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Microorganisms and Processes Involved in the Production of Organic Acids (Citric and Lactic)

A shallow, light-brown ceramic bowl filled with a fine white powder. A wooden spoon is partially submerged in the powder. In the background, two bright yellow lemons are visible, one slightly out of focus. The entire scene is set against a light, neutral background.

Production of Citric Acid and Uses:

- a) Tribasic acid, crystallizes with large rhombic crystals containing one molecule of water of crystallization, which is lost when it is heated to 130°C .**
- b) At 175°C , it is converted to itaconic acid, aconitic acid, and other compounds.**



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**Major food acidulant
used in manufacture
of jellies, jams, sweets
and soft drinks**

**Used for artificial
flavoring in various
foods and soft drinks**

**Sodium citrate is
employed in
processed cheese
manufacture**

**Sodium citrate is used
in prevention of blood
clotting**

**Used in astringent
lotions (aftershave
lotions because of its
low pH)**

**Strong tendency to
form complexes hence
it is widely used in
electroplating, leather
tanning**

**Used in hair rinses
and hair and wig
setting fluids**

**Manufacture of
detergents**

Uses in food industry, medicine and cosmetics



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Biochemical basis of production of citric acid

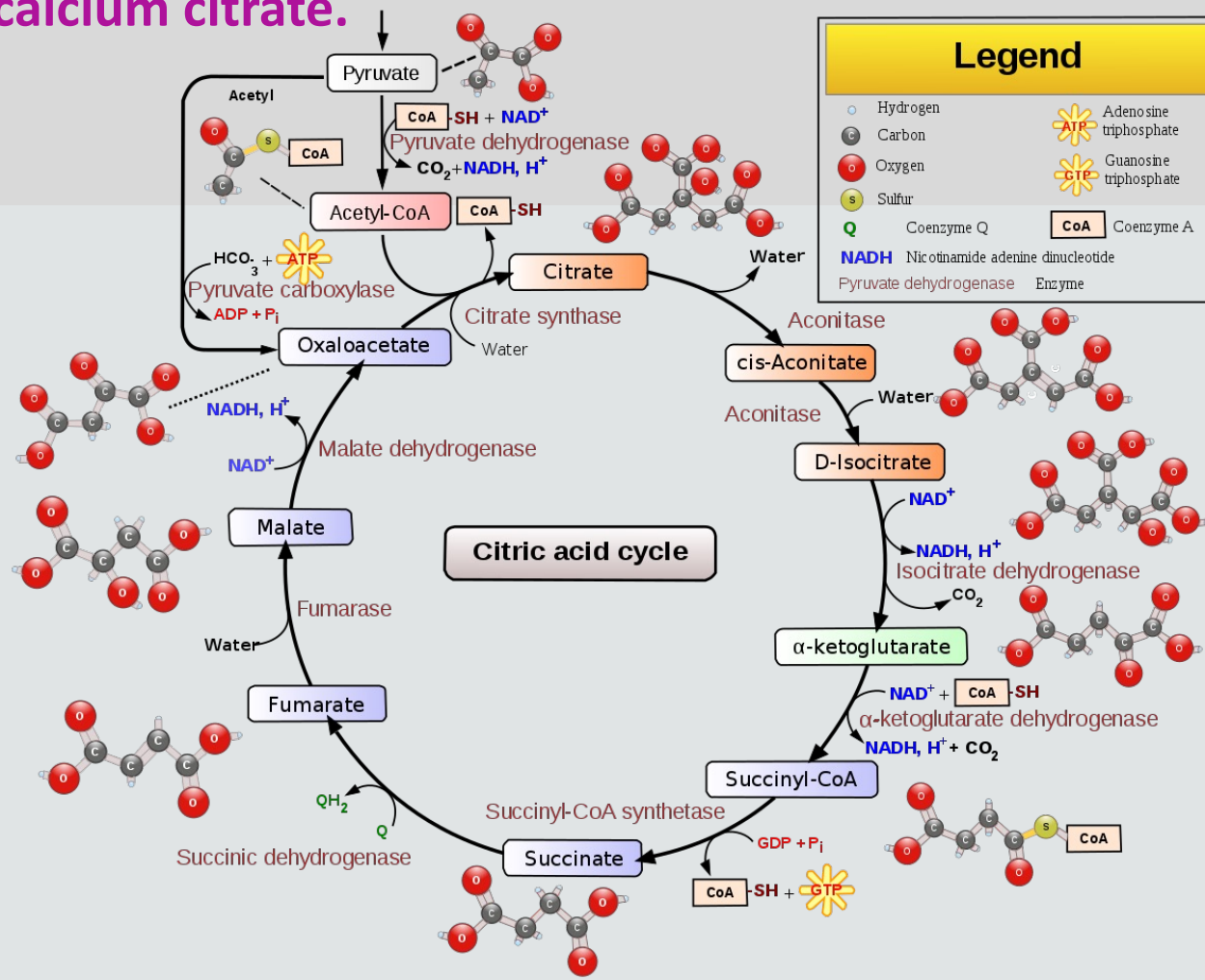
Citric acid is an intermediate in citric acid cycle. Acid can be caused to accumulate by methods:

- **By mutation**-giving rise to mutant organisms which may only use part of metabolic pathway
- **By inhibiting free-flow of cycle through altering environmental conditions** e.g. temperature, pH, medium composition (especially elimination of ions and cofactors considered essential for particular enzymes).
Following environmental conditions are applied to increase citric acid production:
 - (i) Concentrations of iron, manganese, magnesium, zinc, and phosphate must be limited.
 - (ii) Isocitrate dehydrogenase are inhibited by anaerobiosis, hence, limited aeration is done on fermentation so as to increase yield of citric acid.
 - (iii) Presence of citric acid itself inhibits TCA and hence encourages production of more citric acid.

Fermentation for citric acid production

Surface fermentation :

- Surface fermentation using *Aspergillus niger* may be done on rice bran, or in liquid solution in flat aluminium or stainless steel pans.
- Special strains of *A. niger* which produce citric acid despite high content of trace metals in rice bran are used.
- Citric acid is extracted from bran by leaching and then precipitated from resulting solution as calcium citrate.





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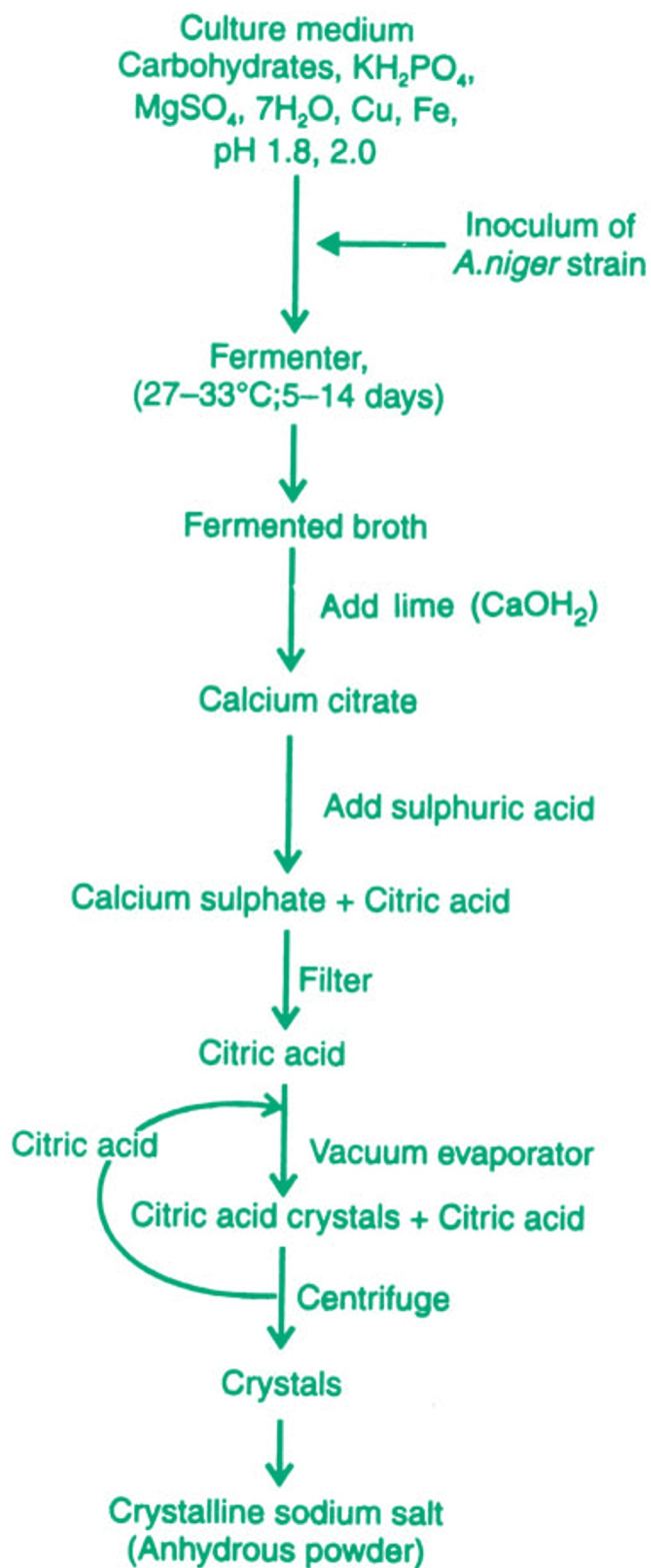
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Submerged fermentation:

- Fermentor is made of acid-resistant materials (stainless steel).
- Carbohydrate sources are molasses decationized by ion exchange, sucrose or glucose.
- The pH is never allowed higher than 3.5.
- Copper is used up to 500 ppm as an antagonist of enzyme aconitase which requires iron.
- 1-5% of methanol, isopropanol or ethanol when added to fermentations containing unpurified materials increases the yield.
- As high aeration is deleterious to citric acid production, mechanical agitation is not necessary and air may be bubbled through.
- Antifoam is added.
- Fungus occurs as uniform dispersal of pellets in medium.
- Fermentation lasts for five to fourteen days.

• Extraction

The broth is filtered until clear. Calcium citrate is precipitated by the addition of magnesium-free $\text{Ca}(\text{OH})_2$. Since magnesium is more soluble than calcium, some acid may be lost in the solution as magnesium citrate if magnesium is added. Calcium citrate is filtered and the filter cake is treated with sulfuric acid to precipitate calcium. The dilute solution containing citric acid is purified by treatment with activated carbon and passing through iron exchange beds. The purified dilute acid is evaporated to yield crystals of citric acid.





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Properties and chemical reactions of lactic acid

Lactic acid is a three carbon organic acid: one terminal carbon atom is part of an acid or carboxyl group; the other terminal carbon atom is part of a methyl or hydrocarbon group; and a central carbon atom having an alcohol carbon group. Soluble in water and water miscible organic solvents but insoluble in other organic solvents

