Glycerol production

In ethanol production by yeast in which:-

glucose → pyruvate → acetaldehyde + CO 2 → ethanol

Acetaldehyde convert to ethanol is known as Neubergs first fermentation. Glycerol is formed as a product through:-

Sulfite processes (Neubergs second fermentation).

Through the addition of sodium hydrogen sulfite which combine with acetaldehyde :-

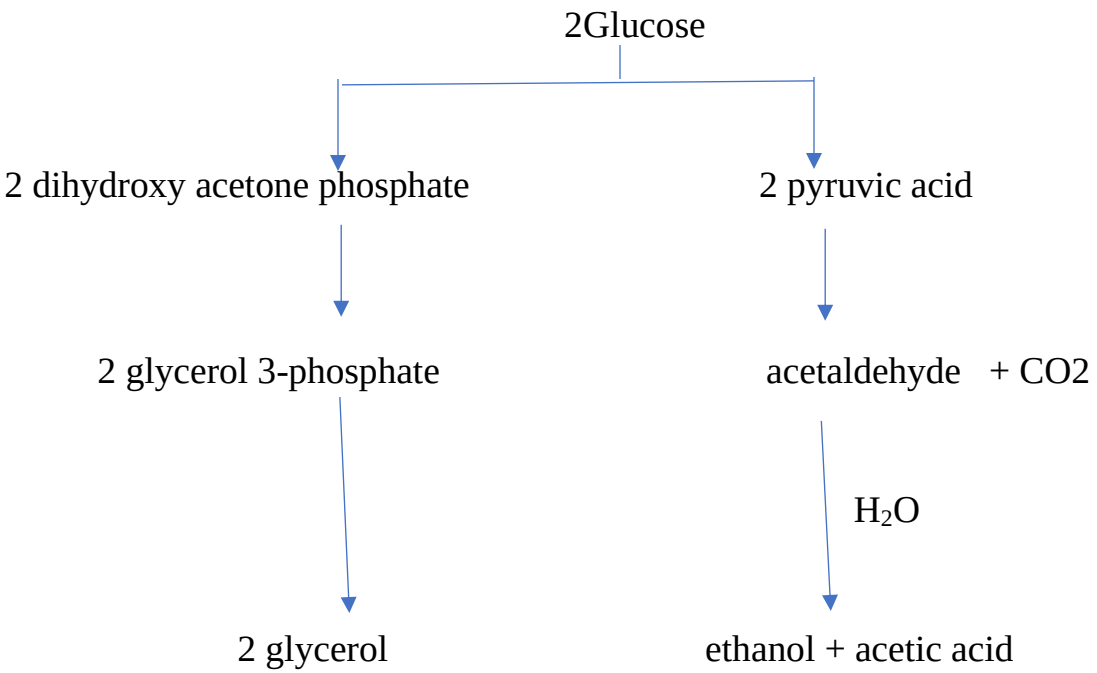
$CH_3CHOH + NaHSO_3 \longrightarrow CH_3CHOH\text{-}SO_3Na + NaOH$

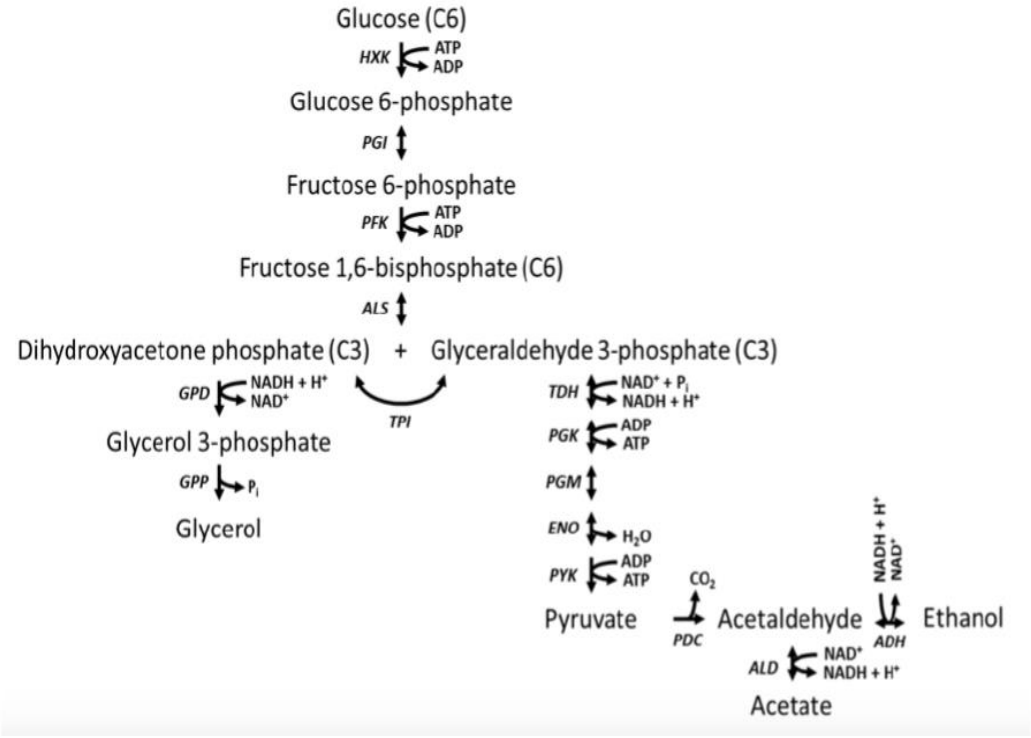
By trapping the acetaldehyde in this way it cannot function as a hydrogen acceptor in place of acetaldehyde , dihydroxy acetone phosphate becomes the hydrogen acceptor which reduced to glycerol-3phosphate which is dephosphorylated to glycerol.

