- **T.Y.B.Sc. CBCS COURSE IN CHEMISTRY CH: 504 Industrial Chemistry**
- **Core Course- (Semester I)**
- **Chapter 1: Fermentation Industry (L: 9, M: 12)**

- **A POWERPOINT PRESENTATION FOR T. Y. B. Sc. CBCS COURSE IN INDUSTRIAL CHEMISTRY ON THE TOPIC**

ENTITLED

"Fermentation Industry"

Seminar-I st

BY

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----- 5, October 2020 -----

Alcohol fermentation: -

All types of alcoholic fermentation require yeast. Not only the alcoholic drinks but also the

rectified spirit and absolute alcohol are produced by alcoholic fermentation. During this process, the polysaccharides are converted into disaccharides which are hydrolyzed into monosaccharides and the monosaccharides are then converted I nto ethyl alcohol and CO_2. All these processes are occurring with the help of specific types of enzymes.

Alcohol is produced by the action of 'zymase' enzyme on certain sugars, the most important is glucose which is found in sweet fruits and honey along with fructose. Zymase is secreted by the microorganism known as yeast. When yeast containing zymase is added to solution of glucose, then glucose is decomposes into alcohol and carbon dioxide.

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} \xrightarrow{\text{Zymase}} \underset{\text{(Ethyl Alcohol)}}{2C_6H_5OH} + 2CO_2$$

It should be noted that all sugars cannot be fermented by zymase. Cane sugar (Sucrose) or beat sugar and malt sugar or maltose is not fermentable with zymase.

These sugars are also fermented by adding yeast into the solution of these sugars. In this case fermentation takes place due to the fact that yeast secretes not only the enzyme zymase but also the enzyme invertase and maltase.

Invertase brings about the conversion of cane sugar or beat sugar or sucrose into glucose and fructose, by process known as hydrolysis.

$$\underset{\text{(Sucrose)}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{Invertase}} \underset{\text{(Glucose)}}{C_6H_{12}O_6} + \underset{\text{(Fructose)}}{C_6H_{12}O_6}$$

$$\underset{\text{(Maltose)}}{nC_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{Maltase}} \underset{\text{(Glucose)}}{2C_6H_{12}O_6}$$

Fermentable sugars can also be obtained from starch which is mainly derived from rice, wheat, barley and maize. The enzyme, diastase is contained in malt.

$$2(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\text{Diastase}} C_{12}H_{22}O_{11} \xrightarrow[H_2O]{\text{Maltase}} C_6H_{12}O_6 + C_6H_{12}O_6$$

(Starch) (Maltose) Glucose Fructose

$$C_6H_{12}O_6 \xrightarrow{\text{zymase}} 2C_2H_5OH + 2CO_2$$

(Glucose) (Ethanol)

Towards the end of a fermentation, the acidity and glycerin increases as follows:

$$2C_6H_{12}O_6 + H_2O \rightarrow C_2H_5OH + CH_3COOH + 2CO_2 + 2C_3H_8O_3.$$

Monosaccharide Alcohol Acetic acid Glycerin

Uses of alcohol: -

1. Absolute alcohol, rectified spirit and denatured alcohol are used as industrial solvents, as thinners and as liquid fuels.
2. Power alcohol is a mixture of ethyl alcohol (20%), benzene and petrol. It is used to generate power.
3. Ethyl alcohol is used as a disinfectant and for making various tinctures used in medicine, such as tincture iodine.
4. It is also used as raw material in manufacture of industrial chemicals such as ethylene, butadiene. Ethylene is used as a raw material for plastics and butadiene is used as a raw material for synthetic rubber.
5. It is also used as preservative, as cleaning agent, as non-freezing solution and in preparation of antiseptics solutions (disinfectant).
6. Ethyl alcohol is used to make ether, Chloroform, vinegar, glacial acetic acid, Chloral hydrate, Celluloid, Collodion, xylonite, transparent soaps.

Theory underlying process of making alcoholic beverages: -

A] Beers: -

Beers are produced mostly from starchy cereals such as barley and additional carbohydrate sources, known as adjuncts are added in varying proportions. Different varieties of Beers are obtained from malt. Malt is germinated wheat or barley. For making beer, short malt is used. Diastase enzyme present in malt hydrolyses the starch in malt into disaccharide. A part of the starch is converted into dextrine.

$$\underset{\text{Starch}}{(C_6H_{12}O_5)_n} \xrightarrow{H_2O} \underset{\text{Dextrine}}{(C_6H_{10}O_5)_{20}}$$

$$\underset{\text{Dextrine}}{(C_6H_{10}O_5)_{20}} \xrightarrow{10\,H_2O} \underset{\text{Maltose}}{10\,C_{12}H_{22}O_{11}}$$

The maltose, dextrin, non-maltose matter, nitrogenous matter goes into solution. This solution is called as wort. It is boiled to destroy diastase. During boiling Hops are added. Hops supply the aromatic oils and give a characteristic taste and flavor to beer. Yeast belonging to the species Saccharomyces Cerevisiae is added to killed malt. Yeast contains many enzymes from these maltase and zymase take part during the conversion of maltose into alcohol.