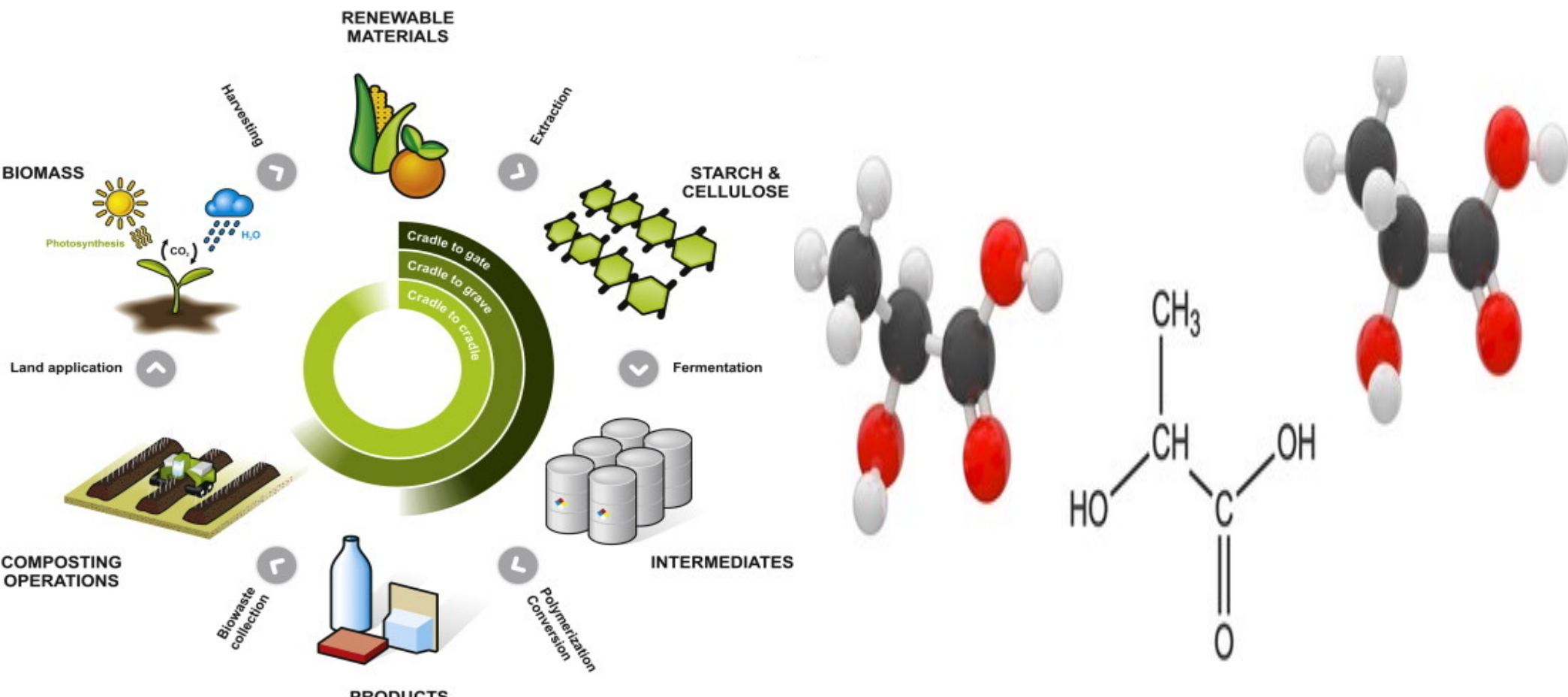


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- Technical grade lactic acid is used as an acidulant in vegetable and leather tanning industries.
- Various textile finishing operations and acid dyeing of food require low cost technical grade lactic acid to compete with cheaper inorganic acid.
- Used in many small scale applications like pH adjustment, hardening baths for cellophanes used in food packaging, alkyl resin modifier and textile printing developers, adhesive formulations, electroplating and electropolishing baths, detergent builders.
- Pharmaceutical and cosmetic applications and formulations in topical ointments, lotions, anti acne solutions, humectants, dialysis applications.
- Calcium lactate can be used for calcium deficiency therapy, and as an anti caries agent.
- Polymers of lactic acids are biodegradable thermoplastics. Degradation can be controlled by adjusting composition, and molecular weight.

- Lactic acid esters like ethyl/butyl lactate can be used as green solvents. High boiling, non-toxic and degradable components.
- Poly L-lactic acid with low degree of polymerization can help in controlled release for large-scale agricultural applications.
- Lactic acid produced commercially by fermentation and the first organic acid to be produced by fermentation.





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Fermentation for lactic acid

1. Homofermentative lactic acid bacteria, *Lactobacillus* spp., especially *L. delbruckii*.
2. In recent times *Rhizopus oryzae* has been used.
3. Both organisms produce L- form of acid, but *Rhizopus* fermentation has advantage of being much shorter in duration.
4. Lactic acid is very corrosive and fermentor, which is usually between 25,000 and 110,000 liters in capacity, is made of wood.
5. Special stainless steel (type 316) may be used.
6. Sterilized by steaming before introduction of broth as contamination with thermophilic clostridia yielding butanol and butyric acid is common.



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7. During step-wise preparation of inoculum, which forms about 5% of total beer, calcium carbonate is added to medium to maintain pH at around 5.5-6.5.
8. Carbon source used in broth includes whey, corn hydrolysates, sulfite liquor, and molasses.
9. For recovery of high quality lactic acid, purified sugar and minimum of other nutrients are used.
10. *Lactobacillus* requires addition of vitamins and growth factors for growth. Requirements of nitrogen are often met with ground vegetable materials such as ground malt sprouts or malt rootlets.
11. Fermentation with *Lactobacillus delbruckii* is usually for 5 to 10 days and *Rhizopus oryzae*, it is about 2 days.
12. Lactic fermentation is anaerobic, organisms involved are facultative, complete anaerobiosis is not necessary.
13. Temperature of fermentation is high around 45°C.

