Natubhai V. Patel College of Pure & Applied Sciences

S. Y. B.Sc. (Industrial chemistry)

C-214: Chemical Process Industries
UNIT 4

SYLLABUS

Fermentation: Introduction, definition, culture development, inoculums preparation, nutrients for microorganism, toxic effect on culture, manufacture of industrial alcohol, absolute alcohol, vinegar.

FERMENTATION

4.0 INTRODUCTION

The term fermentation (Latin fermentare to boil) formally stood for **decomposition of foodstuff** usually accompanied by **evolution of gas**. The fermentation of sugars to alcohols and carbon dioxide by yeast is one of the oldest examples. The term **fermentation** is now applied to changes brought about by microorganisms. Evolutions of gas are not an essential criterion. In our daily life we see many complex chemical reactions; which are brought about by the agency of living organisms. **Examples** are souring and curdling of milk, putrefaction of meal, production of indigo dye from the compound indicate in the food , curing of tobacco, the development of benzaldehyde or oil of biter almonds from the amygdaline contained in the almond seed, conversion of fruit juice into wines etc. All these **processes** are called **fermentation processes**, in which complex organic material is broken down into smaller substances and decomposition it brought about by the action of living organisms, which secretes the enzyme catalyst, suitable to the process.

Although the fermentation of fruits to alcohol and making of beverage out of fruits and grain have been established for centuries, it is only during the past generation that the wider application of fermentation has been recognized. Now man is directing the life process of microorganisms the yeasts, bacterial and moulds to the production of large number of chemicals, such as alcohol, acetone, acetic acid, lactic acid citric acid and many antibiotics which are of great synthetic as well as industrial important.

4.1 DEFINATIONS

1. **Aerobe:** An organism that can use oxygen as an electron accepter at the terminus of an electron-transport chain can grow at a level of O_2 equivalent to or higher than that present in an air atmosphere (21 %) and has a strictly respiratory type of metabolism.
2. **Agar:** A dried polysaccharide extract of red algae, used as a solidifying agent in microbiological media.
3. **Anaerobe:** An organism that does not use O_2 to obtain energy, that cannot grow under air atmosphere, and for which O_2 is toxic.
4. **Culture:** A population of microorganisms cultivated in a medium.
5. **Inoculum:** The substance, containing microorganism or other material that is introduced in inoculation.
6. **Inoculation:** The artificial introduction of microorganisms or other substances into the body or into the culture medium.
7. **Microorganism:** Any organism of microscopic dimension.
8. **Sterilization:** The process of killing of all forms of life.
9. **Medium (media):** A substance used to provide nutrients for the growth and multiplication of organisms.

10. **Lyophilisation:** The preservation of biological specimens by rapid freezing and rapid dehydration in a high vacuum.
11. **Incubation:** The subjecting of cultures of microorganisms to conditions favourable to their growth (e.g. Temp).

4.2 PREREQUISITES OF A GOOD FERMENTATION PROCESS

- A microorganism that formed a desired end product. This organism must be readily propagated and be capable of maintaining biological uniformity, thereby giving predictable yield.
- Economical raw materials for the substrate, e.g. starch or one of several sugars.
- Acceptable yield.
- Rapid fermentation.
- A product that is readily recovered purified.

4.3 NUTRIENTS FOR MICORORGANISMS

All living organisms, microorganisms are the most versatile and diversified in their nutritional requirements. Human and other animals require certain type of complex carbon containing compounds as nutrients, while microorganisms may not. Some microbes can grow with just a few inorganic substances as their sole nutritional requirements, while other microorganisms are like higher organisms in their need for complex organic compounds. But all living organisms share some common nutritional needs, like the need for carbon, nitrogen, and water.

Water is particularly important to microorganisms, besides most of microorganisms can absorb nutrients only when the chemicals are dissolved in water.

4.3.1 Chemical elements as nutrients

All the microorganisms need a variety of chemical elements as nutrients. These elements are necessary for both the synthesis and the normal functions of cellular components. The **main elements** for cell growth include carbon, nitrogen, oxygen, sulphur and phosphorous.

1. Carbon

Carbon is one of the most important chemical elements required for microbial growth. Carbon forms the backbone of three major classes of organic nutrients: Carbohydrates, lipids and proteins. Such compounds provide energy for cell growth and serve as building blocks of cellular material.

2. Nitrogen

All organisms also require nitrogen in some form. This element is an essential part of amino acids that together form proteins.

3. Hydrogen, oxygen, sulphur and phosphorous

Hydrogen and oxygen form part of many organic compounds. Sulphur is needed for the biosynthesis of amino acids cysteine, cystine and methionine. Phosphorous is essential for the synthesis of nucleic acid and adenosine triphosphate (ATP), a compound that is extremely important for energy storage and transfer.

Many **other essential elements** are required, though in smaller amounts than the elements already listed. These may facilitate the transport of materials across cell membranes e.g. Na^+ is required by the permease that transport the sugar meliboise into cells of Escherichia Coli.

4.4 CONDITIONS FAVOURABLE FOR FERMENTATION OR FACORS AFFECT THE RATE OF FERMENTATION OR PHYSICAL CONDITION FOR CULTIVATION OF MICROORGANISMS

Four main conditions influence the physical environment of a microbe are temperature, pH, gaseous atmosphere and osmotic pressure.

The successful cultivation of a various type of microorganisms requires a combination of the proper nutrients and the proper physical environment.

4.4.1 Temperature

Temperature has great influence on the growth of microorganisms. This is not surprising, since all the processes of growth are dependent on chemical reactions that are affected by temperature. Unlike mammalian cells, which grow within a relatively narrow temperature range (close to 37° C), microorganisms require rather broad range of temperature. However, this range may be wider for one than for others e.g. the range for **Bacillus subtilis** is from 8 to 53°C, a 45^0C range; for **Neisseria genorrhoeae** is from 30 to 40°C, a 10° C range

At the most favourable temperature for growth, the **number of cell division** per hour, called the **growth rates** generally **doubles for every temperature increase** of to that of most enzyme catalyzed reactions, supporting the principle that growth is the result of series of integrated, enzyme-based chemical reactions. The temperature at which a species of the microorganisms grow most rapidly is the optimum growth temperature.

For many particular microbes the **three important temperatures** are the minimum, optimum and maximum growth temperature. These are known as the **cardinal temperature** of a particular microbial species may vary with the stage in the life cycle of the microorganisms and with the nutritional contents of the medium.

The optimum temperature for microbial species does not lies midway between the minimum and maximum temperatures. Instead, it is nearer the upper limit of temperature range because the rate of **enzymes reaction increases** with **increasing temperature** until a point where the enzymes are damaged by heal and cells stop growing.

Microorganisms may be divided into **three groups** on the basis of the temperature range in which they grow best.