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{Maltase}} 2\,C_6H_{12}O_6$$

Maltose — Glucose

$$2\,C_6H_{12}O_6 \xrightarrow{\text{Zymase}} 2\,C_2H_5OH + 2CO_2$$

Glucose — Ethyl alcohol

A typical beer contains on an average 3.58% alcohol (ethanol by wt) 1.12% sugar as maltose, 0.33% protein and 2.27 volumes of carbon dioxide in the packaged product. It has blend flavor and P^H of 4.3.

There are four different types of beers i.e. Lager, Ale, Porter and Stout Beers. Mostly throughout the world Lager and Ale beers was preferred to drink.

Brewing i.e. making of beer is the name given to the combined process of preparing the beverage from infusion of grains that actually undergone sprouting (malting) and the fermentation of sugary solution by yeast, where the portion of carbohydrates is changed to alcohol and CO_2.

B] Distilled beverages (Spirit): -

For the preparation of spirit potato, maize, rye is mixed with barley green malt. The fermentation of malt is carried out by using yeast. The alcoholic distilled beverages like whisky or vodka which contains 35 to 40% alcohol, cannot be prepared by simple fermentation. But these are prepared by distilling the fermented liquor to desired concentration. Maximum amount of fermentable sugar maltose should be prepared. It is prepared by the action of diastase on starch. Here long and green malt which contains higher percentage of diastase is used. In this case no dextrin is formed and diastase needs not to be killed by boiling wort. The final fermentation to alcohol is carried out by using distillery yeast.

2) Some of the spirits like Rum, Gin and Brandies are obtained by distillation of Fermented liquors obtained directly from molasses, sugar refuse (bagasse) and fruit juices. In these cases, no diastase is essential. In case of molasses and other cane sugar containing materials, they are directly mixed with yeast containing invertase and zymase enzymes. Thus due to fermentation, cane sugar is first converted into glucose and fructose by invertase enzyme and then these monosaccharides are fermented into alcohol and CO_2 with the help of zymase.

$$\underset{\text{Cane sugar}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{Invertase}} \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

Molasses

$$\underset{\text{Glucose/Fructose}}{C_6H_{12}O_6} \xrightarrow{\text{Zymase}} 2\,\underset{\text{Ethanol}}{C_2H_5OH} + 2\,CO_2$$

Wines: -

Wine can be defined as an alcoholic beverage made by fermentation of fine quality juice of ripe grapes and the usual cellar treatment. Wine fermentation is a process of transformation of sugars present in grapes by yeast under anaerobic conditions into alcohol and carbon dioxide. Although most wine is made from grapes it can also be prepared from other fruits.

There are basically five different types of wines

- Appetizer wine 20 % alcohol Content
- Red table wine 10 to 14 % alcohol Content
- White table wine 10 to 14 % alcohol Content
- Dessert wine 20 % alcohol Content
- Sparkling wine 10 to 14 % alcohol Content

Wines are prepared by fermentation of fruit juice, but the most widely used is grape juice which contains glucose, fructose and various acids like tartaric acid. Skins of grapes contain tannin, essential oils and colouring matter. Generally fermentation takes place with the help of yeast cells present on the grape skin and stalk. This yeast belongs to the species of S. ellipsoideus. The wine is formed by fermentation and the young wine is ripened by storing in stoppered wooden casks. Some aeration takes place and acids and higher alcohols are converted into esters. The aroma and taste of the wine is due to the formation of these esters.

Manufacture of beer: -

The compounds formed in the beer depend largely upon the nature and quality of raw materials used in the production of beer.

•Raw Materials: -

1. Selected barley, malted to develop the necessary enzymes and flavor.
2. Malt adjuncts such as flaked rice, oats, wheat, rice and corn.
3. Hops- dried female flower of hop plant to impart characteristic bitter flavour and aroma.
4. Brewing yeast – Saccharomyces cerevisiae or S. carlsbergensis. (Source of enzymes).

