceticum,* and *Acetobacterium woodii*. Many can also reduce carbon dioxide and other one-carbon compounds to acetate.

Most research has been done with *C. thermoaceticum*. It was isolated from horse manure. It is an obligate anaerobe, Gram-positive, spore-forming, rod-shaped, thermophilic organism with an optimum growth temperature of 55–60 °C and optimum pH of 6.6–6.8. The anaerobic

route should have a lower fermentation cost than the aerobic process. The theoretical yields are higher: 3 mol of acetic acid is produced per mol of glucose consumed (i.e., 1 g acetic acid per g glucose). Actual yields with *C. thermoaceticum* have ranged from 0.85 to 0.90 $g\,g^{-1}$. However, downstream processing costs are higher with the anaerobic process since only 13–20 $g\,l^{-1}$ acetic acid is produced by the wild strain in batch fermentation and 50–60 $g\,l^{-1}$ with mutant strains.

The fermentation of sugars to acetate is a complex process. As shown in **Figure 2**, 1 mol of hexose is metabolized by the Embden–Meyerhoff pathway to yield 2 mol of pyruvate, which is further metabolized to 2 mol of acetate (formed from carbons 2 and 3 of the pyruvate) and to 2 mol of CO_2 (formed from the carboxyl groups). The 2 mol of CO_2 serves as an electron acceptor, where 1 mol of CO_2 is finally reduced to methyltetrahydrofolate (CH_3THF). The CH_3THF then combines with the second CO_2 and coenzyme A (CoA) forming acetyl-CoA and finally the formation of the third mole of acetate. The overall reaction can be written as follows:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 8H^+$$
$$+ 8e^- 2CO_2 + 8H^+ + 8e^- \rightarrow CH_3COOH + 2H_2O$$

The formation of the third mole of acetate involves tetrahydrofolate enzymes, carbon monoxide dehydrogenase (CODH), NADP-dependent formate dehydrogenase (FDH), and a corrinoid enzyme. These enzymes are metalloproteins; for example, CODH contains nickel, iron, and sulfur; FDH contains iron, selenium, tungsten, and a small quantity of molybdenum; and the corrinoid enzyme (vitamin B_{12} compound) contains cobalt. *C. thermoaceticum* does not have any specific amino acid requirement; nicotinic acid is the sole essential vitamin.

In most typical batch fermentations, cell concentration initially increases exponentially and then decreases toward the end of the fermentation. Acetate concentration also increases and then levels off. High glucose concentration inhibits the initial growth of *C. thermoaceticum*. However, after adaptation, the fermentation proceeds rapidly. There appears to be a minimum ratio of nutrient concentration to glucose concentration to produce acetic acid. If glucose is still available but the nutrient is not, the microorganism will produce by-products such as fructose.

Acetate production from glucose by *C. thermoaceticum* generates 5 mol of ATP per mol of glucose consumed. This results in high levels of cell mass per mol of glucose consumed. To maintain productivity, the cells must balance their ATP supply and demand. Since growth consumes more ATP than maintenance, most of the acetic acid produced by *C. thermoaceticum* occurs during the growth phase. When cells use yeast extract as a source of amino acids, nucleotides, and fatty acids, they will need less ATP than if they have to synthesize these compounds using ammonium ions as the starting material. Thus, assimilation of ammonium ions is important if cells are to recycle the ATP generated during production of acetic acid. Therefore, ammonium sulfate (a cheaper nutrient) could partially replace yeast extract without resulting in formation of by-products such as fructose. Medium cost could be lowered further by substituting corn steep liquor for yeast extract.

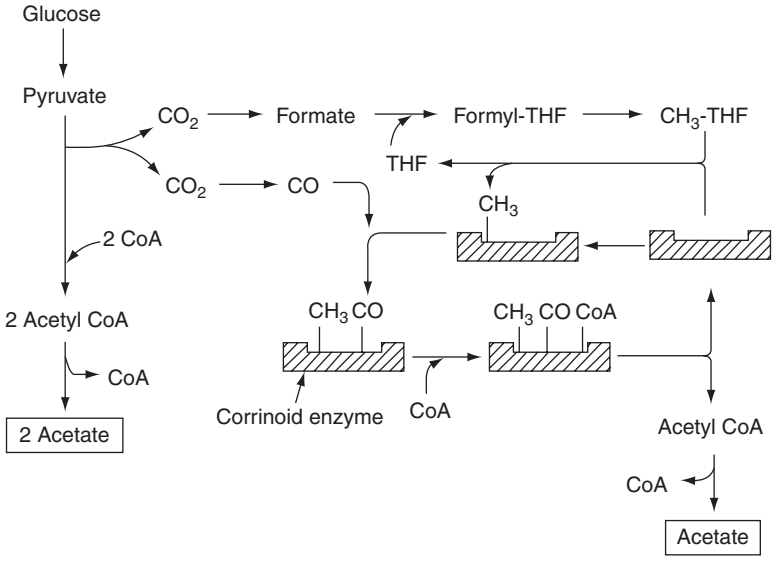

Figure 2 Embden–Myerhoff pathway for production of acetic acid. Reproduced from Cheryan M, Parekh S, Shah MM, and Witjitra K (1997) In: Neidleman SL and Laskin AI (eds.) *Advances in Applied Microbiology*, vol. 3, pp.1–33. New York: Academic Press, with permission.

