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REVIEW ARTICLE

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Abstract

Acetic acid is an important platform chemical. It is mainly produced synthetically and only 10 percent of the world production is manufactured by bacterial fermentation for making vinegar. Several microorganisms can produce acetic acid and a part of them can incorporate CO₂ during the production. In this review we summarized the microbial acetic acid pathways, and the used processes for vinegar production furthermore the optional acetic acid recovery operations to establish a complete possible biotechnological acetic acid production.

Keywords

acetic acid, vinegar, acetic acid bacteria, acetogen, Wood-Ljungdahl pathway, Orleans method, Generator method, Submerged method, recovery

1 Introduction

Acetic acid is an important platform chemical and traditionally used as a food preservative. It is a clear, colorless, corrosive carboxylic acid with sour taste and pungent smell [1]. Acetic acid is produced both synthetically and by bacterial fermentation. Synthetic production mainly depends on petroleum-derived stocks such as methanol, acetaldehyde, butane or ethylene. Today the biological route accounts for only about 10 percent of world production [2]. It remains important for vinegar production, because many of the world food purity laws stipulate that vinegar used in foods must be of biological origin. Vinegar which is mainly a 4-6% diluted acetic acid solution is directly used as a flavoring agent for foods and also as food preservatives [3].

In 2015 the global market of acetic acid stands at 13 million tons, which is forecasted to extend approximately to 16 million tons by 2020. According to 2015 market research, the market price of acetic acid varies within US \$1200 and \$1600 per ton in different countries of the world [4–6]. The largest end uses of acetic acid are the manufacture of vinyl acetate, of acetic anhydride, of acetate esters, of monochloroacetic acid, and the application as a solvent in the production of dimethyl terephthalate and terephthalic acid. Vinyl acetate is used in the production of latex emulsion resins for applications in paints, adhesives, paper coatings, and textile treatment. Acetic anhydride is used in the manufacture of cellulose acetate textile fibers, cigarette filter tow, and cellulose plastics [4].

2 Acetic acid producing microorganism

The need to regenerate the NAD⁺ consumed by glycolysis and to recycle the coenzyme A (CoASH) required in conversion of pyruvate to acetyl-CoA results acetogenesis i.e. the excretion of acetate into the environment. In the case when the full TCA cycle does not operate or when the carbon flux into cells exceeds its capacity, acetogenesis occurs. Thus, acetate excretion befalls anaerobically during mixed-acid fermentation. The Crabtree effect is also this instance under aerobic condition when growth on surplus glucose (or other highly digestible carbon sources) inhibits respiration. There are two distinct groups of acetic acid

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producing microorganism: while acetic acid bacteria apply aerobic pathway, acetogens produce anaerobically [7].

2.1 Aerobes

Acetic acid bacteria (AAB) are well known for the ability to oxidize ethanol as substrate into acetic acid in neutral and acidic media under aerobic conditions, which makes them important to the vinegar industry [8, 9]. AAB are polymorphous, cells are gram negative, ellipsoidal to rod shaped, straight or slightly curved, 0.6 to 0.8 μm long, and occurring singly, in pairs, or in chains. There are nonmotile and motile forms with polar or peritrichous flagella. They are obligate aerobic, and while some produce pigments, others produce cellulose [10, 11]. The above characteristics make them involved in the production of fermented foods, either in a beneficial (chocolate products, coffee, vinegar, and specialty beers) or in a detrimental (spoilage of beers, wines, and ciders) way, and in the production of commercially important fine chemicals as well. *Acetobacter*, *Gluconacetobacter* and *Gluconobacter* are the main genera in AAB for aerobic acetic acid fermentations [9]. Members of the AAB genus *Acetobacter* were historically differentiated from those of the genus *Gluconobacter* by a preference for ethanol and the ability to overoxidize acetate to CO_2 , usually when ethanol is depleted [12].

The taxonomy of AAB has undergone many changes in recent years in accordance with the development and application of new technologies. Several genera and species of AAB have been newly described. AAB are classified into 10 genera and 45 species, namely *Acetobacter* (16 species), *Gluconobacter* (5 species), *Acidomonas* (1 species), *Gluconacetobacter* and *Komagatabacter* (newly called as *Komagateibacter* [13]) (15 species), *Asaia* (3 species), *Kozakia* (1 species), *Saccharibacter* (1 species), *Swaminathania* (1 species), *Neoasaia* (1 species), and *Granulibacter* (1 species), in the family *Acetobacteraceae* [11, 14–16]. The first phylogenetic studies of AAB based on 16S rDNA sequences were published and showed that these organisms formed a cluster within the α -subclass of the *Proteobacteria*. Species were differentiated on the basis of morphology in fluid media, their iodine reaction, and numerous molecular characteristics, such as DNA–DNA hybridizations and polymerase chain reaction (PCR)-based genomic fingerprintings [12].

2.2 Anaerobes

It was discovered that hexose can be converted into three molecules of acetic acid by acetogenic bacteria (acetogens) under anaerobic conditions [17]. Acetogenic bacteria are not identical with AAB. These prokaryotes were initially studied primarily for their novel CO_2 -fixing properties. Up to now, over 100 acetogenic species, representing 23 genera, have been isolated from a variety of habitats [18]. Of the 23 different genera, *Acetobacterium* and *Clostridium* harbor the most known

acetogenic species. While most acetogens like *Clostridium thermoaceticum* (renamed to *Moorella thermoacetica* in 1994) are in the phylum *Firmicutes*, acetogens include *Spirochaetes*, δ -proteobacteria like *Desulfotignum phosphitoxidans*, and acidobacteria like *Holophaga foetida*. All of them have important role in the biology of the soil, lakes, and oceans, respectively. Acetogens have been isolated from varied environments such as the gastrointestinal tracts of animals and termites, rice paddy soils, hypersaline waters, surface soils, and deep subsurface sediments [12].