Biochemical reactions: -

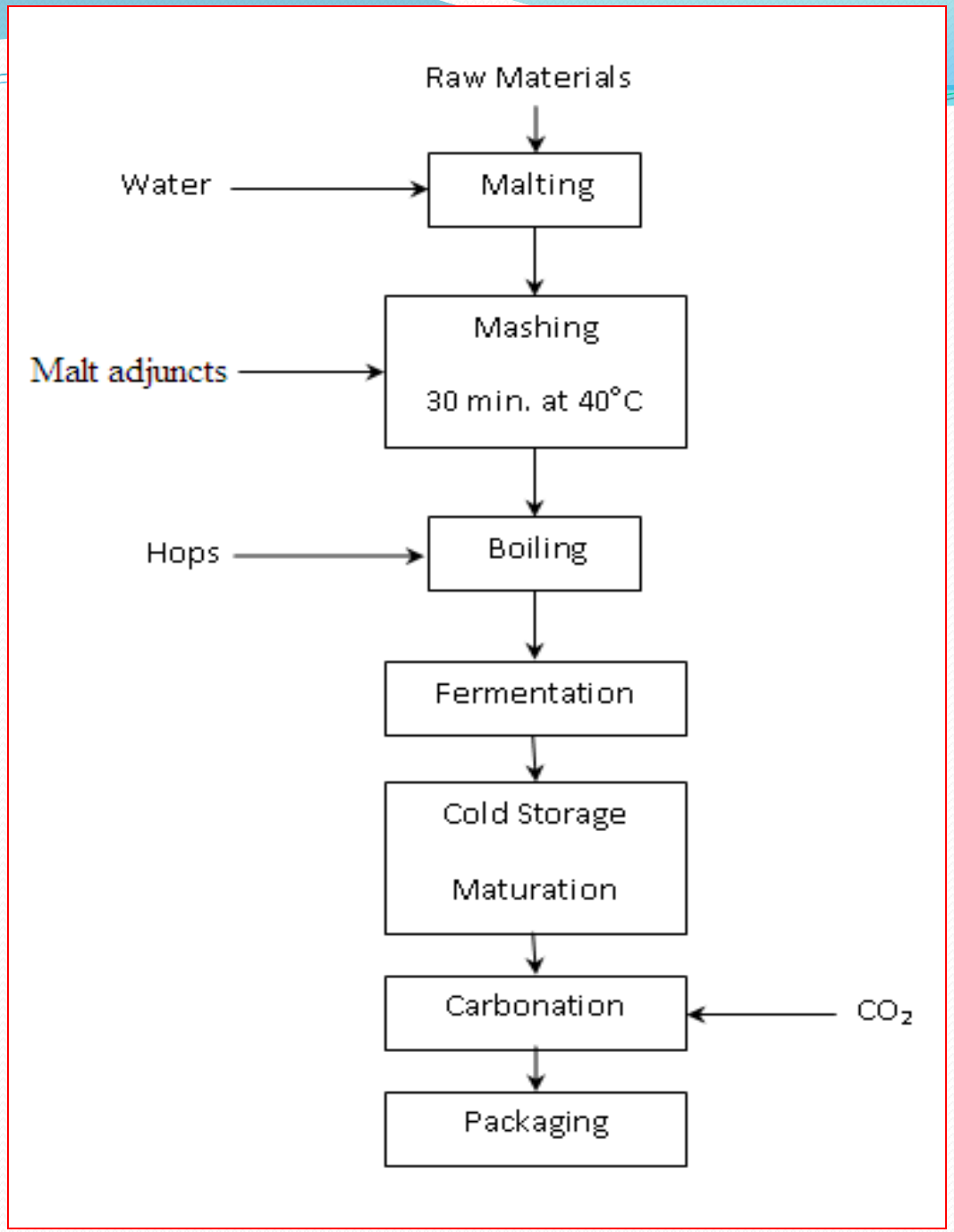
$$\underset{\text{Starch}}{(C_6H_{10}O_5)_n} \xrightarrow[H_2O]{\text{Diastase}} \underset{\text{Dextrine}}{(C_6H_{10}O_5)_{20}}$$

$$\underset{\text{Dextrine}}{(C_6H_{10}O_5)_{20}} + 10H_2O \longrightarrow \underset{\text{Maltose}}{10(C_{12}H_{22}O_{11})}$$

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{Maltase}} \underset{\text{Glucose}}{2\,C_6H_{12}O_6}$$

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{Zymase}} \underset{\text{Ethyl Alcohol}}{2\,C_2H_5OH} + 2CO_2$$

Flowsheet: -


Raw Materials
Water
Malting
Mashing
30 min. at 40°C
Malt adjuncts
Hops
Boiling
Fermentation
Cold Storage
Maturation
Carbonation
CO_2
Packaging

•**Steps involve in beer manufacturing: -**

1) Malting: -

Malt is prepared from the selected barley by soaking it in water so that, infusion of grains takes place and then allowing it to germinate (i.e. undergone sprouting). Then it is dried carefully under regulated conditions. Diastase enzyme is present in malt, which hydrolyses the starch in malt into disaccharides and some part into dextrine.

2) Mashing: -

Grinded malted barley and malt adjuncts are mixed with hot water in a mash tun and incubated for certain period of time (30 minutes at 40^{o}C and 20 minutes at 70^{o}C). During this incubation period, the hydrolytic enzyme in the malt acts on the carbohydrates and proteins in the grain and produces sufficient fermentable sugar, amino acids and peptides. These water soluble products passed into solution. This solution is called as ‘Wort”.

3) Boiling - The mash wort obtained is subjected to boiling in large stainless steel vessels for 1.5-2.5 hours. Hops are added at various intervals during boiling to furnish aromatic oils. Boiling causes sterilization, concentration of wort, inactivation of enzymes, extraction of soluble substances from the hops, precipitation of coaguable proteins and caramellization of the sugar.

After completion of boiling, the hops and the coagulated materials are removed from the wort. This wort is aerated and allowed to cool.

4) Fermentation: -

The aerated mash wort is cooled to 4 to 14^{o}C and inoculated with the yeast. Fermentation is carried out under anaerobic conditions. Fermentation temperature is controlled by cooling coils. During fermentation a large proportion of fermentable sugars are transformed to ethyl alcohol, CO_2, glycerol and acetic acid. Higher alcohols and acids are produced from protein derivatives. The fermentation is complete at about 7 to 12 days.