1. Psychrophiles or cold living microbes.
2. Mesophiles or moderate temperature living microbes.
3. Thermophiles heat living microorganisms

4.4.2 Gaseous atmosphere

Microorganisms in their natural habitats require varying amount of gases such as oxygen, CO_2, N_2 and methane. In order to cultivate microbial, plant and animal cells in the laboratory, the proper gas atmosphere must be present. Some gases are used in cellular metabolism; other may have to be excluded from a culture because they are toxic to cells. CO_2 and O_2 are the two principle gases that affect the growth of microbial cells.

On the basis of their **response to gases oxygen**, microorganisms are **divided** into **four physiological groups** as under.

1. **Aerobic microorganisms**

   Microbes that normally require oxygen for growth and can grow in a standard air atmosphere of 21% O_2 are classified as aerobes. E.g. of aerobic microbes are Mycobacterium and legion Ella.

2. **Facultative microorganisms**

   Facultative microorganisms are those that grow in air atmosphere and can also grow an aerobically. They do not require O_2 for growth, although they may use it for energy- yielding chemical reactions.

3. **Anaerobic microorganisms**

   Anaerobic microorganisms are those which may be poisoned by O_2, cannot grow in an air atmosphere and do not use O_2 for energy yielding chemical reactions e.g. Methanobacterium and Methanospirillum are strict anaerobes.

4. **Microacrophilic microorganisms**

   Microacrophilies are organisms which like aerobes, can use O_2 for energy yielding chemical reactions. However, unlike aerobes, they cannot withstand the level of oxygen (21%) present in air atmosphere and usually grow best at O_2 levels between 1 to 15%.

4.4.3 pH

In contrast to optimum temperature, **optimum pH** for microbial growth lies approximately in the **middle of pH range** over which growth will occur. The optimum pH is usually well defined for individual species. Different species are adapted to grow a various pH values. But to grow in acidic or basic environment microorganisms must be able to maintain its intracellular pH at about 7.5 regardless of the external pH. Living cells has the ability, within limits, to keep a constant internal pH by expelling hydrogen ions or by taking hydrogen ions into the cells. Different **species of microbes** have **different pH tolerance**.

For most **bacteria**, the pH normally is about 7 with pH 9 as the maximum for growth. The optimum pH normally lies between 6 to 8 but some species of bacillus e.g. can grow at pH values as low as 0.5 and are found in acidic drainage water from mines where sulphur and iron are present. **Molds and yeasts** have a broader pH range than the bacteria. In addition their optimum pH for growth is lower than that of bacteria.

However **microorganisms** may have other requirements. An example is the photosynthetic microbes that must have light water, which accounts for 80 to90 % of a cell, is another environmental factor that affects microbial growth.

4.4.4 Concentration

High concentration of a solution renders an enzyme inactive. Thus solution used for fermentation should be sufficient diluted to favours the process

4.4.5 Presence of other substance

Certain inorganic salt solution acts as food for the ferment cell.

4.4.6 Absence of preservatives

Preservative are those substances, which destroy the ferment and retard the fermentation reaction. Hence these substances should be absent.

4.5 CULTURE DEVELOPMENT

Scientists have learned **to cultivate** many types of microorganisms, getting them **to grow and maintain their viability**. As you have already learned microorganisms are activated on media, which provide nutrients. The proper physical environment must be provided for optimal growth. Microorganisms show wide differences with respect to the physical conditions required for growth. Some species grow at temperature near the freezing point of water other grow at temperatures as higher as 110° C. Oxygen is essential to some poisonous to others. Most bacteria grow best at or near neutral pH, but the preferred pH for growth among microbes varies from alkaline to acidic.

Once the chemical and physical requirements are satisfied, it is possible to study the mode of reproduction and growth of species of microorganisms. It is important to remember that the behaviour of the species in pure culture in the laboratory may not be the same as its growth is characterized in nature.

4.6 DEVELOPMENT OF INOCULUM

The culture used to **inoculate for fermentation should satisfy** following criteria.

- It must be in a healthy, active state thus minimizing the length of the lag phase in the subsequent fermentation.
- It must be available in sufficiently large volumes to provide inoculums of optimum size.
- It must be in a suitable morphological form
- It must be free of contamination.
- It must retain its product forming capabilities.

The process adopted to produce an inoculums meeting this criteria is called **inoculums development**. A critical factor in obtaining suitable inoculums is the **choice of the culture medium**. The **quantity** of inoculums normally used is between 3 and 10 % of the medium volume. A relatively large inoculums column is used to minimize the length of the lag phase and to generate the maximum biomass in the production fermentation in as short as possible, thus increasing vessel productivity. Starting from a stock culture, the inoculums must be built up in a number of stages to produce sufficient biomass to inoculate the production stage fermentor. This may involve two or three stages in a sleek flasks and one to three stages in fermentor.

A **typical inoculums development** program will now be described in detail.

- The **master culture** is reconstituted and plated on to solid medium approximately ten calories of typical morphology of high producers are selected and inoculated on to slopes as the **sub-master cultures**, each sub-master culture being used for a **new production run**. At this stage, shake flasks may be inoculated to check the productivity of these cultures, the results of such tests being known before the developing inoculums **eventually reaches the production plant**. A sub master culture is used to inoculate a shake flask (250 or 500 cm^3 containing 50 or 100 cm^3 medium), which in turn is used as inoculums for a large flask or a **laboratory fermentor** which may then be used to inoculate a **pilot-scale fermentor**.
- Culture purity checks are carried out at each stage to detect contamination as early as possible.
- Although the **results** of these tests may not be available before the culture has reached the production plant at least it is known at which stage in the procedure contamination occurred.

4.7 CHARACTERISTICS OF ENZYMES

- They do not take part in the reaction, but act **catalytically** i.e., they are organized catalyst of nature.
- Enzymes, like inorganic catalyst, **do not disturb** the **final state of equilibrium** in a reversible reaction.
- The **rate** of an enzyme reaction is **proportional to concentration of reactant**, provided the concentration is small. At high concentrations, the rate of the reaction is independent of the concentration.
- A **small quantity** of enzyme can bring about the decomposition of large amount of the substrate. For example, urease extracted from soyabean, will catalyst the hydrolysis of urea when present only to the extent of 1 part in 10 million parts.
- Their **action is highly specific**, i.e. a particular enzyme can bring about a particular reaction. For example, urease catalyses the hydrolysis of urea, but it has no effect on the hydrolysis of methyl urea.
- An enzyme is most **reactive** at a particular temperature, called **optimum temperature**. In many cases this temperature is 30-40^{0}C.
- Enzymes, like other catalyst are **influenced** by the presence of **other substances**. Some enzymes, called co -enzymes act as promoters, while HCN, CS_2, etc., poison them.
- They are **destroyed by** the ultra violet light and by heat. At temperature too low (0°C) and too high (70-80° C) the enzyme are most inactive.
- They **bring** about many **complex reactions** e.g. oxidation, reduction and hydrolysis

Some, of the well-known enzymes are **diastase** (origin: malt, liver), **catalase** (origin: plant juice, blood) **zymase** (origin: yeast), **urease** (origin: soybean), **invertase** (origin: small intestine yeast), **lactic bacilli** (origin: curd).