In anaerobic production of acetic acid, *Moorella thermoacetica* was used to elucidate the mechanism of homofermentation of acetic acid, which converts 1 mol of glucose into 3 mol of acetic acid. Among all the homoacetogens known to date, only *M. thermoacetica* has been previously considered to have industrial application and, thus, is the only one having been extensively studied for anaerobic acetic acid fermentation.

Acetogenic bacteria are a specialized group of bacteria that are able to produce acetate from two molecules of carbon dioxide (CO_2) via the Wood–Ljungdahl pathway (WLP) (Fig. 2). Acetogens are facultative autotrophs that can grow by the oxidation of a large variety of organic substrates, including hexoses, pentoses, alcohols, methyl groups and formic acid, or by the oxidation of inorganic substrates such as hydrogen (H_2) or carbon monoxide (CO), which is usually coupled to the reduction of CO_2 [1]. Acetogens are sometimes called as “homoacetogen” (meaning that they produce only acetate as their fermentation product) or as “ CO_2 -reducing acetogen”. They have the ability to convert glucose almost stoichiometrically to three moles of acetic acid [2].

3 Acetic acid producing pathways

3.1 From ethanol by two step

AAB can convert ethanol to acetate in two consecutive steps using membrane-bound quinoproteins (Fig. 1) (ethanol dehydrogenase and acetaldehyde dehydrogenase) [10, 19].

Ethanol is oxidized to acetaldehyde by alcohol dehydrogenase and then aldehyde dehydrogenase oxidizes it to acetic acid. *Acetobacter* uses pyrroloquinoline (PQQ) as the preferred hydrogen acceptor that transfers electrons generated from these reactions. Electrons are initially transferred to ubiquinone, which will be reoxidized by a membrane-associated oxidase. Finally, oxygen is the last electron acceptor, resulting in the formation of H_2O and a proton motive force necessary for energy production through a membrane-bound adenosine triphosphatase (ATPase). As such, AAB are thought to have absolute requirement for oxygen and, hence, are described as obligate aerobes [8].

This ethanol oxidation occurs in all AAB, except the genus *Asaia*. In addition to the set of membrane-bound dehydrogenases catalyzing oxidations, a second set of dehydrogenases, using NAD(P) as cofactor, is located in the cytoplasm [20].

While acetic acid is cytotoxic, it was also identified that the proteins aconitase and putative ATP-binding cassette (ABC)

transporter derived from *Acetobacter aceti* are involved in acetic acid resistance. AAB kill competing organisms by secreting acetic acid, a membrane-permeable organic acid that acidifies the cytoplasm of susceptible microorganisms, poisoning them and disrupting their proton gradients. During this process, the *A. aceti* cytoplasm also becomes acidic, but the cells continue growing and oxidizing ethanol even as the cytoplasmic pH drops to as low as 3.7 [21, 22].

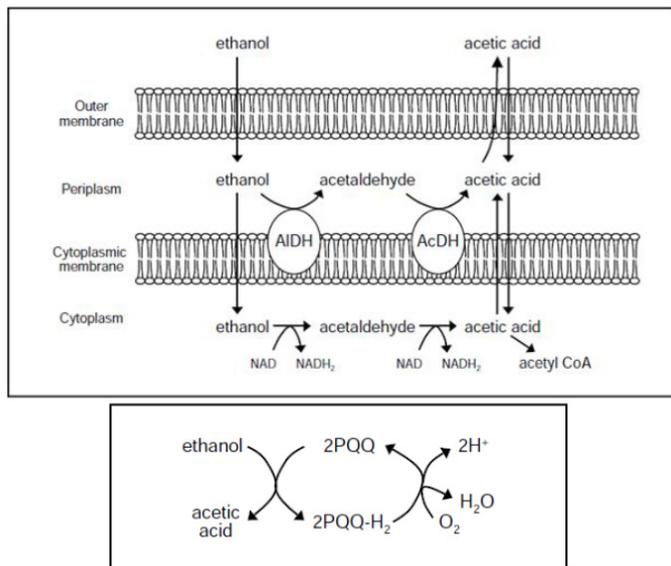
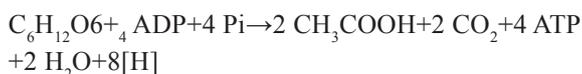


Fig. 1 The formation of acetic acid from ethanol by AAB. PQQ: pyrroloquinoline [23].

3.2 Through Wood-Ljungdahl pathway

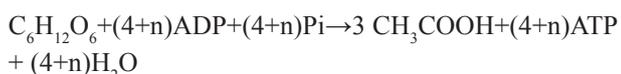
Hexose fermentation in acetogens is passed through glycolysis to form pyruvate, which is then oxidized to acetyl-CoA and CO₂. Acetyl-CoA is then converted to acetyl phosphate by phosphotransacetylase and then to acetate by acetate kinase. This metabolic alternative enables the production of four ATP molecules instead of the two normally produced in glycolysis.



The reducing equivalents and the 2 moles of CO₂ that are gained in this reaction are shuttled to the Wood Ljungdahl pathway (WLP) and form the third molecule of acetate:



In sum, glucose is oxidized to 3 mole of acetate:



The reducing equivalents derive from the oxidation of sugars, but they can also derive from the oxidation of H₂, making acetogens facultative autotrophs that can convert H₂ and CO₂ [2, 18, 24]



3.2.1 Description of the Wood-Ljungdahl Pathway

Fig. 2 shows the key reactions in the Wood-Ljungdahl pathway of CO₂ fixation described to consist of a methyl and a carbonyl branch. One molecule of CO₂ undergoes reduction by six electrons to a methyl group in the methyl branch, while the carbonyl branch involves reduction of the other CO₂ molecule to carbon monoxide. This is followed by condensation of the bound methyl group with CO and coenzyme A (CoA) to make acetyl-CoA. Acetyl-CoA is then either incorporated into cell carbon or converted to acetyl phosphate, whose phosphoryl group is transferred to ADP to generate ATP and acetate, the main growth product of acetogenic bacteria.