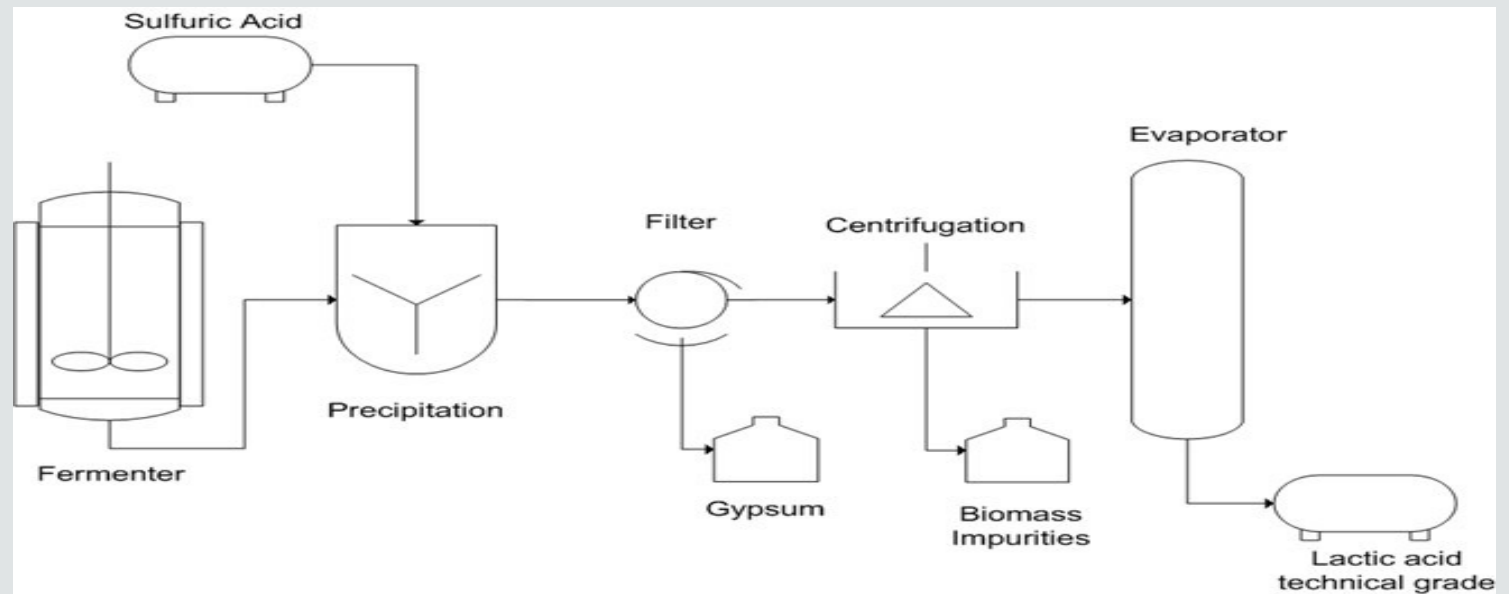


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Extraction

1. Lactic acid is crystallized with great difficulty and in low yield.
2. Purest forms are usually colorless syrups which readily absorb water.
3. At end of fermentation when the sugar content is about 0.1%, beer is pumped into settling tanks.
4. Calcium hydroxide at pH 10 is mixed in and the mixture is allowed to settle.
5. Clear calcium lactate is decanted off and combined with filtrate from slurry. It is then treated with sodium sulfide, decolorized by adsorption with activated charcoal, acidified to pH 6.2 with lactic acid and filtered.
6. Calcium lactate liquor may then be spray-dried.





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7. The fermentation broth is treated with sulfuric acid to precipitate calcium sulfate which is filtered. Filtrate containing free organic acid is evaporated to obtain pure LA.
8. It is 44-45% total acidity.
9. Food grade acid has a total acidity of about 50%. Made from fermentation of higher grade sugar and bleached with activated carbon.
10. Metals especially iron and copper are removed by treatment with ferrocyanide and then filtered.
11. Plastic grade is obtained by esterification with methanol after concentration.
12. High-grade lactic acid is made by steam distillation under high vacuum, solvent extraction etc.

Vinegar

Product resulting from conversion of alcohol to acetic acid by *Acetobacter* spp. Although acetic acid is major component of vinegar, material cannot be produced simply by dissolving acetic acid in water. When alcoholic fermentation occurs and later during acetifications many other compounds are produced, and some of these find their way into vinegar.