4.8 INDUSTRIAL ALCOHOL

Industrial alcohol was an outgrowth of alcoholic beverages, but now it has become important by virtue of its **economically useful properties** as a solvent and for synthesis of other chemicals. Alcohol is sold as tax-paid alcohol or, much more widely, as non-taxed denatured alcohol. The completely denatured formulas are admixtures of substances which are difficult to separate from the alcohol and which smell and taste bad, all this being designed to render the alcohol non-potable.

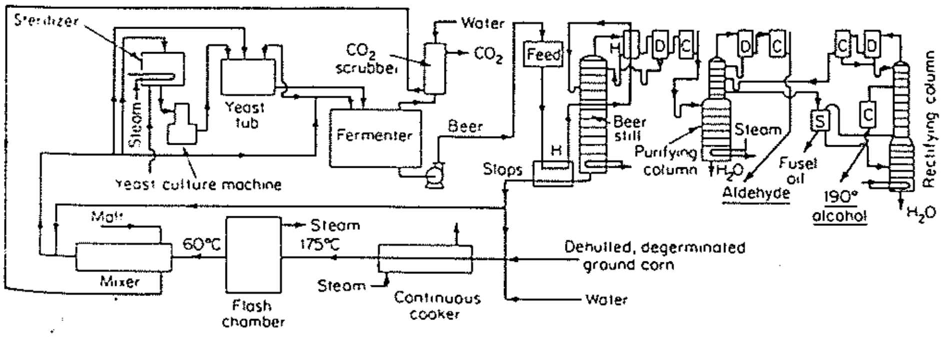
4.8.1 Raw material

Ethylene, cellulosic material like wood, wood waste and sulphite liquor are the basic raw material for industrial alcohol. Corn is considered to be the most promising raw material for fermentation to alcohol, especially for gasohol.

4.8.2 Manufacture

Manufacturing Sequence of industrial alcohol by fermentation is as follows

- Transplantation of corn or molasses to the plant.
- Storage of corn or molasses
- Grinding of corn
- Hydrolysis by beating of cornmeal with acid to make mash
- Growth of culture
- Fermentation of diluted inverted molasses or of corn mash
- Distillation of alcohol from beer
- Rectification and purification of alcohol.
- Recovery of by-product, e.g. CO_2, feed, potash salts



Flowsheet for industrial alcohol. KEY: C, condensor; D, dephlegmator; H, heat exchanger; S, separator.

The flow chart in figure shows the various operations involved in changing **corn to alcohol.** The **corn** is degerminated, dehulled and milled, either in wet or dry. The milled corn is conveyed to the cooker. **Cooking** is necessary to gelatinize the ground grain so that the barley malt amylases can convert the starch to fermentable sugars. The cookers may be batch or continuous and are operated under pressure. In the continuous process the grain is precooked for **1 to 5 minutes** with water and stillage (the dealcoholized, fermented beer that is discharged from the bottom of the beer still). The mash is continuously fed to a steam heater that instantaneously raises the temperature to **175^0C**. The mash is passed through a series of pipes and discharged through a relief valve into a flash chamber. Time in the cooker is about **1.5 min** and pressure is maintained at **60 to100kPa gages**. The temperature of the mash is drops to about 60^0C in the flash chamber.

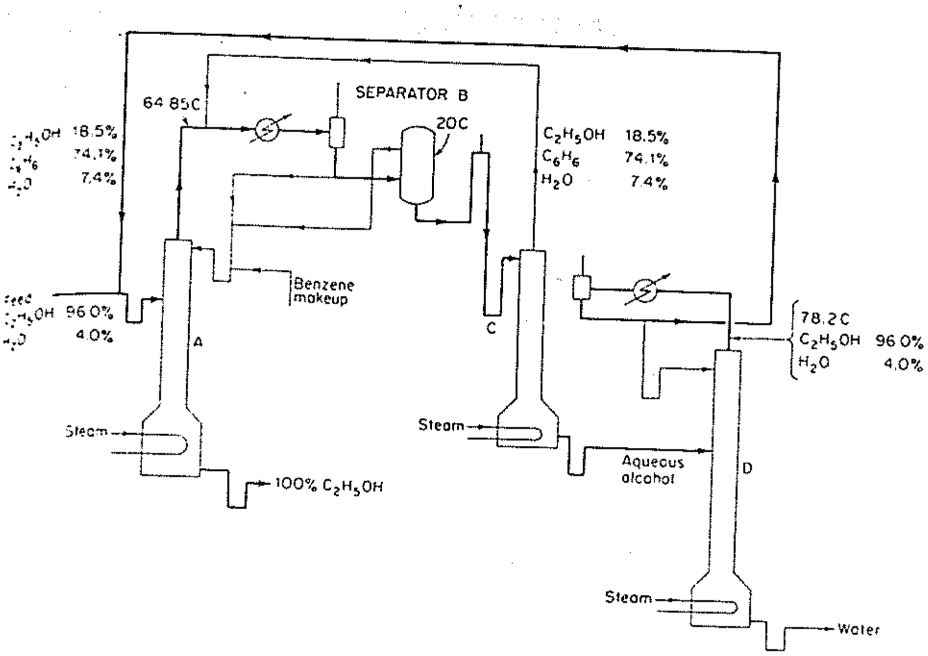
The gelatinized (cooked) **grain** is mixed with malted barley and water. The mix is pumped through a pipe to the s through pipe coolers. The **starch** is **hydrolyzed** to about **70% maltose and 30% dextrin** in the short time in the converter. **Stillage** (20 to 25% of the final mash volume) from the beer still is added to the converted grain mash prior to fermentation to lower the pH, furnish nutrients for the yeast and to add buffering action. The initial **pH** is adjusted to 4.8 to 5.0 with sulfuric acid and/or stillage. As the reaction indicates, **fermentation is exothermic**, so **cooling** may be **necessary** to ensure that maximum **temperature** does **not exceed 32°C**. The time of the fermentation may vary from **40 to 72 hr.**

The **liquors in the fermentors**, after the action is finished are called **beer**. The alcohol is separated by distillation. The beer containing (5.5 to 11% alcohol by volume is pumped to the upper section of the **beer still**. As the beer passes down the column, it loses its lighter boiling constituents. The liquid discharged from the bottom of the still through heat exchanger is known as Stillage. The overhead containing alcohol, water, and aldehydes passes through **heat exchanger** to the **partial condenser, or dephlegmator**, which **condenses 50% alcohol**, containing volatiles or aldehydes. This **condensate** is conducted into the **aldehyde column**, from which the low boiling impurities are separated as an overhead. The effluent liquor from bottom of the aldehyde column flows into the rectifying column.