In the carbonyl branch of the WLP, one molecule of CO₂ is reduced to CO via the carbon monoxide (CO) dehydrogenase/acetyl-CoA synthase (CODH/ACS) (EC 2.3.1.169).

The first reaction in the methyl branch is the conversion of CO₂ to formate by a formate dehydrogenase (EC 1.2.1.43). The formyl group is then bound to tetrahydrofolate (THF) by a formyl-THF synthetase (EC. 6.3.4.3.), which generates formyl-THF in a reaction that requires the hydrolysis of ATP [2].

The next two steps in the Ljungdahl-Wood pathway are catalyzed by formyl-THF cyclohydrolase (EC 3.5.4.9.) and methylene-THF dehydrogenase (EC 1.5.1.15). Thus formyl-THF is first converted to methenyl-THF by formyl-THF cyclohydrolase, followed by NAD(P)H-dependent reduction of methenyl-THF to form methylene-THF, which is catalyzed by methylene-THF dehydrogenase. In the next reaction methylene-THF is reduced to methyl-THF via the methylene-THF reductase (EC 1.1.99.15.) with the use of NAD(P)H as an electron donor [24].

Finally, a methyltransferase (EC 2.1.1.245) transfers the methyl group from methyl-THF via a corrinoid iron-sulphur protein (CoFeSP) to the CODH/ACS. This bifunctional enzyme reduces CO₂ to CO in the carbonyl branch and fuses it with the methyl group from the methyl branch and with CoA to form acetyl-CoA. It is used by a phosphotransacetylase (E.C. 2.3.1.8) to generate acetyl phosphate, and then turned into acetate via an acetate kinase (E.C.2.7.2.1.) [25].

3.3 Through the glycine synthase pathway

It should be mentioned that purine fermenting bacteria including *Clostridium acidurici*, *C. cylindrosporium*, *C. purinolyticum*, and *Eubacterium angustum* have a pathway of synthesis of acetate from CO₂ and one-carbon compounds (like second CO₂ on Fig. 2) almost exclusively dependent on tetrahydrofolate [17].

The CO₂ fixation through glycine synthase pathway, shown in Fig. 2, serves only as an electron sink to recycle reduced electron carriers that are generated during the fermentation of purines and amino acids. The pathway recycles the electron carriers by reducing two CO₂ molecules to glycine, which is then converted to acetate and secreted from the cell. The glycine