Glucose + HydrogenSulphite → glycerol +Aetaldehyedesulphite + CO_2

Eoff processes (Neubergs third fermentation)

The addition of alkali ($NaHCO_3$, Na_2HPO_4) to fermenting yeast extracts also leads to the formation of glycerol because acetaldehyde undergoes dismutation to ethanol and acetate and hence does not function as a hydrogen acceptor .


2Glucose
2 dihydroxy acetone phosphate
2 pyruvic acid
2 glycerol 3-phosphate
acetaldehyde + CO2
H2O
2 glycerol
ethanol + acetic acid


Glucose (C6)
HXK
ATP
ADP
Glucose 6-phosphate
PGI
Fructose 6-phosphate
PFK
ATP
ADP
Fructose 1,6-bisphosphate (C6)
ALS
Dihydroxyacetone phosphate (C3) + Glyceraldehyde 3-phosphate (C3)
GPD
NADH + H+
NAD+
TPI
TDH
NAD+ + Pi
NADH + H+
Glycerol 3-phosphate
PGK
ADP
ATP
GPP
Pi
PGM
Glycerol
ENO
H2O
PYK
ADP
ATP
CO2
NADH + H+
NAD+
Pyruvate
PDC
Acetaldehyde
ADH
Ethanol
ALD
NAD+
NADH + H+
Acetate

Acetic acid fermentation

Raw material is alcoholic solution which could be prepared from several methods:-

1- Starch materials (In this case these materials must be first treated by enzymes or acids in order to hydrolyze the starch to a fermentable sugar hexose sugar)

2- Dried fruits (by addition of water to it)

3- Fresh fruits (by extracting the juice)

4- Other materials which is high in sugar content such as molass (diluted with water).

Vinegar

is a product resulting from the conversion of alcohol to acetic acid by acetic acid bacteria, *Acetobacter spp*. The name is derived from French (Vin = wine; Aigre-sour or sharp).

Although acetic acid is the major component of vinegar, the material cannot be produced simply by dissolving acetic acid in water.

When alcoholic fermentation occurs and later during acidifications many other compounds are produced, depending mostly on the nature of the material fermented and some of these find their way into vinegar.

The other compounds include non-volatile organic acids such as malic, citric, succinic and lactic acids; unfermented and unfermentable sugars; oxidized alcohol and acetaldelyde, acetoin, phosphate, chloride, and other ions.

Furthermore, reactions also occur between these fermentation products. Ethyl acetate, for example, is formed from the reaction between acetic acid and ethanol. It is these other compounds which give the various vinegars their bouquets or organoleptic properties.

USES

(1) Ancient uses: The ancient uses of vinegar which can be seen from various records include a wide variety of uses including use as a food condiment, treatment of wounds, and a wide variety of illnesses such as plague, ringworms, burns, lameness, varicose veins. It was also used as a general cleansing agent. Finally, it was used as a cosmetic aid.

(2) Modern uses: Vinegar is used today mainly in the food industry as;
(a) a food condiment, sprinkled on certain foods such as fish at the table.