In this **third column** the alcohol is brought to the strength and finally purified to 95 to 95.6%.

4.9 ABSOLUTE ALCOHOL

Absolute alcohol made by **absorbing 4-5% water** present in 95-96% industrial alcohol by **azeotropic distillation** at 101kPa using **benzene as third component**. Column (A) has 95% alcohol fed into it. The ternary azeotrope is taken overhead in this column and absolute alcohol is obtained as a bottoms product. The overhead vapours are condensed and passed to separator (decanter) (B), in which two liquid layer form. The upper layer rich in benzene is returned to column (A) as reflux, and the lower layer is fed to column (C), which produces the ternary azeotrope as the overhead product and benzene - free aqueous alcohol as the bottoms product. This latter product is fed to column (D), which produces by ordinary distillation, an overhead product of 95% alcohol and a bottom product of nearly pure water. The benzene is recycled continuously in this system and it is necessary only to make up the benzene losses from the system. This withdrawing agent is used over and over again with a loss that should not exceed 0.5 percent of the volume of the anhydrous alcohol produces.



4.10 ETHYL ALCOHOL FROM ETHYLENE BY CATALYTIC HYDRATION

4.10.1 Raw material

Ethylene (97%)	515 kg
H_3PO_4	Small quantity
NaOH	Small quantity

4.10.2 Reaction

$CH_2 = CH_2 + H_3PO_4 + H_2O \longrightarrow C_2H_5OH$

Ethylene — 95% Yield

4.10.3 Manufacture

The most recent synthetic alcohols process is the direct hydration of ethylene using H_3PO_4 as catalyst. In recent year this process has dominated in manufacture of industrial alcohol.

The **process** has **following sequence**

- Liquefaction of petroleum gas contains ethylene
- Rectification to produce ethylene and ethane
- Dehydrogenation of ethane to ethylene
- Hydration over a catalyst
- Distillation of alcohol from partially converted ethylene.

➢ Rectification & purification of alcohol.

An colggines stream containing (97%) **ethylene** compress to**1000 psi**. This combined **ethylene** to 0.6 mole **H_2O**. The **mixture** is vaporised by **heat exchanger** and passes through a **reactors**, where it is heated at about **300^0C**. In the reactor the deron-flow feed posses over a **catalyst H_3PO_4 (85–90%)**. As the product stream leaves the reactor, it is partially **condensed** by heat exchanger (condenser). Dilute **NaOH** solution is added to **neutralise** H_3PO_4 acid vaporised from catalyst. The alcohol water portion condensed in **second heat exchanger**. After then liquid and vapour are separated in high pressure **separator**. The recycled gas cooled treated with H_2O in a column to recovered last portion of alcohol in the gases.

The alcohol-water solution from separator passes through a **striping column**. Where H_2O is removed from bottom and concentrated alcohol from the overhead **if the ethane** are **present** in a starting material **as an impurity**. It gives rise to **acetaldehyde** by hydration process. Therefore, starting material ethylene can be purified. It means it is free from methane, ethane, acetylene and higher olefins.

4.11 ETHYL ALCOHOL FROM ETHYLENE BY ESTERIFICATION & HYDROLYSIS

4.11.1 Raw Material

Basis: 1000 litre ethyl alcohol (95% purity)
Ethylene= 480 Kg
H_2SO_4 = 72 Kg H_20 = 240m^3
NaOH=15.5Kg   Steam = 2400 Kg %

4.11.2 Reactions

1. $CH_2=CH_2 + H_2SO_4 \longrightarrow C_2H_5OSO_2OH$
   Mono Ethyl Sulphate
2. $2CH_2=CH_2 + H_2SO_4 \longrightarrow C_2H_5OSO_2OC_2H_5$
   Diethyl sulphate

Hydrolysis

3. $C_2H_5OSO_2OH + C_2H_5OSO_2OC_2H_5 + 3H_2O \longrightarrow 3C_2H_5OH + 2H_2SO_4$
4. $2C_2H_5OH \longrightarrow C_2H_5OC_2H_5 + H_2O$
   Diethyl ether (90-95% yield)

4.11.3 Manufacture

Synthetic ethyl alcohol produced by scrubbing **ethylene with H_2SO_4** to gives a **mixture of ethyl-sulphate**. Which are then **hydrolyse to crude alcohol** and dilute H_2SO_4. The alcohol is **purified by distillation** and acid is concentrated.

The simplified flow diagram of the manufacture process is as shown in figure.

The purified **ethylene** passes to bottom of the **absorber**, **counter current** to 90% **H_2SO_4.** The unabsorbed gas passes out from the top in the form of fuel gas. The resulting liquid is mixture of **monoethyl sulphate and diethyl sulphate** removed from bottom of column containing small amount of acid and H_2O. Temperature of exothermic reaction is controlled by rate of feed of two reactants. The mixed ester obtained from column, purified and sent to **hydrolyser**. Here, at small residence time of **1.5 to 2 hr. at 70^{0}c**. The sulphates are hydrolysis to ethanol, dilute H_2SO_4 and small amount of ethyl ether. The **crude alcohol passes** through a **striping column**. Where, alcohol ether and acid separates. Dilute H_2SO_4 withdrawn from bottom of stripping column cooled and stored for recovery. The crude alcohol vapour leaves from stripping column are treated **(Neutralize) by NaOH** solution. The crude alcohol passes through **ether column**, where ethyl ether is removed by stream. The alcohol has **95% purity** in vapour form are condense & collected in special types of equipment.

4.12 <u>ETHYL ALCOHOL FROM SUGAR (CARBOHYDRATES) BY FERMENTATION PROCESS</u>

4.12.1 Raw material

Basis: 1000 litre (95% pure) ethyl alcohol

Molasses (black straps)	2400 lit.	H_2O	10,000 lit
H_2SO_4	20kg	Cooling H_2O	42,000 lit.
$(NH_4)_2SO_4$	1.8kg	Stream	5990 kg