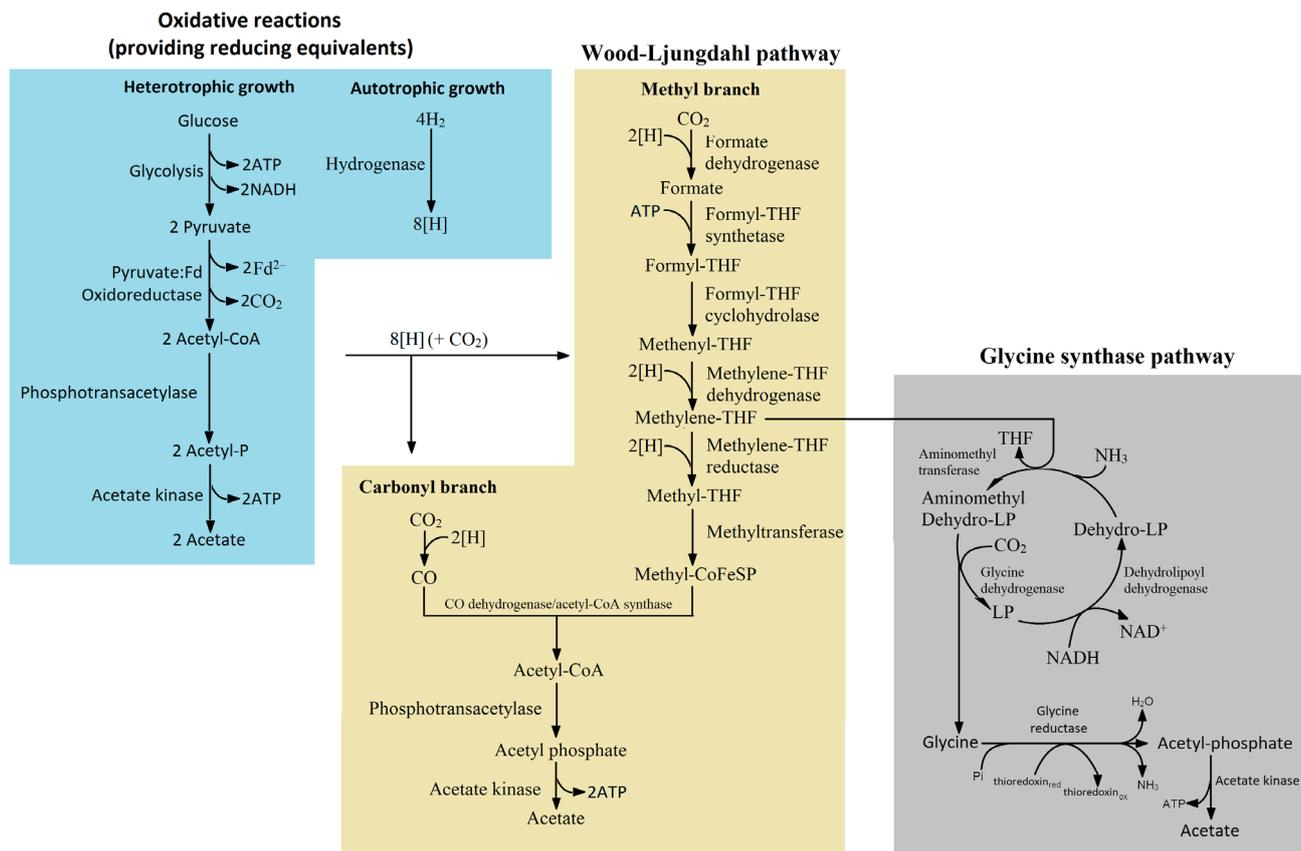


Fig. 2 The Wood–Ljungdahl pathway of acetogenesis and the glycine synthase pathway. THF: tetrahydrofolate Fd²⁻: reduced ferredoxin; LP: lipoyl-protein CpFeSP: corrinoid iron-sulphur protein [H]: reducing equivalent (= 1e⁻ + 1H⁺) [18]

cleavage system, the core of the glycine synthase pathway, is a multi-protein complex that occurs throughout the tree of life and catalyses the reversible synthesis of glycine [17, 25].

The biochemical pathway used for glycine dismutation to CO₂ and acetate has been worked out in detail in the past, and is probably used as well in the reductive direction, as suggested before for some non-homoacetogenic clostridia: CO₂ is reduced via formate, ATP-dependent linkage to tetrahydrofolate, and subsequent reduction to methylene tetrahydrofolate. The methylene derivative is reductively carboxylated and aminated to form glycine which is subsequently reduced to acetate, releasing ammonia. This latter step also releases one ATP in a substrate-level phosphorylation reaction, thus recovering the ATP invested before [17, 25].

Notably, the reductive acetyl-CoA pathway and the glycine synthase pathway are quite similar in their general reaction sequence. Despite this apparent similarity, the usage of the reductive acetyl-CoA pathway is much more versatile than that of the glycine synthase pathway. The reductive acetyl-CoA pathway is used for autotrophic growth, energy conservation, and as an electron sink. However, the glycine synthase pathway serves only as an electron sink. Energy conservation is probably not possible through the glycine synthase pathway [25, 26].

4 Fermentation processes

Acetic acid has been produced from ethanol as vinegar since ancient times by the souring of wine and beer [27].

There is a two-stage fermentation process to produce vinegar, consist of alcoholic and acetous fermentation. Alcoholic fermentation proceeds rapidly and usually depletes most sugars within the first 3 weeks. Fermentable sugars are converted into ethanol by the action of yeasts, normally strains of *Saccharomyces cerevisiae*. In acetous fermentation, the AAB are mainly members of the genus *Acetobacter* and can further oxidise ethanol into acetic acid. Alcoholic fermentation is carried out under anaerobic conditions, whereas acetous fermentation is carried out under aerobic conditions [3].

From a technological point of view, there are two well defined methods for vinegar production: slow processes (as Orleans method and generator method) and quick methods (such as submerged method and method using immobilized cells). The first one is the so called surface culture fermentation, where the AAB are placed on the air–liquid interface in a direct contact with atmospheric air (oxygen). The presence of the bacteria is limited to the surface of the acidifying liquid and hence, it is also considered as a static method. Nowadays, this method is employed for the production of traditional and

selected vinegars and a very long period of time is required to obtain a high acetic degree (acetic acid concentration (%) in vinegar). As a consequence, production time and costs are increased. These systems permit simultaneous acetification and ageing [3, 28, 29].

Some example of higher acetic acid fermentation results are shown in Table 1. Traditional vinegar production generally use indigenious starter culture instead of applying selected (monophyletic) starter culture (SSC), because of the low price of the product. However a recent study shows, that a systematic process development with SSC can confirm the feasibility of applying selected strain [30].

4.1 Orleans method

The Orleans process (Fig. 3) is one of the oldest and well known methods for the production of vinegar. It is a slow, continuous process, which originated in France. High grade vinegar is used as a starter culture, to which wine is added at weekly intervals. The vinegar is fermented in large (200 liter) capacity barrels. Approximately 65 to 70 liters of high grade vinegar is added to the barrel along with 15 liters of wine. After one week, a further 10 to 15 liters of wine are added and this is repeated at weekly intervals. After about four weeks, vinegar can be withdrawn from the barrel (10 to 15 liters per week) as more wine is added to replace the vinegar. Acetous fermentation is slow, taking effect only at the surface of the liquid, where there is sufficient dissolved oxygen, which ensures the conversion of alcohol to acetic acid. One of the problems encountered with this method is that of how to add more liquid to the barrel without disturbing the floating culture. This can be overcome by using a glass tube which reaches to the bottom of the barrel. Additional liquid is poured in through the tube and therefore does not disturb the bacteria. This fermentation lasts between

8 to 14 weeks depending on various factors, such as the fermentation temperature, the initial composition of the alcoholic solution, the nature of the microorganisms and the sufficiency of the oxygen supplied [27, 12, 3, 11].

Improvement of the “Orleans-method” are focusing on thin microbial film and large surface for good oxygen absorption [31, 32].