(b) for pickling and preserving meats and vegetables; vinegar is particularly useful in this respect as it can reduce the pH of food below that which even spore formers may not survive.

(c) It is an important component of sauces especially renowned French sauces many of which contain vinegar.

(d) Nearly 70% of the vinegar produced today is supplied to various arms of the food industry where it finds use in the manufacture of sauces, salad dressings, mayonnaise, tomato productions, cheese dressings, mustard, and soft drinks. Most of the vinegar used in industry is the distilled or concentrated type.

ORGANISMS

The bacteria converting alcohol to acetic acid under natural conditions are film forming organisms on the surface of wine and beer.
The film was known as ‘mother of vinegar’ before its bacteriological nature became known.
The bacteria were first described as Mycoderma (viscous film) in 1822. Later other workers classified them in M. vini (forming film on wine) an M. acetic (forming film on beer).
Pasteur confirmed that acetic acid is produced only in the presence of the bacteria, but he did not identify them.
The genus name Acetobacter was put forward by Beijerinck in 1900.

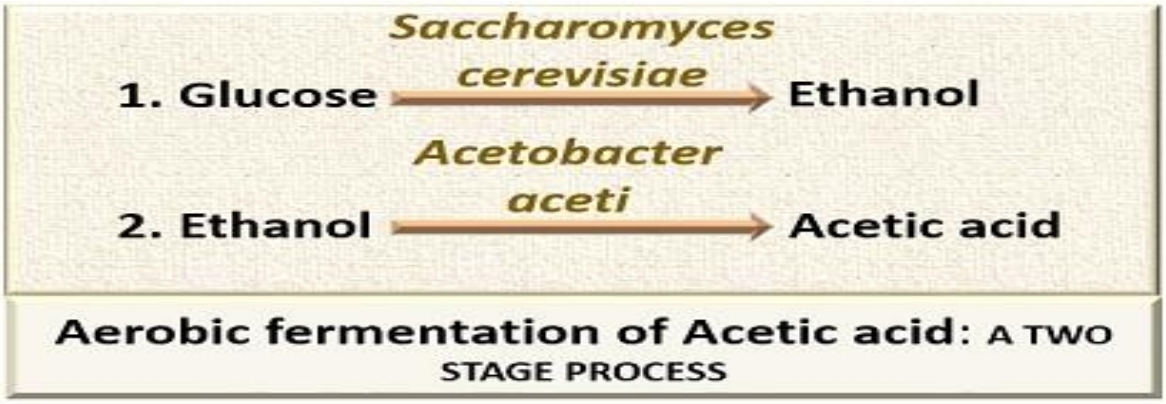
Strains of acetic acid bacteria to be used in industrial production should

a) tolerate high concentrations of acetic acid;

b) require small amounts of nutrient;

c) not overoxidize the acetic acid formed; and

d) be high yielding in terms of the acetic acid produced.



Aerobic fermentation of Acetic acid: A TWO STAGE PROCESS

Fermentation conditions

1- Anaerobic conditions in alcoholic fermentation (temp. 23-27 oC)
(Saccharomyces cerevisiae)

2- Aerobic conditions in acetic acid fermentation (temp. 27-30 oC)
(Acetobacter spp. (aceti))

Acidification

10-25% strong vinegar (6%) is added to the alcoholic solution :-

1- Favor the presence of acetic acid bacteria.

2- Retard the growth of undesirable microorganisms.

MANUFACTURE OF VINEGAR

The three methods used for the production of vinegar are

1-The Orleans Method (also known as the slow method),

2-The Trickling (or quick) Method

3-Submerged Fermentation.

The last two are the most widely used in modern times.

The Orleans (or Slow) Method

The oldest method of vinegar production is the 'let alone' method in which wine left in open vats became converted to vinegar by acetic acid bacteria entering it from the atmosphere. Later the wine was put in casks and left in the open field in the 'fielding process'. A small amount of vinegar was introduced into a cask of wine to help initiate fermentation. The introduced vinegar not only lowered the pH to the disadvantage of many other organisms but also introduced an inoculum of acetic acid bacteria.The casks were wooden and of approximately 200 liter capacity. It was never filled beyond about two-thirds of its capacity so that there was always a large amount of air available above the wine.

A thick film of acetic acid bacteria formed on the wine and converted it in to vinegar in about five weeks. About 10-20% of the vinegar was drawn off at weekly intervals and replaced with new wine. The withdrawal and replenishment were done from the bottom of the cask so that the film would not be disturbed. Often a series of casks was present and the transfer was done from one cask to another.