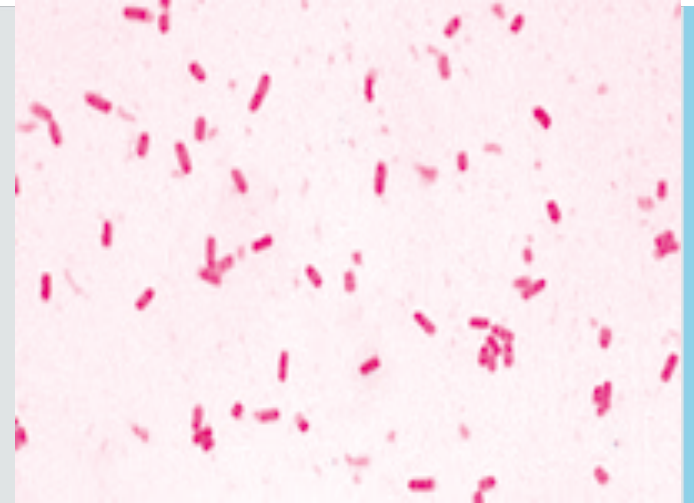
Ethyl acetate is formed from reaction between acetic acid and ethanol. Other compounds include non-volatile organic acids such as malic, citric, succinic and lactic acids; unfermented and unfermentable sugars; oxidized alcohol and acetaldehyde, acetoin, phosphate, chloride, and other ions.





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Uses

1. Use as a food condiment, treatment of wounds, and a wide variety of illnesses such as plague, ringworms, burns etc.
2. Used as general cleansing agent.
3. Pickling and preserving meats and vegetables.
4. Can reduce pH of food below that which even sporeformers may not survive
5. Nearly 70% of vinegar produced today is supplied to various arms of food industry where used in manufacture of sauces, salad dressings, mayonnaise, tomato productions, cheese dressings, mustard, and soft drinks.



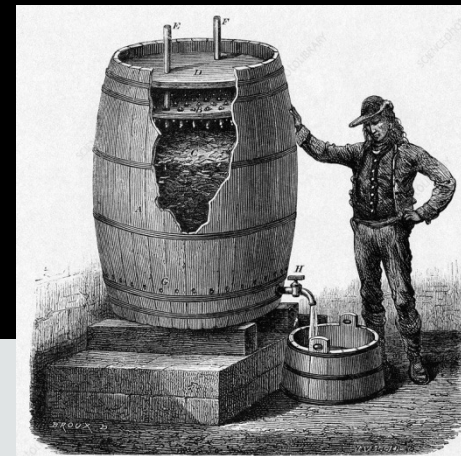
Substrate

1. Wine vinegar made from grapes is common in continental Europe and other vine growing countries, malt vinegar is common in United Kingdom.
2. Rice vinegar is common in far Eastern countries of Japan and China and pineapple vinegar is used in Malaysia.
3. In some tropical countries, vinegar has been manufactured from palm wine derived from oil/palm.

Pure cultures are hardly used, except in submerged fermentation because of difficulty of isolating and maintaining organisms. *Acetobacter xylinum* produce slime. 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 over-oxidize the acetic acid formed; d) high yielding of acetic acid produced.

Manufacture of vinegar

- 1. Three methods used for production of vinegar are Orleans Method (slow), Trickling (quick) Method and Submerged Fermentation (last two widely used).**
- 2. Common feature in all submerged vinegar production is that aeration must be very vigorous as shortage of oxygen because of the highly acid conditions of submerged production, would result in the death of the bacteria within 30 seconds.**
- 3. As lot of heat is released (over 30,000 calories are released per gallon of ethanol) an efficient cooling system must be provided.**
- 4. Submerged vinegar is turbid because of high bacterial content and have to be filtered.**





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Processing of vinegar

Clarification and bottling:

- Vinegar for retailing is clarified by careful filtration using a filter aid such as diatomaceous earth.
- Vinegar from trickling generators are less turbid than submerged fermentations because a high proportion of bacterial population responsible for acetification is held back on shavings.
- After clarification it is pasteurized at 60-65°C for 30 minutes.

Concentration of vinegar:

- Vinegar can be concentrated by freezing; thereafter the resulting slurry is centrifuged to separate the ice and produce the concentrate.
- With this method 200° grain (i.e., 20% w/v) acetic acid can be produced.