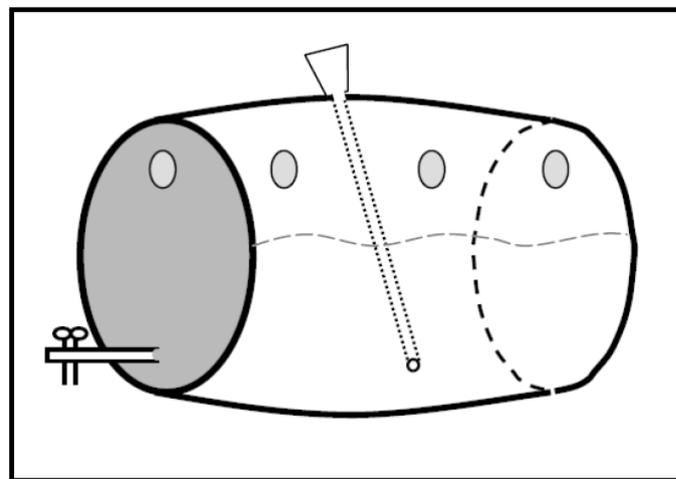


Fig. 3 The Orleans method of vinegar production [23]

4.2 The generator method

Generator processes, also called as “trickling” or “German” processes because they were first introduced in Germany and have been in general use for almost 200 years. In order to achieve faster rates of vinegar production, this method increase the acetification surface contact by using wood shavings as a bacterial supporting material. This method uses a generator, which is an upright tank filled with beech wood shavings or grape stalks and fitted with devices which allow the alcoholic solution to trickle down through the shavings (Fig. 4). This

Table 1 Some example of higher acetic acid fermentation result.

Microorganism	Volume, Substrate	Operation mode	Acetic acid titer (g*L ⁻¹)	Productivity (g*L ⁻¹ *h ⁻¹)	Yield (g*g ⁻¹)	Ref.
Acetobacter sp.	12000L, alcohol	Two stage process	164	1.55	0.96	[38]
Acetic acid bacteria	18000L, alcohol	continuous batch process with recycle	206	n.a	n.a	[39]
Acetobacter aceti 10-8S2	2.5L, ethanol	Fed batch	105	0.70	0.94	[40]
Acetobacter aceti 10-8S2 m60k-1	2.5L, ethanol	Fed batch	111.7	0.89	n.a.	[21]
Acetobacter senegalensis	200L, ethanol	semi-continuous	70	0.09	0.94	[41]
Acetobacter senegalensis	15L, ethanol	semi-continuous fermentation	100.8	1.08	n.a.	[42]
Acetobacter pasteurianus CICIM B7003-02	8L, alcohol	semi-continuous in Frings Acetator	93.09	1.83	0.94	[43]
A. pasteurianus CICIM B7003-02	50 mL, alcohol	repeated batch fermentation	103.81	0.65	n.a.	[44]
Acetobacter pasteurianus FS1	6L, alcohol	semi-continuous	70.25	1.82	0.91	[45]
Acetobacter pasteurianus CICC7015	10L, ethanol	Two stage process	66	0.367	0.31	[46]
L. lactis and C. formicoaceticum	2.6 L, corn meal hydrolyzate	co-cultured immobilized cell fermentation	76	3.2	>0.95	[35]

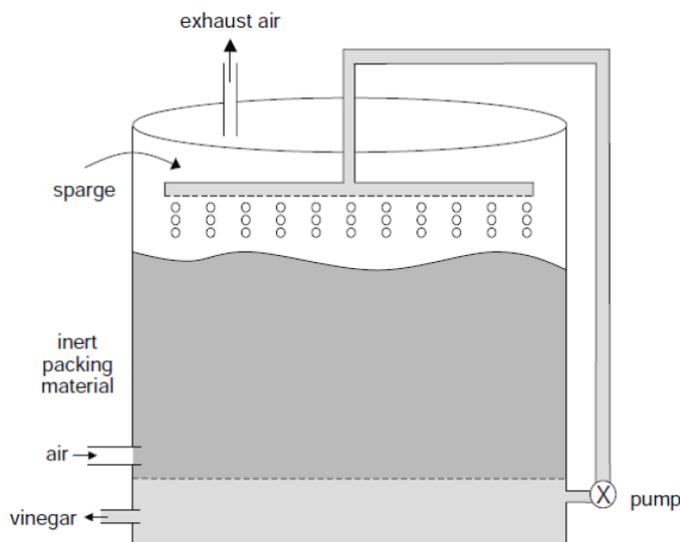


Fig. 4 Generator for vinegar production [23]

process is principally a surface process in which the microbial population is immobilized on wood shavings. The cylindrical tank has a perforated false bottom supporting beech-wood shavings or similar material that will help to increase the flow of air from this bottom to the exit at the top [3, 11, 28].

The liquor is prepared of an adjusted solution of alcohol acidified with acetic acid and special nutrients for the growth of AAB. The liquid is applied in a trough at the top of the chamber and allowed to trickle down over the shavings. The liquid is collected at the bottom of the generator and is re-circulated over the shavings resulting in further oxidation of alcohol until vinegar of the desired concentration is obtained. Oxidation of alcohol by bacteria may result in the increase of temperatures high enough to kill them. In order to keep the temperature down to 25 to 30°C, cooling coils need to be provided [11, 12, 27].

The inconvenients of these method include accumulation of dead AAB over the wood shavings, the growth of cellulose producing bacteria (*Acetobacter xylinum*) on the shaving woods, the infection of the vinegar with anguillulas (vinegar eels), and the difficulties of controlling temperature, oxygen supply as well as the evaporation of the substrate (ethanol) [29].

4.3 Submerged method

The submerged culture system implies that the AAB are suspended in the acetifying liquid in which a strong aeration is applied to assure the oxygen demand. This method was introduced around 1952 for the production of vinegar.