4.12.2 Reactions

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6$$

Sucrose

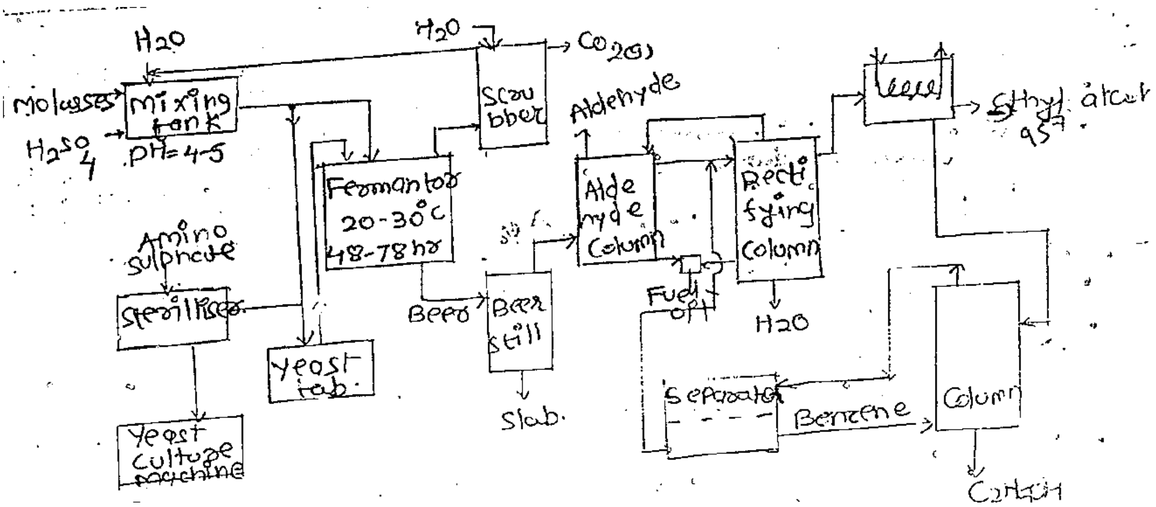
$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_{2(g)}$$

Dextrose (Glucose) → Ethanol (90% Yield)

4.12.3 Manufacture

Ethyl alcohol is produce from sugar containing material like molasses by fermentation process using **yeast** to convert the **sugar into alcohol**

The most widely use raw material is **black-strap molasses** after the extraction of crystalline sugar. It contains about **55% total sugar** by weight. This molasses are treated with **dilute H_2SO_4** in a mixing tank keeping **pH = 4.0 to 5.3**. The **diluted acidified molasses** is called a **mash** is posses to fermentor which are made from wooden or steel tank, where a selected stream of **yeast** is added. The temperature in fermentor is maintained at **20-30°C** through cooling coils which are inserted or arranged to cooled the reaction mixture of **exothermic** reaction. The **time** required for fermentation process **28-72 hours** to produce **alcohol** having concentration **8-10%**. The yeast are supplied cultivated in a yeast tub during fermentation $CO_{2(g)}$ evolved which convert mono saccharine to alcohol. The $CO_{2(g)}$ goes out from top portion of column and alcohol water purified using activated carbon. **After fermentation reaction** the **liquor** is known as **Beer** containing **8-10%** alcohol passes through a column and a **heat exchanger condenser**. This condensate contains **50-60%** alcohol. It is known as **high wine** from which **impurities** such as aldehyde removed during rectification, 95% ethyl alcohol obtained yield based on sugar content of molasses.



4.12.4 Properties

- its colourless liquid of characteristics odour
- Specific gravity 0.7893 at 20°C.
- Miscible with water, ether, chloroform and in organic compounds.

4.12.5 Uses

- As a solvent second to water
- As raw material for making hundreds of chemicals such as acetaldehyde, Acetic acid, ethyl acetate, glycols, ethylene dibromide, ethyl esters.
- 99.5% alcohol will mix with gasoline for gasohol; this requires costly extra processing, because simple distillation will not produce ethanol above 95% concentration.
- Use for cosmetics
- As thinners, detergents, flavours
- As disinfectants

4.13 BEERS, WINES, AND LIQUORS

Alcoholic beverages are divided into **three groups**: malt liquids, fermented wines, and distilled liquors. **Beer** require malted (germinated) grain to make the carbohydrates fermentable, **wine** are produced by the action of yeast on the sugar of fruit, and **distilled liquors** are fermented liquor which are then distilled to increase the alcoholic content.

Grains and **fruits** supplying carbohydrates are the basic raw materials. The variety of grains and fruits employed is wide, changing from country to country or from beverage to beverage. Russia ferments potatoes and by distillation obtains vodka (type of wine). Raw materials for fermentation are the cereals, corn, barley, rice, and grapes.

4.14 BEER

Beer and allied products are beverages of **low alcoholic content** (2 to 7%) made by **brewing** various **cereals with hops**, usually added to impart a more or less bitter test and to control the fermentation that follows.

4.14.1 Raw materials

The **cereals** employed are **barley**, malted to develop the necessary enzymes and the desired flavour as well as malt adjunction such as flaked rice, oats, and corn; wheat is used in **Germany** and rice and millet in **china**. Brewing sugars and syrups (cone sugar or glucose) and **yeast** complete the raw materials. For beer the most important cereal is barley, which is converted in to malt by partial germination. Most brewers buy finished malt from suppliers rather than making their own.

4.14.2 Malt preparation

To make **malt** the **barley** is steeped in **cold water** and spread out on floors or in special compartments and regularly turned over for from 5 to 8 days, the layers being gradually thinned as the germination proceeds. At the proper time, when the enzymes are formed, growth is arrested by heat. During growth, oxygen is absorbed, carbon dioxide is given off, and the enzyme diastase is formed. This enzyme is the biological catalyst that changes the dissolved starch into the disaccharide maltose, which, after transformation into the monosaccharide glucose by maltase, is directly fermentable by yeast.

4.14.3 Manufacture

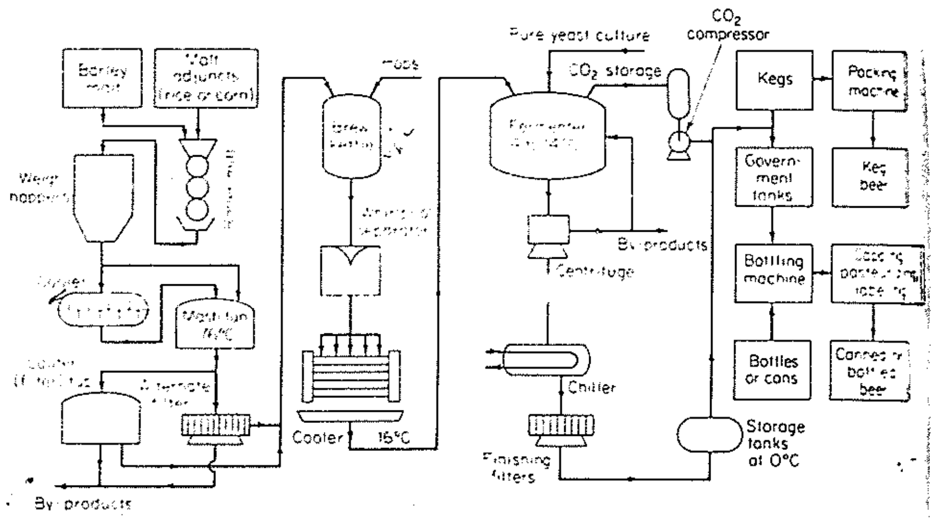
The flowchart **for beer manufacture** may be divided into **three groups** of procedures:

1. Brewing of the mash through to the cooled hopped wort
2. Fermentation
3. Storage, finishing and packaging for market.

Mashing is the extraction of the valuable constituents of malt, malt adjuncts, and sugars by macerating the ground materials with 190 to 230 L of water per 100 kg of materials and treating with water to prevent too high a pH, which would tend to make a dark beer. In the **pressure cooker**, the insoluble starch is converted into liquefied starch, and the soluble malt starch into dextrin and malt sugars. The resulting boiling cooker mash, mixed with the rest of the malt in the mash tub, which raises the temperature to **75°C**, is used to prepare the brewers wort. This is carried out in the **mash tub**. After all the required ingredients have been dissolved from the brewing materials, the entire mash is run from the insoluble spent grains through a slotted false bottom and run into the copper wort cooker. For complete recovery of all substances in solution, a spray of decarbonated water at 74^{0}C is rained through the grains. This is called **sparging**.

The **wort** is cooked for approximately **1 to 1.5 hr,** during part of which it is in contact with hope. The **purpose of boiling** is to concentrate the wort to the desired strength, to sterilize it (15 min) and destroy all the enzymes, to coagulate certain proteins by heat (82°C), to modify its malty odour and to extract the hop resins tannin and aroma from the hops, which are added during the cooking process. **At the end** of the time the spent hops are separated from the boiling wort very quickly in a **whirlpool separator**. Since the spent hops retain 770 L of wort per 100kg of hops, they should also be sparged. The wort is then ready to be cooled.

The **cooling step** is not only to reduce the temperature but also to allow the wort to absorb enough air to facilitate the start of fermentation. The wort is then cooled in a plate heat exchange to **48°C** and **then aerated**. Slight concentration due to evaporation, occurs. This operation is performed, **under controlled condition** to prevent contamination by wild yeasts. Frequently, sterilized air is used.



Flowchart for the production of beer.

The cooled **wort** is mixed with **selected yeast** in the line leading to the starting tubes, between 285 to 380 gm of yeast being used per 100 L of beer. The initial fermentation temperature is **4 to 6°C** but, as the fermentation proceeds, the temperature **rises to 14°C**. This is easily explained by the fact that the conversion of the sugar to carbon dioxide and ethyl alcohol by the enzymes of the yeast **generate 650 kJ/Kg** of maltose converted. The temperature is partly controlled by coolers inserted in the fermentors. The mixture is skimmed to remove the foreign substances that the evolved carbon dioxide brings to the top. The **carbon dioxide** evolved is collected by using closed fermentors and is stored under 17,000 kPa of pressure for subsequent use in carbonating the beer.

The **yeast** gradually settles to the bottom of the fermentation tank in about **7 to 10 days**. The liquid is very opalescent in appearance, under a cover of foam. As the beer leaves the fermentors, it contains in suspension hop resins, insoluble nitrogenous substances and a fair amount of yeast. It is then sent to the **lagering** (aging) tanks where, usually, a second fermentation occurs. The **temperature** is kept **high enough** to initiate the second fermentation and then is **cooled 0 to 2°C** to large the beer. Lagering consists of storing fermentation and then is **cooled** to 0 to 2°C for **1 to 4 months**. During this time the taste and aroma are improved and tannins, proteins and hop resins are removed by setting. Highly hopped beers require more lagering time than beers containing less hops. Near the end of the period the beer is saturated with CO_2 at 50 to 70kPa. In the United States, public demand favours a brilliant beverage, so the beer is filtered through a pulp filter under CO_2. About 92L of beer is produced per 100L of wort in the starling tubes. **After bottling** the beer is **pasteurized at 60°C**.

4.15 <u>WINE</u>

Wine has been made for several thousand years by fermentation of the juice of grape. The quality of the product is largely related to grape, soil, and sun resulting in a variation in flavour, bouquet, and aroma. The colour depends largely upon the nature of the grapes and whether the skins are pressed out before fermentation.

Wines are classified as **natural** (alcohol 7 to 24%), **fortified** (alcohol 14 to 30%), **sweet or dry**, **still or sparkling**. Fortified wines have alcohol or brandy added. In the sweet wines some of the sugar remains unfermented.

4.15.1 Manufacture

For the manufacture of dry red wine, **red or black grapes** are necessary. The grapes are run through a **crusher**, which macerates them but do not crush the seeds and also removes part of the stems. The **resulting pulp** or must is pumped into 11000 to 38000 L tanks where **sulphurous acid** is added to check the growth of wild yeast. An **active culture** of selected and cultivated **yeast** equal to **3 to 5 %** of the volume of juice is added. During fermentation, the temperature rises, so that cooling coils are necessary to maintain a temperature **below 30°C**. The **carbon dioxide** evolved carries the stems and seeds to the top, which is partly prevented by a grating floated in the vat. This allows extraction of the colour and the tannin from the skins and seeds. When the fermentation slows up, the juice is pumped out of the bottom of the vat and back over the top. The **wine** is finally run into closed tanks in the storage cellar, where, during a period of **2 to 3 weeks**, the yeast ferments the remainder of the sugar. The wine is given a **cellar treatment** to clear it, improve the taste and decrease the time of aging. Bentonite or other diatomaceous earth may be used for clearing 20 to 185 gm being stirred into every 100 L of wine.

4.16 <u>DISTILLED SPIRITS</u>

Various fermented products, upon distillation and aging, yield distilled liquor. **Brandy** is distilled from wine or from the marc, which is the left by racking or straining. Making **beer** from a grain mixture containing at least 51 % corn and distilling and aging it yield **bourbon whiskey**. Similarly, **rye whiskey** must start with 51% rye in the grain to be mashed and fermented. **Scotch whisky** uses barley dried with peat, which gives it its distinctive flavour. In modern liquor plants the equipment, up to the stills, is of steel, and the stills are of copper or stainless steel. By law, the again of bourbon or rye whisky of claimed age must take place in charred new **whiteoak barrels** of approximately of 65 to 70% usually for **1 to 5 years**. The aging whiskey also extracts colour and other products from the charred whiteoak. **Whiskey** must be fermented from whole grains, so that the germs (containing the corn oil) and the husks are suspended in the liquor from the beer still in whisky manufacture. This discharge liquor is known as slop, or stillage.