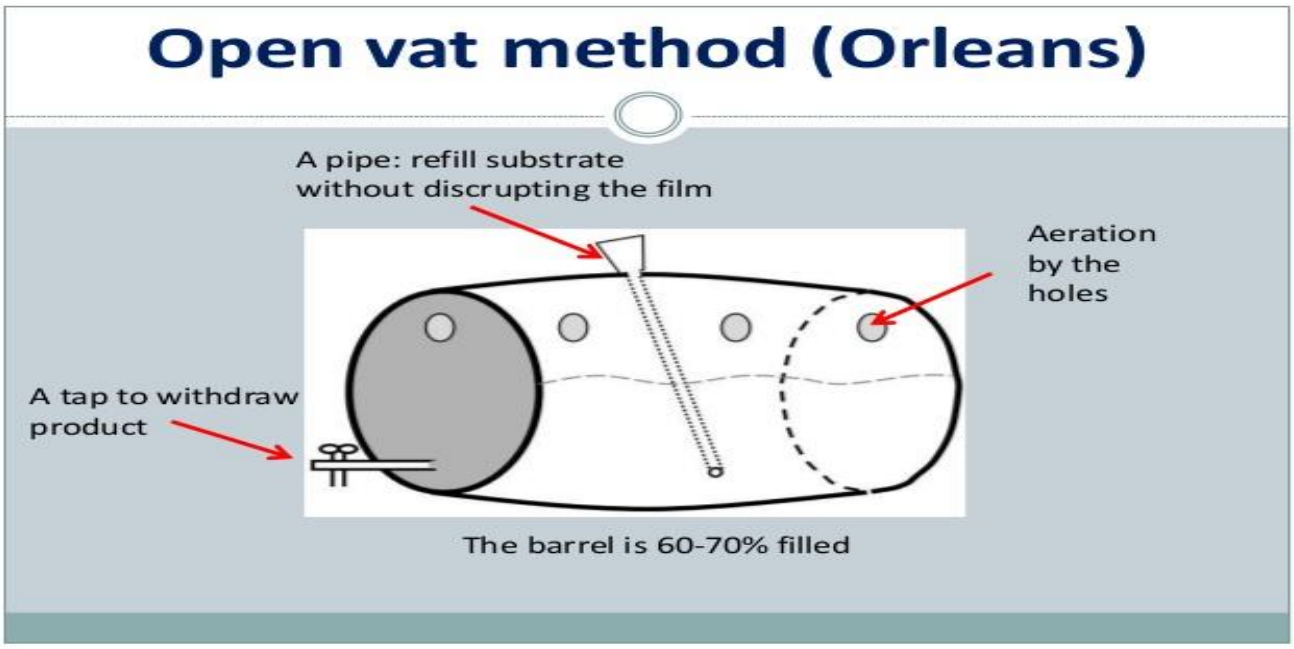
Often due to its thickness and consequent weight and sometimes due to disturbance, the film sank. When this happened the whole process had to be restarted, since acetic acid bacteria are aerobic. Following Pasteur's (1868) suggestion, the film of bacteria often developed in wooden rafts is placed in the cask for this purpose. Later on the casks were stored, especially in the Orleans district of France, in a heated building or in an underground cellar to speed up the process. The process now derives its name from the district.

The process had a number of disadvantages:

(a) It was slow in comparison with later methods, taking up to five weeks sometimes as against days, hence it is also known as the slow method.
(b) It was inefficient, yielding 75-85% of the theoretical amount.
(c) The 'mother of vinegar' usually gradually filled the cask and effectively killed the process.

Despite these disadvantages the product was of good quality and it continued to be used in many European countries long after the introduction of the Quick Process, described below.

Modern vinegar production uses mainly the Trickling (quick) and submerged methods to be described below. There are fewer and fewer of the Orleans equipment in use today.



The Trickling Generators (Quick) Method

Credit for devising the fore-runner of the modern trickling generator is usually given to the Dutch Boerhaave who in 1732 devised the first trickling generator in which he used branches of vines, and grape stems as packing. Improvements were made by a number of other people from time to time. Later ventilation holes were drilled at the bottom of the generator and provided a mechanical means for the repeated distribution of the alcohol acetic acid mixture over the packing.