This system basically consists of stainless steel fermentation tanks with a capacity of 10,000–40,000 L, air supply system, cooling system, foam controlling system, loading and unloading valves (Fig. 5). The batch method consist of three steps, first the loading of the raw material and inoculation into the fermentation medium, the fermentation and finally the complete unload of fermented medium. A semicontinuous process is similar, but here part of the finished product is unloaded and

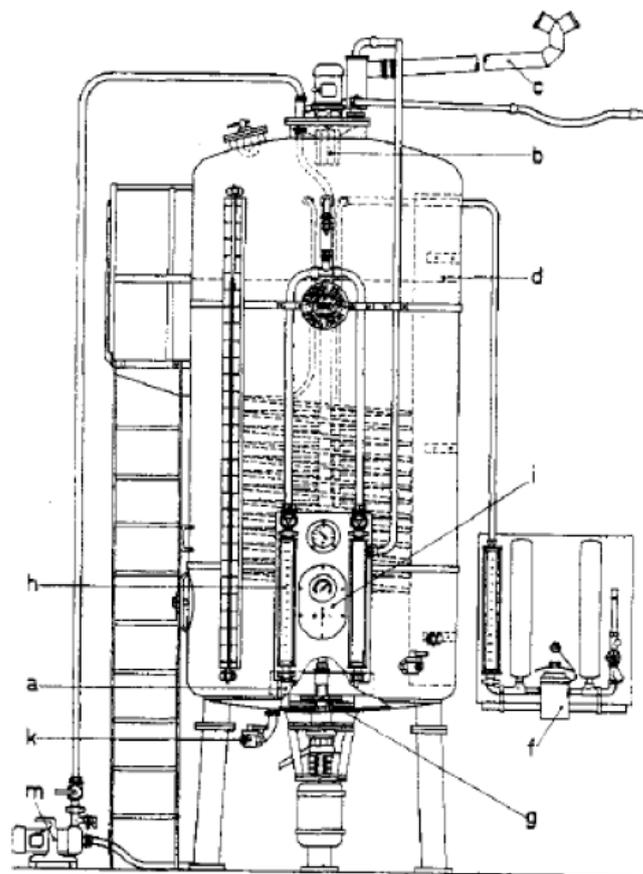


Fig. 5 The Frings Acetator. a) air line, b) defoamer, c) air exhaust line, f) cooling water valve, g) aerator and motor, h) rotameter, i) thermostat controlling, m) charging pump [33]

another part is left in the vessel and it is used to inoculate the next cycle. A continuous process is unloading continuously a small aliquot of the biotransformed product which represents a constant composition in time and a need of continuous substrate supply with the aim of maintaining the volume of the fermentation medium in the bioreactor constant. An important factor is the maintenance of the bacterial culture in the exponential growth phase. At this time it is necessary to provide nutrients and oxygen for the survival of the bacteria [3, 27, 29].

This process allows to reach high acetification rate (increase in acetic degree along time), around 8–9 acetic degrees within 24–48 h. Several methods and different types of bioreactors for submerged acetification have been described and patented, such as Frings Acetator, Cavitator, Bubble column fermenter, or fermenters with different aeration systems like Jet or Effigas Turbine (vinegator). Each system offers very different values for oxygen mass transfer coefficients. Basically these fermenters assure highest oxygen transfer coefficients and are not equipped with additional agitation system. Actually Bubble column fermenter and Frings Acetator are widely used in vinegar producing industries.

The bubble column fermenter consists of a column with a diameter height ratio of 1:5 (or generally more). The upper part of the column has wider diameter which permits the reduction of the foam height at the surface and will facilitate the sedimentation

of the bacterial culture in order to reduce the removal of these along the finished product. The air is supplied from a compressor and passes through a diffuser (perforated plate) at the base of the column by which aeration and mechanical agitation are attained.

In the case of Frings Acetator it is completely automated where rapid acetification and homogeneous product is guaranteed. It consists of a stainless steel equipped with aerator, charging pump, alkalograph, cooling water valve, thermostat cooling, rotameter, cooling coil, air line, air exhaust line and defoamer [11, 19, 29]. Aerator is the most specific part of Acetator, since this special impeller is self-aspirating, and through effective dispersion provide the highest oxygen transfer, described detail elsewhere [27].

4.4 Immobilized cells fermentation

Acetic acid production with immobilized cells was typically carried out by fibrous-bed bioreactor (FBB) (Fig. 6). The immobilized cell bioreactor was made of a (jacketed) glass column packed with spiral-wound terry cloth or cotton towel and had a working volume about 0.4L-1L that was adjustable depending on the strains. The bioreactor was connected to a 5L recirculation reactor containing about 4L of the basal medium. The recirculation reactor was mainly used for pH control. One major advantage of using the immobilized cells is the high reactor productivity resulting from high cell density. The reactor was shown to have cell densities $>30 \text{ g} \cdot \text{L}^{-1}$ and was self-renewing, and eventually established a dynamic steady state due to the balance between the growth of new cells and the removal of dead cells [34–36].

Out of the above fermentations several different vinegar and balsamic vinegar production exits and are reported in a recent study [37]. In these processes ageing has special role to obtain desired quality and composition.

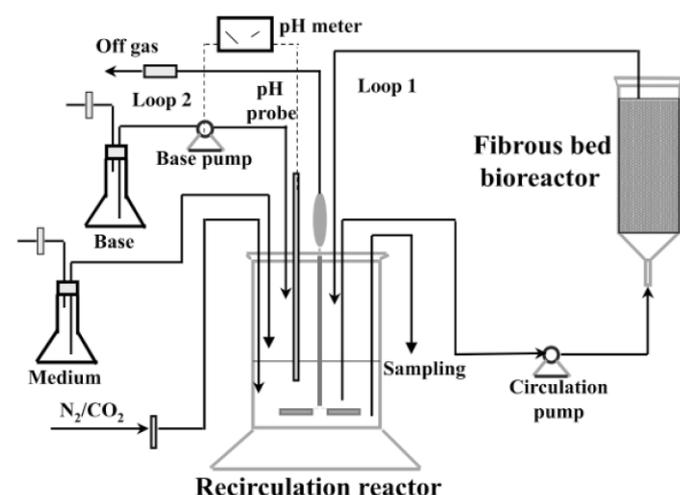


Fig. 6 Schematic illustration of the FBB system used in immobilized cell fermentation [35]