4.17 ACETONE FROM ISO-PROPYL ALCOHOL

4.17.1 Raw materials

Isopropyl alcohol, air and Brass as a catalyst

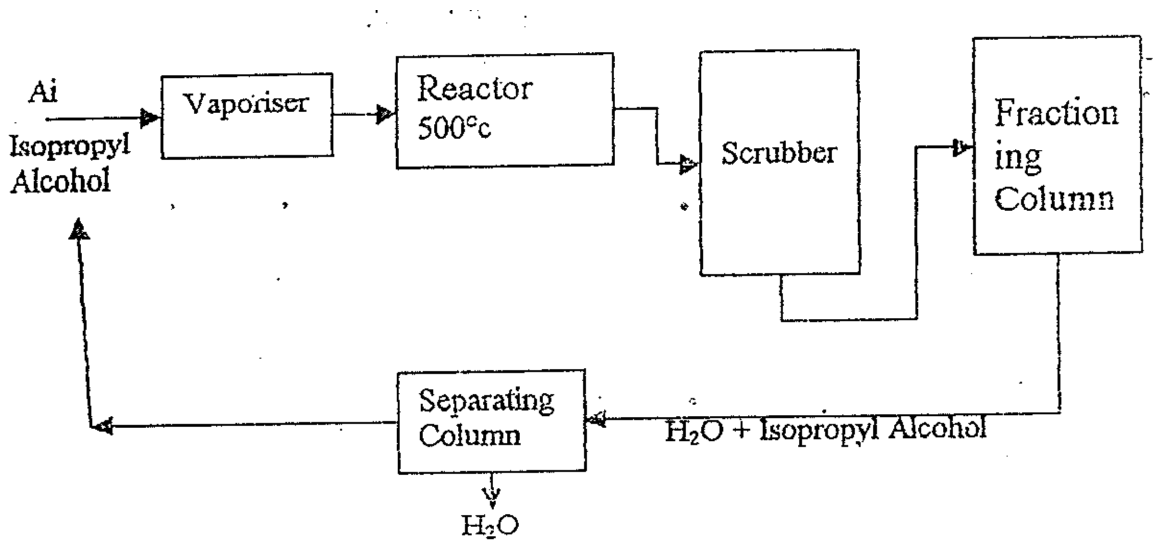
4.17.2 Reactions

$(CH_3)_2CHOH + ½ O_2 \longrightarrow CH_3COCH_3 + H_2O$
Isopropyl alcohol

OR

$(CH_3)_2CHOH + ½ O_2 \longrightarrow CH_3COCH_3 + H_{2(g)}$
(85-90% yield)

4.17.3 Manufacture



Isopropyl alcohol along with **air** is brought into **vaporiser** in required proportion. Then the vapours are transferred to reactor where **brass** is added as **catalys**t. Reaction conditions are **500°C** temperature and **40-50 psi** pressure. The heated reaction gases from reactor contain acetone, isopropyl alcohol and hydrogen passes through a scrubber, where it is treated with H_2O. Here, traces of isopropyl alcohol and acetone are removed from non-condensable hydrogen. The isopropyl alcohol-water solution from bottom of column passes through a fractionating column from which acetone is taken out from the top and Isopropyl alcohol-waste mixture removed from bottom of column. Isopropyl alcohol and water is a binary mixture having constant boiling point from which isopropyl alcohol separated, Purified and reused as starting materials.

4.17.4 Properties

Acetone is a colourless. Highly volatile, highly flammable liquid with an ether odour having MW = 56.08 gm/mole and sp. Gravity=0.791 at 20°C. It can be transported and stored in tanks, drums, cans & in bottles.

4.17.5 Uses

Acetone is used for production methyl methacrylate (MMA) methyl-isopropyl ketone. Methyl acrylic, methyl isobutyl carbonel, Bisphenol A and also used in pharmaceuticals industry, as a solvents, in laboratory.

4.18 BUTYL ALCOHOL (BUTANOL)

4.18.1 Raw materials

Butyl alcohol, acetone and ethyl alcohol are produced by selective bacterial fermentation of carbohydrates containing material such as molasses & grain.

4.18.2 Reaction

$$(C_6H_{10}O_5)_x \xrightarrow{H_2O} C_6H_{12}O_6 + CH_3COCH_3 + CH_3(CH_2)_3OH + C_2H_5OH + H_{2(g)} + CO_{2(g)}$$

Starch Glucose Acetone Butyl alcohol

4.18.3 Manufacture

Molasses diluted with H_2O to a concentration of approximately 5% sugar, sterilize, cooled to 30°C pumped to **fermentor**. After fermentation have been proceeds to **36-48 hrs.** The beer containing 1.5 to 2.5% mixed solvents are taken out from Beer column % slob (residue) obtained from bottom of column. During fermentation $CO_{2(g)}$ & $H_{2(g)}$ evolves. The beer treated in a batch column from which H_2O can be removed & ethyl alcohol, Acetone removed from top of column. The butanol section containing about 50% H_2O taken out from middle portion of column are created in a separator column from which butanol obtained as a bottom product.

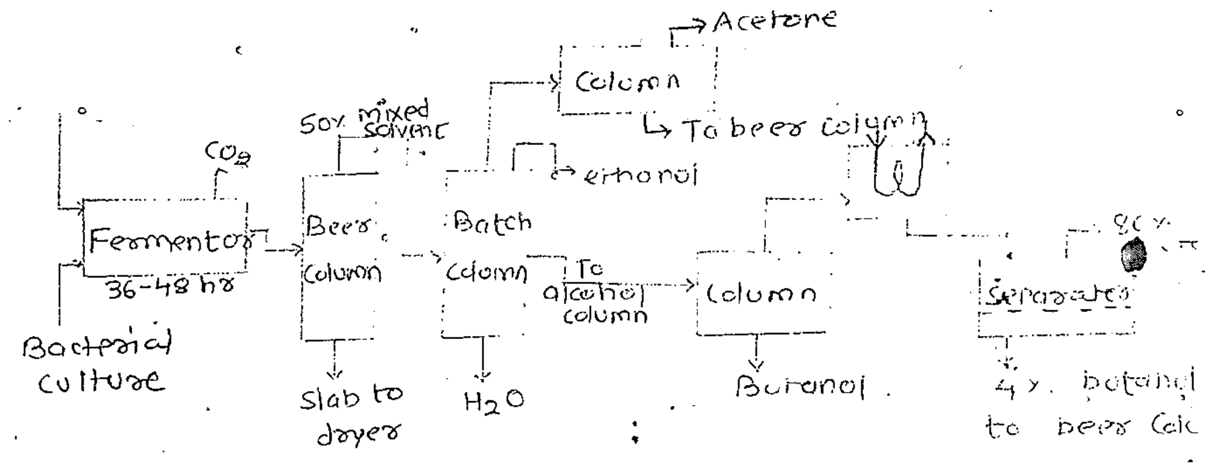


Figure: flow diagram for manufacture of Butanol

4.18.4 Properties

Butylalcohol is a colourless liquid possessing vinegar odours, having MW=74.12 gm/mole. Sp gravity=0.811 at 20°c and B.P.=117.25^{0}C. It is a soluble in ethanol and ether at room temp, slightly soluble in H_2O transported and stored in a tanks-car, drum (steel-drum) & bottles.