5) Cold storage maturation: -

After fermentation, the beer is run into large tanks in which it is stored at 0–3^{o}C for periods of several weeks or months. This is known as lagering, which provides an opportunity for a slow secondary fermentation to take place. Because of lagering, the taste and aroma of the beer becomes decidedly more mellow. The beer is also clarified to some extent as a result of the precipitation of proteins. At the end of lagering period, the beer is filtered.

6) Carbonation: -

The filtered beer is then carbonated by saturating it with CO_2 under pressure.

7) Packaging: -

The carbonated beer is filtered by passing it over diatomaceous earth and then it is packaged in pasteurized bottles, cans, barrels and kegs.

Manufacture of Spirit: - In the production of spirits from maize, potato, rye etc., the enzyme diastase is provided by green malt. The dry barley or wheat is steeped for 2-4 days in presence of dilute milk of lime to prevent growth of bacteria and moulds. The steeped barley is then allowed to germinate for about 20 days to produce maximum amount of diastase.

The starchy materials (potato, maize or rye) are gelatinized in cooker by high pressure steam for about 2-3 hours at 2 atm. pressure. This gelatinized starch is called mash, is then cooled to 55^{o}C and is introduced in a vat. Here 4-5% green malt is mixed in case of potato and 9-10% in case of other grains, such as maize or rye. The mixture is kept for 15 minutes at this temperature. The mash is saccharified and some more mash is added and temperature is controlled at 65^{o}C. At this temperature whole starch gets liquified. The temperature is then increased upto 67.7^{o}C to kill the bacteria completely and wort is formed within one hour. The diastase remains almost unaffected under these conditions.

The wort is made slightly acidic by adding lactic acid or hydrofluoric acid and allowed to cool at 26.7^{o}C and mixed with distillery yeast (S. cerevisiae). Again the temperature is lowered to 14^{o}C and then allowed to raise upto 29.4^{o}C by the heat of fermentation. The temperature is regulated untill the fermentation is over. The diastase and yeast transfer the dextrine into alcohol. The fermentation requires about 72 hours. The product obtained by this fermentation, contains 10-12% alcohol. Finally it is distilled to obtain spirit of containing desired (25-60%) alcohol concentration.

Manufacture of alcohol from cane sugar molasses (Blackstrap molasses): -

Blackstrap molasses: -

Molasses is a dark coloured liquid left after the crystallization of cane sugar from cane juice. It contains about 50 – 55 % total sugars (cane sugar, glucose, and fructose) of which 35 – 40% is cane sugar or sucrose. Black strap molasses is concentrated cane juice, which contains enzymes invertase and zymase responsible for fermentation.

A] Raw materials: -

i) Blackstrap molasses (it contains about 55% total sugar by weight of which 35 to 40% is sucrose and 15-20% is invert sugars(glucose and fructose).

ii) Selected strains of yeast (viz. S. cerevisiae)

iii) Sulphuric acid

iv) Ammonium sulphate, ammonium phosphate.

v) Process water and cooling water

Biochemical reactions: -

i) Inversion of sucrose:

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{Invertase}} \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

Sucrose

(Monosaccharides)