5 Product Recovery and Purification

Energy budget studies and economic analyses have indicated that product separation is the most energy intensive and expensive step for the manufacture of bulk chemicals and liquid fuels through fermentations. The optimum pH range for most acidogenic fermentations is usually 6.0–7.5, which means that the acids are in the ionized state and hence are completely nonvolatile. Reduction of the pH to around the pKa (4.76) values of the acetic acid is necessary before sufficient concentrations of free unionized acid are generated. Fermentation broths, which contain substrates, microbial biomass, inorganic salts, colloids, dissolved ammonia and carbon dioxide, and other organic nonelectrolytes, in addition to acetic acid, which is usually present in concentrations around $100 \text{ g} \cdot \text{L}^{-1}$. Ideally, a process is required to separate the acids alone and recycle the remaining materials back to earlier stages in the process. The separation of acetic acid from water has been explored for many years, with various technologies being developed such as fractional distillation, azeotropic dehydration distillation [47], solvent extraction [48], combination of the above methods, extractive distillation [49, 50], and adsorption [51].

The following sections give a brief review for various methods used for recovery of acetic acid. Some recovery results are summarized in Table 2.

Table 2 Possible operation result of acetic acid recovery

Method	Volume	Recovery	Ref
Adsorption	50mL	82%	[51]
Extraction	40mL	96%	[52]
Extraction	750mL	88%	[53]
Extractive distillation	372mL	68%	[49]
Nanofiltration	800mL	99%	[68]
Reactive distillation	n.a.	57.6%	[67]
Solvent extraction	200mL	96.3%	[48]

5.1 Liquid-Liquid Extraction

In the design of an extraction process three main aspects play a role. First of all the pH should be regulated in order to have the acid in undissociated form, as only undissociated acid is extracted to the solvent phase. Secondly, the extraction solvent should be chosen such that it has a high partition coefficient of the carboxylic acid, meaning it has a high preference for the organic phase over the aqueous phase, and a high selectivity for extraction of carboxylic acid over water, in order to limit the co-extraction of water. And thirdly, the extraction system should be reversible, so the solvent should be well recoverable [52, 53].

The three main groups of extraction solvents are (I) the carbon-bonded oxygen bearing extractants such as alcohols and ethers, (II) organophosphorous extractants such as trioctylphosphine oxide (TOPO) and tributylphosphate (TBP), and (III)

aliphatic amine extractants such as tri- *n*-octylamine (TOA). The types of extractants ((II) and (III)) show strong complexation and are usually used in one or more diluents, which mainly act as solvent for the complexes that these extractants form with the carboxylic acid and to adjust the viscosity and density of the organic phase. For the first group (I) the distributions are relatively low for the recovery of acids from the dilute aqueous acid solutions found in most fermentation streams [54].

A new type of solvents proposed recently concerns ionic liquids, which are seen as potential green solvents. Attractive properties of ionic liquids are their temperature stability and practically zero vapor pressure [55, 56].

For intermediate concentrations (10–50%), liquid–liquid extraction is employed and is usually followed by azeotropic distillation, as low-molecular-weight solvents (esters, ethers, and ketones) have sufficiently high distribution coefficients for acids at low concentrations. An economically attractive scheme for the recovery of acids from a dilute aqueous stream by extraction with a hydrocarbon, followed by distillation, has been developed by Helsel [57].

The inherent advantages of this technology are as follows: (1) a high distribution coefficient of acetic acid in very dilute aqueous solution allows a small solvent usage; (2) good phase separation reduces the size of the extraction equipment; (3) the stability and high boiling point of the solvent enable a small volume of acid to be recovered from a much larger solvent flow; and (4) the low solubility of the solvent in water permits higher selectivity and obviates the need for raffinate stripping. These advantages represent significant energy and capital savings compared with other recovery processes, especially when the acetic acid concentration is less than 5% [58].

5.2 Adsorption

Acetic acid may be recovered by adsorption on solid adsorbent such as activated carbon [51, 59], Anatase TiO(101) [60] and cross-linked polymer adsorbent of pyridine skeletal structure. The adsorbents need to have good selectivity and high adsorption capacity for acetic acid even in the presence of inorganic salts [59]. The selected elutants were aliphatic alcohol, aliphatic ketones and carboxylic esters [61]. Acetic acid recovery by adsorption is not enough effective and also uneconomical, due to not used in the industry.

5.3 Precipitation

Precipitation is a classical method for the recovery of organic acids from broth, which has been used for the isolation of lactic acid and citric acid industrially since the last century. Precipitation can recover organic acids from a bulk of fermentation broths efficiently, which makes it more competitive, especially in preliminary purification.

Taking calcium precipitation as an example, organic acids are often separated by four steps. First, the fermentation liquid

is filtered to obtain the mother liquor and to remove impurities, and then $\text{Ca}(\text{OH})_2$ or CaCO_3 is added to the mother liquor beside agitating. Second, the calcium salt of organic acid is filtered off from the fermentation. Third, the calcium salt is treated with a high concentration of sulfuric acid to free the desired acid. Afterwards, the purified acid is obtained by further purification processes [62].

After years of development, precipitation is now a well-established technique. The main advantages of precipitation are that it is highly selective, has no phase transition, and has high product purity. Meanwhile, finding proper precipitants for the products is the key factor for this method. As for the industrial-scale calcium precipitation process, when a one molar amount of organic acid is converted, an equal amount of $\text{Ca}(\text{OH})_2$ / CaCO_3 and H_2SO_4 are consumed, and low valuable calcium sulfate is formed [63]. Despite the wide application of calcium based precipitation technique, for acetic acid purification other precipitants should be applied, because the solubility of the Ca-acetate is relatively high, which results almost no precipitation at the reached acetic acid concentration via fermentation.