4.18.5 Uses

Butyl alcohol can be used as solvent and also used for production of butyl acetate, glycol ester and ester. Also, used as plasticizes and in the production of Amine-resins.

4.19 CITRIC ACID FROM MOLASSES BY FERMENTATION PROCESS

4.19.1 Raw materials

Basis: 1 metric ton Citric-Acid

Molasses = 4000 kg H_2SO_4(95%)= 700 kg Nutrients = 5215 kg

4.19.2 Reactions

$$C_{12}H_{22}O_{11} + H_20 + 30_2 \longrightarrow 2C_6H_80_7 + 4H_20$$

Sucrose Citric acid

$$C_6H_{12}O_6 + 1.5\ O_2 \longrightarrow C_6H_8O_7 + 2H_2O$$

Dextrose Citric acid

4.19.3 Manufacture

Beet molasses containing 48-50 % sugar by wt sugar from cane-molasses (black strap) is introduced into a mixture, where, it is treated with **H_2O** and **dil. H_2SO_4**. To adjust the pH of molasses between 5.5 to 6.5, phosphorous, potassium and nitrogen in the form of acid or salts are added as nutrients or for proper mold growth. The amount depends upon nature of raw material used. The culture is prepared in laboratory, passes in a fermentor, cooled to **30°C**. The fermentation process required **8 to 12 days**. The medium becomes more acidic, temperature of medium during fermentation maintained 28-32°C. Even with careful control of temperature.

The selection of high citric acid producing organisms oxalic acid and citric acid from purified air is generally circulated through a fermentor to maintain humidity between 40-50 after completion of each run the fermentation chamber **sterilize** using H_2O and dilute formaldehyde solution. During fermentation process liquor is obtained, precipitated by addition of small amount of lime. The resulting calcium oxalate and liquor heated to **95°C filter** through vacuum, calcium-citrate obtained which on acidified by H_2SO_4 filtered, **filtrate** contains **citric acid**, which can be decolourize by charcoal treatment, concentrated by using evaporator white citric acid crystal obtained in form of monohydrates.

4.19.4 Properties

Citric acid is generally produced in anhydrous form by direct crystallization commercially available as monohydrate in form of colourless translucent crystal or powder. It is odourless has an acid test having MW 92 gm/mole, Sp. Gravity = 1,665 at 20°C M.P. - 153°C. Soluble in cold H_2O, absolute alcohol & in cold ether transported & stored in begs boxes, piler-drums & in bottles.

4.19.5 Uses

Citric acid used in production of beverages, food and candy industries. Also, used in pharmaceuticals industry sodium salt & ester production and also used in cosmetic industries.

4.20 VINEGAR

Many weak alcoholic beverages such as **wine become sour** when exposed to some time to the air. The souring is **due to the oxidation** of alcohol to acetic acid by air in presence of certain fungi and bacteria e.g. mychoderm aaceti and bacterium aceti. This process is used in the production of vinegar. **Vinegar** is a dilute solution of acetic acid and contains about **5.5-10% acetic acid**. Vinegar stronger than 14% cannot be prepared, because bacteria causing the fermentation are killed.

4.20.1 Raw material

- Any fruit such as apple, grapes etc containing more than 9% of sugar can be fermented to vinegar in two steps. Sugar syrup, cornstarch, beer, etc
- Vinegar is usually manufactured from malt, which is mashed and then fermented with diastase as in the preparation of alcohol.

4.20.2 Reaction

Oxidation of **ethyl alcohol to acetaldehyde**

$CH_3CH_2OH + ½O_2 \longrightarrow CH_3CHO + H_2O$

Oxidation of **acetaldehyde to acetic acid**

$CH_3CHO + ½O_2 \longrightarrow CH_3COOH$

4.20.3 Manufacture

Anaerobic oxidation of both ethanol and acetaldehyde is also possible by acetic bacteria in the presence of methylene blue, in which there is a direct participation of molecular oxygen in the hydrogen abstraction process.

- Temperature for the fermentation is 30- 35^{0}C.
- Alcohol concentration should be 12-14% (because in the concentration solution bacteria are killed)
- Alcohol should be contaminated with 10-12% acetic acid, because slightly sour media causes bacteria cell multiplication.
- **Nutrients** such as magnesium sulfate, ammonium sulfate.etc
- The **fruit extract** or diluted molasses containing 12% sugar is inoculated with **saccharomyces ellipsoids as special wine yeast**, which imparts a good flavour to fermented liquor and ultimately goes to enrich the flavour of vinegar formed in the second phase of fermentation.
- The **wort is then fermented** by yeast and alcohol percentage is adjusted (between 10 to 13%) and along with nitrogenous matter and salts necessary for the growth of the bacteria is sprinkled over large wooden vat provided with perforated bottom and packed with basket work. Air is permitted in vat through the holes, provided at one side of the vat near the bottom.
- **Pure culture** or bacteria aceti is added to alcoholic liquor, which is sprinkled over the basket work and air is allowed to pass through the hole.
- The **bacteria** quickly grow on the basket work and alcohol liquor is spread over a large surface while exposed to the action of bacteria and **air oxidation** of alcohol to acetic acid is rapidly takes place. The temperature and air supply are carefully controlled.
- If the **amount of air** is **large** then the **acetic acid** formed may be **oxidized** further into **CO_2** and **H_2O** resulting in the loss of acetic acid.
- If the amount of **air** admitted is **small** then **slimy matter** is **formed** which hinders the entry of air and ultimately stops the oxidation.
- The liquid is drawn off from the bottom of vat and passed repeatedly over the basket work, until alcohol is completely converted into acetic acid. Some amount of alcohol should be present in acetic acid, in order to prevent oxidation and destruction of acetic acid. The acetic acid or vinegar thus formed contains about **6% of acetic acid.**

4.21 <u>EXERCISE</u>

1. Define the term
    - Fermentation
    - Aerobe
    - Inoculam
    - Inculation
2. Enlist basic prerequisites of a good fermentation process
3. Write a notes on nutrients for micro-organism
4. Discuss the effect of various physical condition during cultivation of micro-organism
5. Write a notes on
    - Development of inoculums
    - Characteristic of enzymes
6. With the help of flow diagram explain manufacture of following
    - Industrial alcohol
    - Beer
    - Absolute alcohol

- Acetone
- Butyl alcohol
- Citric acid
- Ethyl alcohol from ethylene by catalytic dehydration
- Ethanol from ethylene by esterification and hydrolysis
- Ethanol from sugar by fermentation process

7. Discuss the manufacture of vinegar

4.22 FURTHER READING

1. Industrial chemistry by B. K. Sharma
2. Shreve's chemical process industries by George T. Austin
3. Industrial microbiology by A. H. Patel