Fermenter Designs

Industrial fermentation processes have evolved from the simple 'let-alone' method involving a partially filled open container of wine exposed to air to the 'field' fermentation in which a series of casks are filled with wine and inoculated in series by the vinegar produced in the previous casks. In the 'Orleans' method, holes are bored into the casks and a glass tube is inserted to allow the addition and removal of vinegar. The 'trickling' or 'German' process is a surface fermentation in which the microbial population is attached to an appropriate support (usually beechwood shavings) and the wine is trickled down while a large volume of air is sparged up through the bottom of the tank. This process was the basis for the manufacture of the trickling generator that incorporates forced aeration and temperature control. The partially converted solution collects at the bottom and is cooled, pumped back up to the top, and allowed to trickle down until the reaction is complete. Ethanol conversion into acetic acid is 88–90%; the rest of the substrate is used in biomass production or lost by volatilization. Advantages of this process include low costs, ease of control, high acetic acid concentrations, and lower space requirements. The costs of the wood shavings, long startup time, loss of ethanol by volatilization, and production of slime-like material by the *Acetobacter* (e.g., A. *xylinum*) are some of the disadvantages. Furthermore, there are often local zones of overoxidation, uneven aeration, and heat development.

The next technological advance occurred in 1949 when Hromatkar and Ebner applied submerged fermentation techniques to oxidation of ethanol to acetic acid. The level of gas-phase oxygen is crucial to this process, and thus, efficiency is based on broth aeration with oxygen. For industrial processes, 10–18% ethanol and 5 times the nutrients used for surface fermentation are the starting conditions for fermentation. When the concentration of ethanol reaches 0.4–2.4 $g\,l^{-1}$, 50–60% of the solution is removed and replaced with fresh substrate containing 10–18% ethanol. There is usually ~80 mg of dry bacterial solids per liter. The productivity is 1.7–2.1 g acetic acid per liter per hour, and the process is operated in a semicontinuous manner that helps to minimize variation in the product. During refilling, charging is slow with consistent mixing to prevent bacterial damage and/or death. Dead cells cause foaming; hence, mechanical defoaming techniques are used to eliminate this problem. Compared to surface fermentation, submerged fermentation results in higher productivity, faster oxidation of ethanol, smaller reaction volumes, low personnel costs due to automation, fewer interruptions due to clogging by shavings, and lower capital investment per product amount, even though the ratio of productivity to capital investment is higher.

Much of the work done with this fermentation has been performed with batch fermenters, in which all the carbohydrate and nutrients are added at the start of the fermentation. With fermentations that are substrate inhibited, a better method is to use the fed-batch mode of operation. This significantly improves the performance of the *C. thermoaceticum* fermentation. Continuous fermentation with immobilized whole cells has been used to increase the productivity of homoacetogenic fermentations. However, cell immobilization has been plagued by oxygen-transfer problems.

In contrast, cell-recycle fermenters using a membrane module as the separation device have been shown to vastly increase the productivity of several anaerobic fermentations, such as ethanol and lactic acid, and may have some advantages over immobilized cells, such as higher concentration of free cells, no diffusion limitation, excellent mixing in the bioreactor, and a cell-free product stream. The greatest advantage is that cell concentrations far in excess of normal levels can be used with no danger of cell washout. However, high productivity and high product concentration are mutually exclusive in such high-rate fermenters. The yield of acetate was 0.85–0.9 $g\,g^{-1}$ glucose consumed.

A 'draw-and-fill' bioreactor in combination with a membrane appears to be the optimum design. In this design, the reaction vessel is operated as a batch fermenter. At the end of the fermentation, a portion of the fermentation broth is withdrawn through the membrane module. The cells are recycled, and the reaction vessel is charged with fresh substrate.

In batch fermentation without cell recycle, acetic acid production is proportional to the amount of yeast extract and trace salts supplied in the medium. For all types of bioreactors studied, increasing dilution rate increases volumetric productivity but decreases specific productivity (grams acetate produced per gram cells). Thus, in cell recycle bioreactors, the nutrient supply should be increased in proportion to cell concentration to realize the full potential of the microorganism.

Downstream Processing

Downstream processing refers to the series of unit operations used to isolate, purify, and concentrate the product. Downstream processing often determines the economic feasibility of the process. The first operation is cell separation, which can be done by cross-flow microfiltration. When a microfilter or ultrafilter is combined in a semi-closed loop configuration to the bioreactor or fermenter, it becomes a powerful tool to dramatically improve the productivity of the fermentation while simultaneously providing a cell-free broth for subsequent downstream processing. Other membrane technologies,

such as nanofiltration and electrodialysis, are useful in subsequent stages of downstream processing to separate and perhaps concentrate the acid. However, other techniques will have to be used if a purified industrial-grade acetic acid is required.

Depending on the physical and chemical nature of the fermentation products, the cell-free broth is subjected to chromatography, electrophoresis, crystallization, precipitation, extraction, distillation, and/or membranes. Solvent extraction with azeo-tropic distillation is the preferred method for chemically derived acetic acid, whereas freeze concentration is used for vinegar. Both require substantial amounts of energy since a change in phase of the solvent is required. Simple distillation, although technically feasible, may not be economical since the fermentation broth typically consists of 90–95% water. Furthermore, if the acetate is required in the free acid form, there will be additional cost to convert the salt form produced in the anaerobic fermentation into the free acid form.

Liquid–liquid extraction has been used to recover acetic acid from the chemical manufacture of cellulose acetate, vinyl acetate, and other acetate products. Extraction solvents are ethers, ketones, or alcohols. In addition, the relative amounts of dissociated and undissociated acid in the feed solution are important. Extraction efficiency is high when the organic acid is present in the undissociated (acid) form (i.e., at a low pH). This makes it difficult to use with the anaerobic acetate process unless the fermentation broth is acidified or subjected to bipolar electrodialysis.

See also: Bioreactors; Clostridia; Fermentation

Further Reading

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