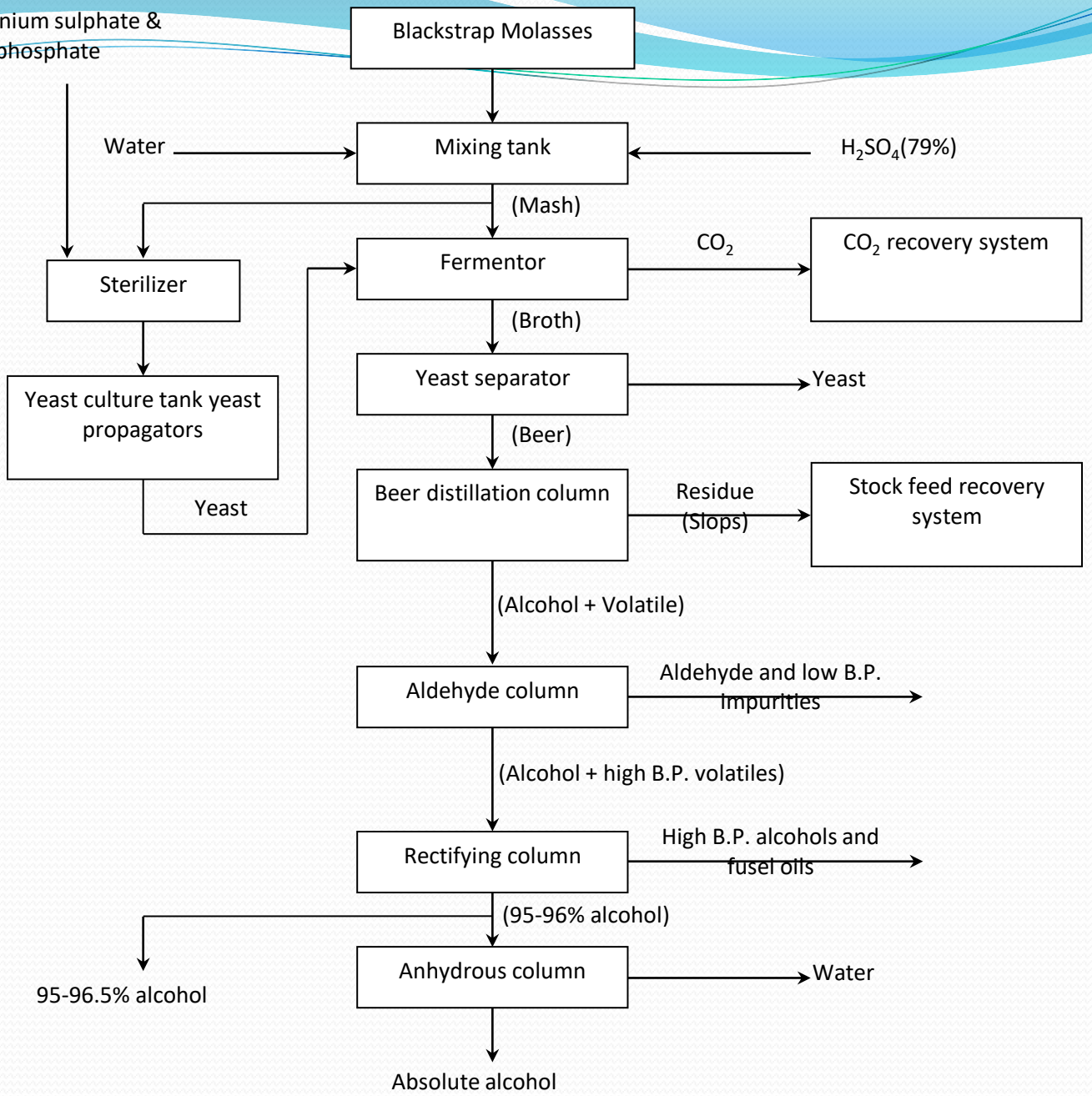
Fermentation of monosaccharides:

$$C_6H_{12}O_6 \xrightarrow{\text{Zymase}} 2\,C_2H_5OH + 2CO_2$$

iii) Side reactions:

$$2C_6H_{12}O_6 + H_2O \longrightarrow \underset{\text{Alcohol}}{C_2H_5OH} + \underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Glycerine}}{2C_3H_8O_3} + 2CO_2$$

Flowsheet: -


Ammonium sulphate & phosphate
Blackstrap Molasses
Water
Mixing tank
H_2SO_4(79%)
(Mash)
Sterilizer
Fermentor
CO_2
CO_2 recovery system
(Broth)
Yeast culture tank yeast propagators
Yeast separator
Yeast
(Beer)
Yeast
Beer distillation column
Residue (Slops)
Stock feed recovery system
(Alcohol + Volatile)
Aldehyde column
Aldehyde and low B.P. impurities
(Alcohol + high B.P. volatiles)
Rectifying column
High B.P. alcohols and fusel oils
(95-96% alcohol)
95-96.5% alcohol
Anhydrous column
Water
Absolute alcohol

Procedure: -

i) Preparation of mash: -

Blackstrap molasses is charged into a mixing tank and diluted with warm water to give sugar concentration of 10–15%. The P^H of molasses is above 5 and is reduced in between 4 to 5 by addition of sulphuric acid (79%). This diluted acidified molasses is commonly known as mash. The microbial contaminants in the medium are inhibited by the low pH and high sugar conc.

ii) Preparation of inoculum (Starter or growing of yeast): -

A selected strain of yeast (S. cerevisiae) is grown by adding the sterile mash with additional nutrients (Ammonium sulphate and ammonium phosphate) under controlled conditions of P^H 4–5 and temp 25–30^{o}C. During preparation of inoculum the yeast cells are slightly aerated to provide rapid cell multiplication.

iii) Fermentation: - The mash is taken into the large wooden or steel fomenter. Then about 5% inoculum is added with the mash. Anaerobic fermentation reaction takes place at 20 to 30^{o}C.

The enzyme invertase supplied by the yeast, catalyses the hydrolysis of sucrose to glucose and fructose. While, the zymase produced by yeast, catalyses conversion of glucose and fructose to alcohol and CO_2. The fermentation of monosaccharides is exothermic and hence heat evolved during fermentation raises the temperature but it is controlled by cooling coils. The fermentation requires 28–72 hours to produce an alcohol of 8–10% concentration.

iv) Separation of yeast: - After the fermentation, the yeast in fermentation broth is separated mostly by settling. The separated yeast may be recycled or used for other purpose.

v) Distillation of beer: - The fermented liquor (known as beer) containing 8-10% alcohol is subjected for distillation. The distillate contains 50-60% alcohol. The residue known as slop or stillage is discharged from the bottom of the beer column.

vi) Removal of low boiling volatiles: -

Aldehydes and other low boiling volatiles from the distillate are removed by fractional distillation as overhead of the aldehyde column. The remaining liquid is subjected to rectification.

vii) Rectification of alcohol: -

The azeotropic alcohol water mixture (95 – 96.6% alcohol) is obtained near the top of the rectifying column.

viii) Dehydration of alcohol: -

In order to obtain anhydrous or absolute alcohol a third component such as benzene is added to the rectified alcohol (i.e. 95% alcohol) and the mixture is distilled.