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Frings acetator

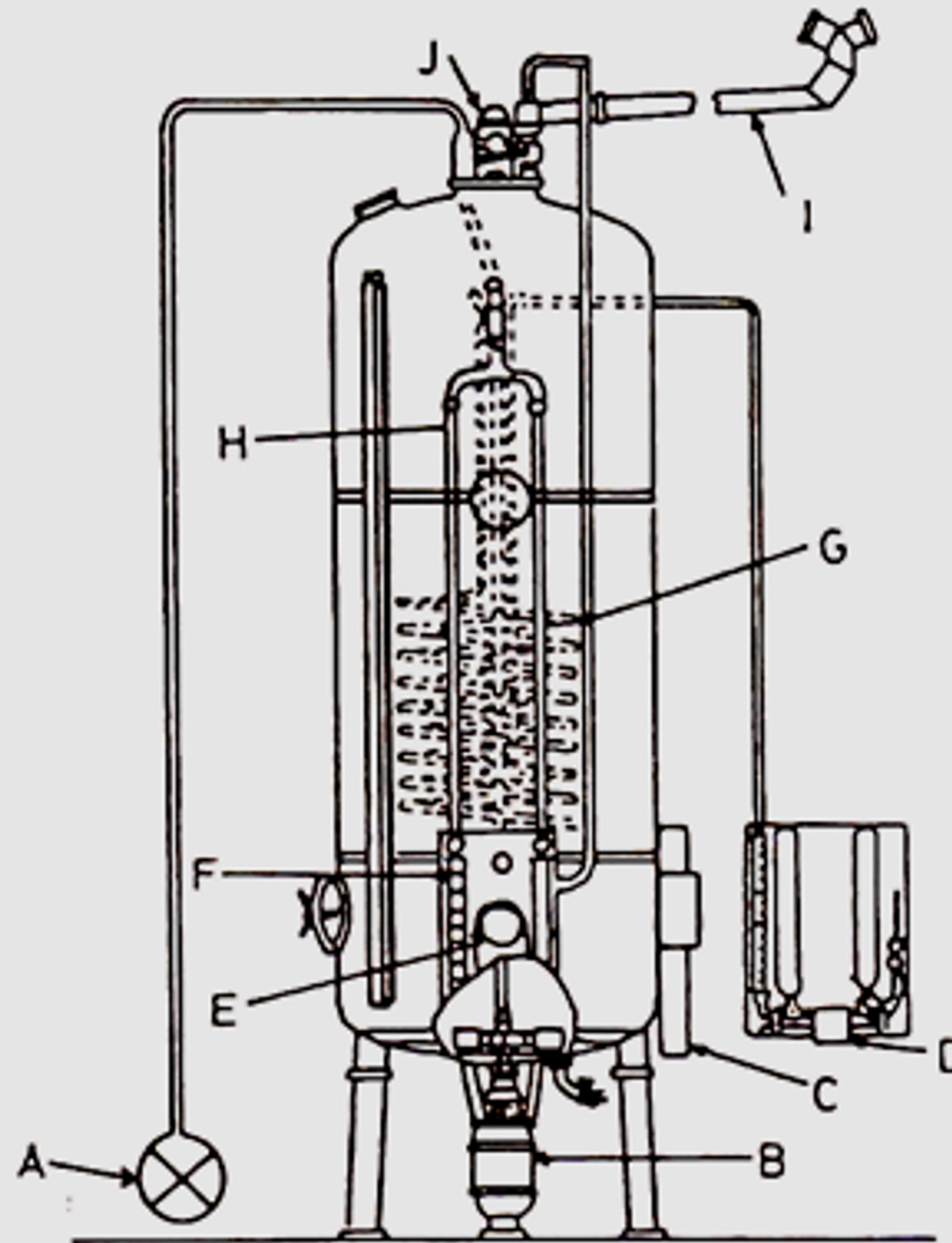
- Consists of a stainless steel tank fitted with internal cooling coils and a high speed agitator fitted through the bottom.
- Air is sucked in through an air-meter located at top.
- It is then finely dispersed by the agitator and distributed throughout the liquid.
- Temperature is maintained at 30°C.
- Foaming is interrupted with an automatic foam breaker.
- Operated batchwise and cycle time for producing 12% vinegar is about 35 hours.
- Frings alkograph automatically monitors the alcohol content and signals the end of the batch when alcohol content falls to 0.2% (v/v).
- At this stage about one third of product is pumped out and fresh feed pumped to original level. Aeration must continue throughout period of the unloading and loading.
- Fermentation cycle takes 24 to 48 h.





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The Frings Acetator. A, charging pump; B, aerator and motor; C, alkograph; D, cooling water valve; E, thermostat controlling D; F, rotameter; G, cooling coil; H, air line; I, air exhaust line; J, defoamer