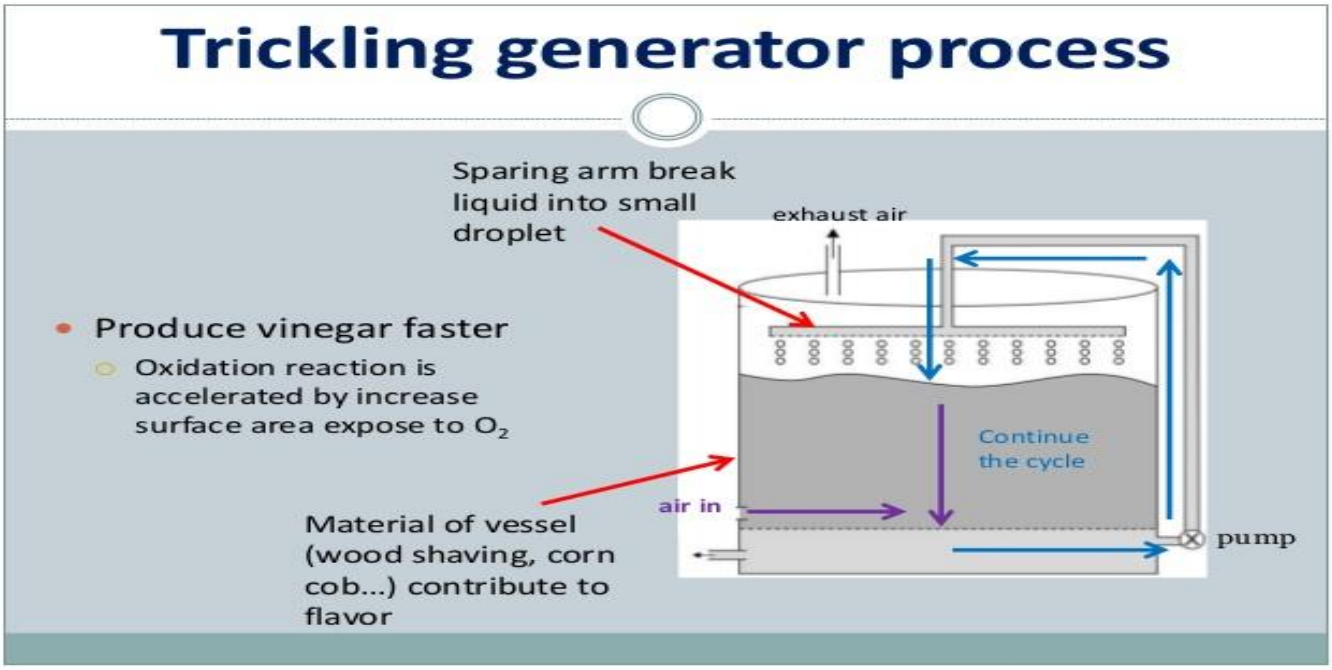
The heat generated by the exothermic reaction in the generator caused a draft which provided oxygen for the aerobic conversion of alcohol to acetic acid. This latter model of the quick method (sometimes called the German method) enabled the production of vinegar in days instead of in weeks. It remained in vogue unmodified for just over a century when several modifications were introduced in the

Frings method, including: (a) forced aeration (b) temperature control (c) semicontinuous operation.

The modern vinegar generator consists of a tank constructed usually of wood preferably of cypress and occasionally of stainless steel. A false bottom supports the coils of Birchwood shavings and separates them from the collection chamber which occupies about one fifth of the total capacity of the generator.

A pump circulates the alcohol-acetic acid mixture from the reservoir through a heat exchanger to the top of the generator where a spray mechanism distributes it over the packing in much the same way as a trickling filter functions in waste-water treatment . Air is forced through the false bottom up through the set-up. The cooling water in the heat exchanger is used to regulate the temperature in the generator so that it is between 29°C and 35°C; this is determined with thermometers placed at different levels of the generator.The top of the generator is covered but provision exists for exhaust air to be let out.

Meters measure three parameters: (a) the circulation of the mash (b) the flow of cooling water through the heat exchange (c) the amount of air delivered through the system.



Theoretically, 1 gm of alcohol should yield 1.304 gm of acetic acid but this is hardly achieved and only in unusual cases is a yield of 1.1 attained.

2 ethanol (46) 2 acetic acid (60) (130.4%)

Glucose (180) 2 acetic acid (60) (66.67%)

Over-oxidation can occur and it is undesirable.
In over-oxidation acetic acid is converted to CO_2 and H_2O.
It occurs when there is a lack or low level of alcohol.
It occurs more frequently in submerged fermentations that in the trickle processes.