Theory of fractional distillation: -

The liquid to be distilled may be consists of the following types; -

•**Binary liquid:** A mixture of two components can be completely separable by fractional distillation.

•**Binary azeotrope:** A mixture of two liquid components which forms a constant boiling mixture or azeotropic mixture, which is separable into the azeotrope and the pure liquid (water and alcohol forms a binary azeotrope).

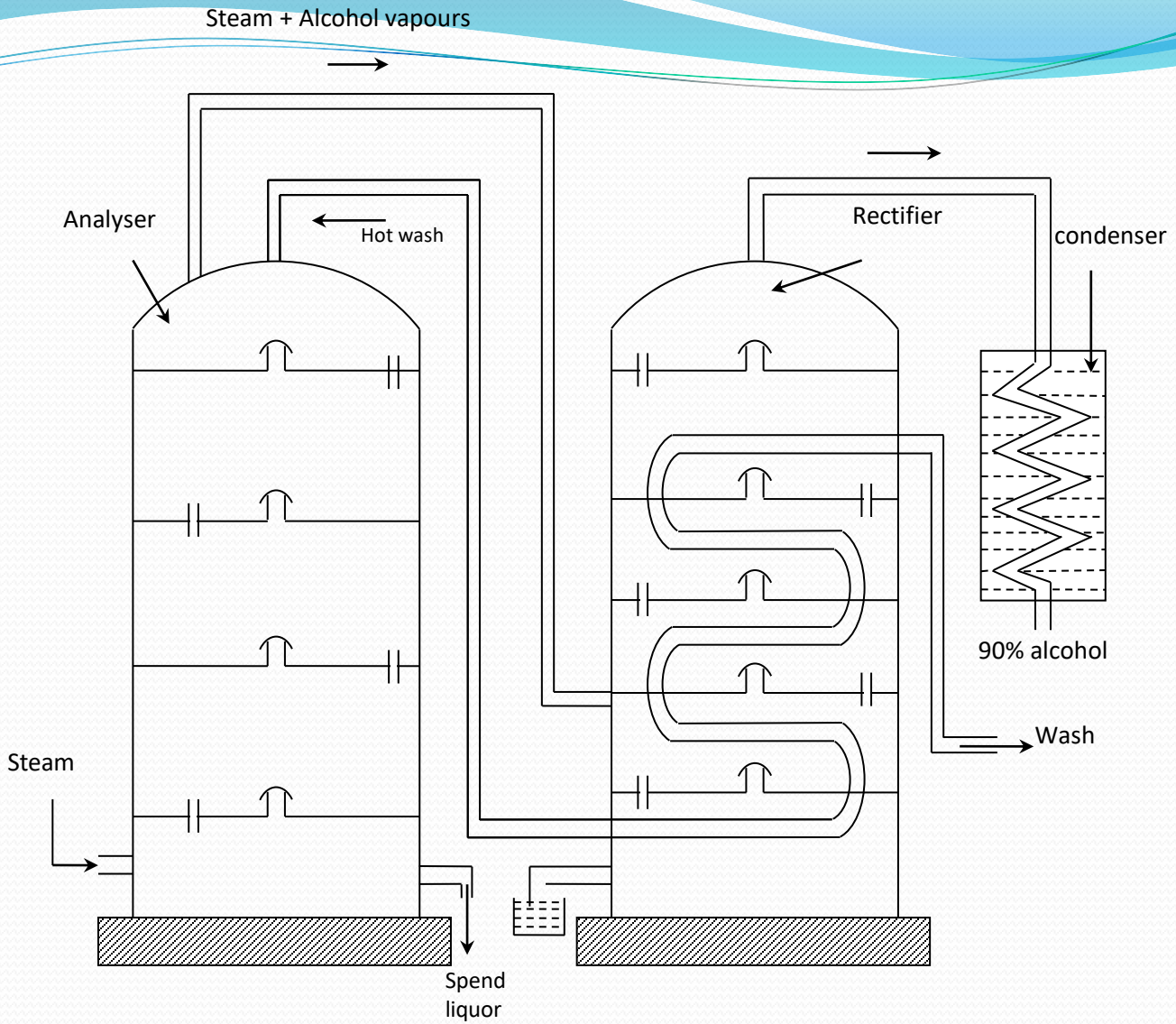
•**A ternary liquid:** A mixture of three liquids which forms a ternary azeotrope separable into ternary azeotrope and one of the pure liquids by fractional distillation (water, alcohol and benzene forms a ternary azeotrope).

•A liquid mixture containing several pure liquids of different volatility can be separated by fractional distillation. e. g. Crude petroleum.

Any type of liquid mixture can be separated into different fractions by using a still fitted with a fractionating column and a condenser. i. e. In modern plants, both distillation and rectification are carried out in one operation.