5.4 Distillation

Water is the lower boiling component and relative volatility of water to acetic acid is very low. Although acetic acid and water do not form an azeotrope, it is necessary to have a large number of equilibrium stages and a very high reflux ratio to obtain glacial grade acid by simple distillation [49]. As an alternative to fractionation, to reduce energy consumption, azeotropic dehydration can be employed with addition of another liquid. In this technique, the entrainer carries the water overhead in the distillation column with the mixture being phase separated after condensation and entrainer being returned to the column. It is effective only for high concentration of acids [58, 61].

In the method proposed by Othmer [64], a water-insoluble ‘withdrawing’ liquid, called the entrainer, is added, which decreases the effective boiling point of water relative to that of acetic acid by the formation of a low boiling point azeotrope. Low molecular weight esters such as butyl acetate are generally used as entrainers [12].

In extractive distillation, countercurrent washing of mixed vapors in a distillation column takes place via a descending stream of a high boiling point liquid, which is preferentially solvent for one of the components. This method was first developed by Suida [65] in Austria for the removal of acetic acid from pyroligneous acid, which requires more expensive equipment and consumes a greater amount of steam than other methods.

5.5 Reactive distillation

Reactive distillation (RD) holds dominance over conventional physical separation methods such as distillation and extraction. Distillation is associated with the high costs involved in vaporizing the more volatile water that exists in

high proportions and possesses a high latent heat of vaporization. Extraction is limited in view of the distribution of the components in the reacting system [50].

The execution of RD reduces capital and operating costs, and allows a wider range of operating conditions. RD is receiving increasing attention and holds a huge potential for the recovery of acetic acid. RD is the combination of chemical reaction and distillative product separation in single piece of equipment, offers several dividends over conventional processes in which the reaction and the product separation are done in series, especially in that reactions which are limited by equilibrium constraints. Improved selectivity, increased conversion, better heat control, effective utilization of reaction heat, scope for difficult separations and the avoidance of azeotrope are a few of the advantages that RD offers. The combination of reaction and separation by distillation in one unit allows a continuous production, with reduced processing time. This leads to constant high product quality and at the same time simpler maintenance and process control, which is especially valuable for larger production capacities. Well-defined and narrow residence time at gentle conditions throughout the whole plant minimizes degradation of the fatty acids and fatty acid esters. No neutralization, separation or recycling of catalyst is necessary. There is no necessity of emptying and cleaning the equipment, reducing the waste streams to the absolute minimum. The energy consumption of the RD process is only half of the conventional batch one. Also the size of the plant could be drastically reduced [66, 67].

5.6 Membrane Processes

Membrane technologies have been used in the recovery process of organic acids because of their adaptability and selectivity. With the development of recovery processes and material technology, membrane separation attracts more attentions, especially in the in situ product removal technology. A membrane is essentially a thin artificial or natural barrier, which permits selective mass transport of solutes or solvents across the barrier to achieve the physical separation and enrichment purposes. High purity and high yield can be obtained by membrane separation for acetic acid. The main membrane filtrations used for the separation of organic acids includes microfiltration, ultrafiltration, nanofiltration (NF) [68], reverse osmosis, pervaporation, and electrodialysis [69].

5.7 In situ product removal

In situ product removal (ISPR) is the fast removal of organic acid, thereby preventing its subsequent interference with cellular or medium components. It couples fermentation with separation, including extraction, resin, and membrane, to realize a continuous process. Moreover, the remove of products can minimize the toxicity of metabolites to microorganisms.

The suggested extraction methods are ion exchange, solvent extraction, and membrane separation. Solvent extraction by TOPO may be very useful in removing the acids preferentially for concentrations ranging from a low level of 1–3% to the medium of 20–25%. It was reported that high efficient production and purification of propionic acid were attained by using a novel extractive fermentation process with advantages of including better pH control and a purer product [12].

In these processes, the problem of membrane fouling exists, which requires frequent cleaning of the dialyzer. It gives a higher extent of acids separation but with increased power and energy consumption. The drawbacks are being of hindered implementation, mainly complexity of operation and swelling in liquid surfactant membrane and supported liquid membrane often suffers from membrane instability [61].

For the final purification, the acid is distilled in the presence of KMnO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$, or similar oxidants. KMnO_4 is more expensive but oxidizes a wider range of impurities. Glacial acetic acid is obtained at the top. The bottom product is passed through a solvent extraction system containing toluene or butyl acetate, and the oxidized organic impurities are removed with the solvent layer, while the MnO_4^- stays in the water layer to be reused [12].

6 Conclusion

Fermentation has been found to be a promising method for the production of acetic acids from renewable biomass. However, it is still a challenge to efficiently separate the organic acids from a mix of multiple diluted components and reduce the impurity of other organic acids with similar properties to a minimum. Current methodologies used in recovery processes all have their limitations and their improvements are especially needed with regard to yield, purity, and energy consumption. Therefore, there is a need to develop a process that should ideally be simple to carry out and allow the purification of acetic acid directly from the fermentation broths. Besides this, the emergence of new materials and the development of technologies would boost the recovery processes, which would make the biological process more competitive than the chemical routes and promote the development of green chemistry [62]. A major decrease in the capacity to synthesize industrial acetic acid from methanol and CO or by other chemical processes may occur at the end of the next century due to depletion of natural gas and petroleum resources together with an increasing demand for these materials worldwide. With price increases accompanying this situation, bacteria-based processes can certainly become major players in the glacial acetic acid market [32].

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