In case of large fractional distillation, the fractionating column is known as rectifier is used. When a liquid mixture is boiled, the vapour phase consists of higher portion of more volatile component and lower portion of less volatile component. When these vapours are scrubbed (meets) with the liquid mixture, then higher percentage (%) of less volatile components passes into the liquid state and lower percentage of higher volatile components are condensed and passes into the liquid state. Hence, when a mixture of vapours rising up meets a down coming mixture of the same liquid, in the rectifying column, then the vapour mixture becomes richer and richer in the more volatile component, and the down coming liquid mixture becomes richer in the less volatile component.

If the rectifying column is sufficiently tall, the components may be completely separated. The most volatile portion goes out from the top and least volatile portion collected at the bottom of rectifying column. Sometimes, the fractionating column is fitted with plates at different levels, which are provided with pipes for vapours and to drain the condensate from upper plate to lower plate.


Steam + Alcohol vapours
Analyser
Hot wash
Rectifier
condenser
90% alcohol
Wash
Steam
Spend
liquor

Aeneas coffey in 1831 deviced a continuous still for fractional distillation of wash (dilute alcohol) in order to make spirits. Instead of distillation flask, he used steam counter currently in his still.

Constuction: - Coffey's still consists of two tall fractionating columns called as analyser and rectifier. These columns are made up of wood and lined with copper plates. The analyzer and rectifier are divided into series of chambers by horizontal plates which are perforated with holes and furnished with valves opening upwards. The two columns are placed side by side on the same level. The analyzer is connected with a pipe at its top to rectifier near bottom. A zig-zag pipe is fitted inside the rectifier, whose inlet enters on the right side and the outlet going out through the left side near the bottom and becomes connected to the top of the analyzer. There is a steam inlet pipe on the left side near the base of the analyzer.

Working: - The coffey's still works on counter current principle as the steam and alcohol travels in opposite direction through the still.

Initially, the steam is introduced in the analyzer and it saturates the analyzer. Then the steam enters the rectifier and fills it up and goes out through the top.

The wash containing 6 – 10% alcohol is pumped into the zig-zag pipe, in the rectifier, where it gets heated nearly to its boiling point and is then discharged at the top of the analyzer, to act as the reflux liquid. Steam passes up the analyser and takes away the vapours of alcohol from the down coming wash. The mixture of alcohol and steam vapours leaves the analyser from the top and enters the rectifier near the bottom.

In the rectifier, condensation take place and most of the steam is condensed because of higher boiling point of water (100^{o}C) than that of ethanol (78.3^{o}C). The uncondensed alcohol vapours escape from the top and are condensed with the help of condenser. The alcohol thus obtained is about 90% pure. The residue left in the still is used as cattle feed.

Rectified spirit (Ordinary commercial alcohol): -

It is an azeotropic mixture of 96.5% alcohol and 3.5% water by volume.

Rectified spirit is also known as neutral spirits or rectified alcohol which is highly concentrated ethanol that has been purified by repeated distillation process called as rectification.

The distilled beverages like Rum, Gin, Whiskey, Brandy and Vodka etc. are known as **spirits**. The spirit is diluted with water for about 40-45% and is boiled in a still provided with a 45 feet long rectifying column. On boiling the spirit, and after passing through rectifier, the rectified spirit gets condensed. As an azeotrope, it gives a vapour of the same composition and hence it cannot be further concentrated by fractional distillation, no matter how efficient fractionating column is used. Rectified spirit is the ordinary commercial alcohol.

The rectified spirit is used as solvent for many organic reactions. It is also used to manufacture industrially the absolute alcohol.

Absolute alcohol (Anhydrous alcohol): -

It is the alcohol which is entirely free from water. Absolute alcohol means anhydrous alcohol.

Alcohol is made anhydrous by an azeotropic distillation of rectified spirit involving the use of benzene as a third added liquid. The benzene is added to rectified spirit and the mixture is fractionally distilled. The first fraction consisting of ternary mixture distills over at 64.8 ^{o}C containing water 7.4%, alcohol 18.5% and benzene 74.1%, until all the water is eliminated. Then the binary mixture passes over at 68.2 ^{o}C consisting of 80.2% benzene and 19.8% of alcohol until all benzene has been removed. The last fraction distilling over at 78.3^{o}C is absolute alcohol.

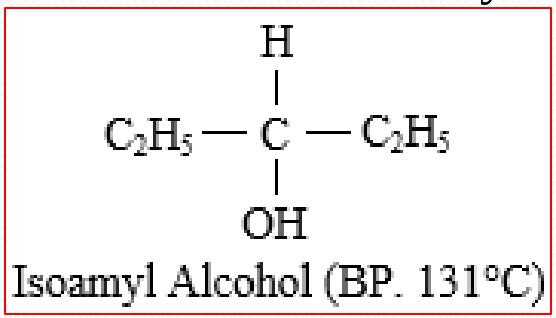
In laboratory, absolute alcohol is obtained by digesting the rectified spirit over quicklime for long time and then distilling.

The absolute alcohol is used in the manufacture of acetaldehyde and preparation of esters. It is also used as solvent.

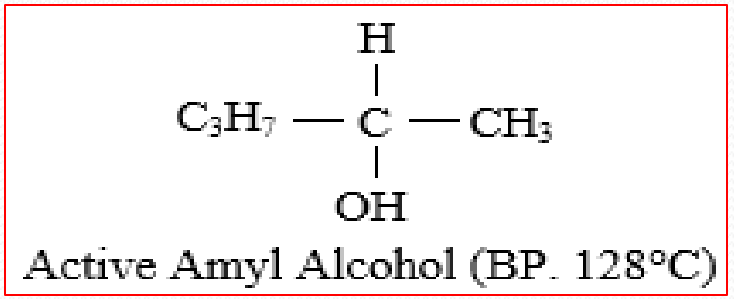
Fusel Oil: - Fusel oil is also called as Fusel alcohols or fuselol. It is a mixture of several alcohols produced as a by-product from alcoholic fermentation process. The word Fuse is German used for "Bad liquor".

Fusel oil (German meaning inferior liquor) is the last running in the distillation of crude spirit (between 125^0c and 140^0c). i.e. It is obtained as byproducts when rectified spirit is obtained by distillation of wash. It is a bright yellow oily liquid with a nauseating odour. It is insoluble in water and lighter than water.

It consists of a mixture of isomeric amyl (pentyl) alcohols, mainly isoamyl alcohol and active amyl alcohol, alongwith small quantities of n-propyl, n-butyl, isobutyl alcohols, hexyl alcohols and esters. Pure isoamyl alcohol and active amyl alcohol are obtained from fusel oil by fractional distillation.



Isoamyl Alcohol (BP. 131°C)



Active Amyl Alcohol (BP. 128°C)

It also contains about 3% ethanol. The proportion of fusel oil in raw spirit varies from 0.2 to 0.6% by volume. Fusel oil has a much higher price than the alcohol itself. It is mainly used in the production of amyl alcohol and for the preparation of amyl acetate which is valuable solvent for varnishes and lacqueres. Amyl acetate possesses pineapple essence due to which it is used in confectionary items.

Proof spirit: -

Formerly strength of alcohol was determined by pouring it over a gun powder and then setting fire to it. The weakest possible alcohol which would allow gun powder to catch fire was termed as proof spirit. The proof spirit concentration is a upper limit of dilution of spirit which can fire gun powder.

If the specific gravity of the alcohol sample is known, then the % of alcohol in the sample can be found out by referring to the standard tables. Special types of graduated hydrometers are used so as to read directly the % of alcohol in terms of proof spirit. This hydrometer is called “Alcoholometer” and is graduated in 10^{th} and 100^{th} of proof spirit, instead of specific gravity.

Proof spirit is now legally defined as an alcohol-water mixture which contains 57.1% of alcohol by volume or 49.28% by weight of alcohol at 15^{o}C with a specific gravity of 0.91976 at 15^{o}C.

The strength of alcohol (liquor) is expressed as degrees under proof (^{o}U. P.) or degrees over proof (^{o}O. P.) according as it is weaker or stronger than proof spirit respectively.

e.g.10^{o} under proof means that 100 volumes of sample contains as much alcohol as 90 volumes of proof spirit. Similarly 10^{o} over proof means that 100 volumes of sample contains as much alcohol as 110 volumes of proof spirit.

The tax is levied on the basis of proof spirit. This determination of strength (%) of alcohol is called alcoholometry. Absolute alcohol is 75.25 degrees over proof, with specific gravity of 0.7949 at 15^{0}c. Water is 100 degrees under proof.

Denatured alcohol (Methylated spirit): - The process which makes alcohol undrinkable is called as denaturing of alcohol. Ordinary rectified spirit is called as industrial alcohol. For external pharmaceutical preparations and industrial applications methylated or denatured spirit may be used.

The manufacture and sale of ethyl alcohol and alcoholic beverages is controlled by the government. Heavy excise duty is levied on them. Pure alcohol is subjected to heavy excise duty, but denatured alcohol which is used for industrial purpose is sold duty free or with nominal duty. It is therefore made unfit for drinking by adding poisonous and nauseating materials like methanol, pyridine, mineral naphtha, wood naphtha, methyl violet etc. the process is known as denaturing. The added substances give the spirit disagreeable taste. The denatured alcohol is generally sold in the market under the name “Methylated spirit”, which is used for the preparation of varnishes and domestic purposes. The completely denatured formulas are admixtures of substances which are difficult to separate from the alcohol.

The composition of a highly denatured spirit which can be sold openly to the public by licensed dealers is as follows –

Component	Composition by volume
1. Spirit	90 parts
1. Wood spirit (Wood naphtha)	9.1 parts
1. Crude pyridine	0.5 parts
1. Petroleum naphtha	0.4 parts
1. Methyl violet	0.005 (traces)

In India, rectified spirit is generally denatured by the addition of Caout-Choucine (obtained by distilling vulcanized rubber) and 0.5% pyridine bases. Some other chemicals that are added to alcohol for denaturing are pyronate, acetaldol, methyl isobutyl ketone and kerosene.

Thank *You*.