



## \* Basic requirements for fermentation: →

### ① Micro-organism: →

Micro-organism that forms a desired end product is the most essential requirement for the fermentation process. These micro-organisms must be readily propagated and capable of maintaining biological uniformity, thereby giving predictable yields. The suitable micro-organisms are yeasts, bacteria and moulds.

### ② Raw materials: →

Must be cheap and easily available in uniform composition.

③ Time: → Time required for fermentation process should be less.

④ Recovery: → The recovery of the product in the pure form and should be easy.

⑤ Economy: → The process should be more economic than the chemical synthetic process.

## \* Factors favourable for fermentation: →

### ① Temperature: →

80°C enzymes become inactive.  
below 20°C fermentation is very slow.  
Optimum temperature 30 to 50°C.

② Aeration: → fermentation process usually proceed well only in presence of air.

### (111) ③ Concentration: →

High concentration of a sol<sup>n</sup> renders an enzyme inactive.

(114)

### ④ Presence of other substances: →

Certain inorganic salt solutions acts as a food for ferment cell.

### ⑤ Absence of Preservatives: →

Preservatives are those substances which destroy the ferments and retard the fermentation reaction. Hence it should be absent.

### ⑥ pH conditions: →

- Optimum pH 6.5 to 7.5 of the medium in the basic requirement for better fermentation result.

- Acidic and Basic substances produced by the micro-organism during fermentation can alter the pH. This inhibits the growth of the microorganism. So various buffers are used to control the pH.

### \* Fermentation operations:

- ① Preparation of inoculum.
  - ② Preparation of medium.
  - ③ Sterilization.
  - ④ Fermentation.
  - ⑤ Recovery of fermentations products.
- ⑥ Recovery of microbial cells.

### ① Preparation of inoculum :→

It involves the development of selected strain of micro-organism in flask culture and in seed tank. It requires sterile conditions. It involves number of successive stages with increasing volume.

### ② Preparation of Medium :→

Natural medium is chosen and its conc<sup>n</sup> and pH are adjusted. Sometimes a synthetic medium consisting of all nutrients such as carbon, nitrogen, vitamins and other growth factors such as buffers, pressures etc in appropriate amount in water is prepared. Conc<sup>n</sup> of nutrients and pH of medium is properly adjusted in mixing tank.

### ③ Sterilization :→

To avoid contamination of undesirable microorganisms, the equipment, medium and air is sterilized.

### ④ Fermentation :→

The inoculum is added to sterilized medium. The several process variables such as temp., pH, aeration, foaming etc are adjusted to get desirable yield of fermentation products. This operation is carried out in fermentation tank.

### (111) ⑤ Recovery of fermentations products: →

When fermentation is complete, the products of fermentation are recovered by various separation techniques such as precipitation, solvent extraction, adsorption on ion exchange resin or activated carbon and distillation. (112)

### ⑥ Recovery of microbial cells: →

It is carried out by filtration, centrifugation or gravity settling technique.

### \* Manufacture Industrial Alcohol: →

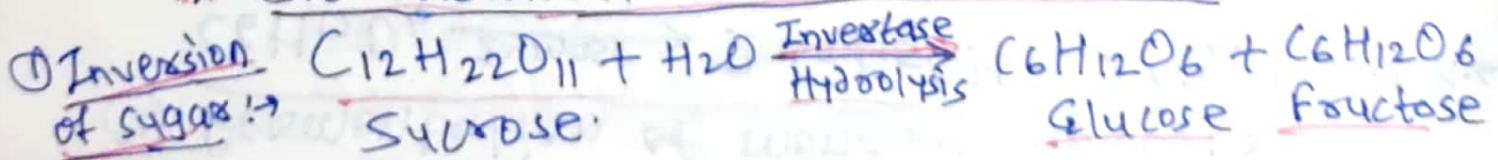
From ① Molasses ② food grains and ③ Hydrocarbons.

### ① Ethyl Alcohol from Molasses: →

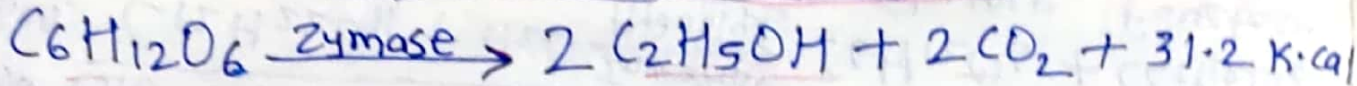
#### \* Raw Materials: →

- ① Blackstrap Molasses.
- ② selected strain of yeast.
- ③ Sulphyric acid 75%
- ④ Ammonium sulphate.
- ⑤ Ammonium Phosphate.
- ⑥ Process water, cooling water, electricity etc.

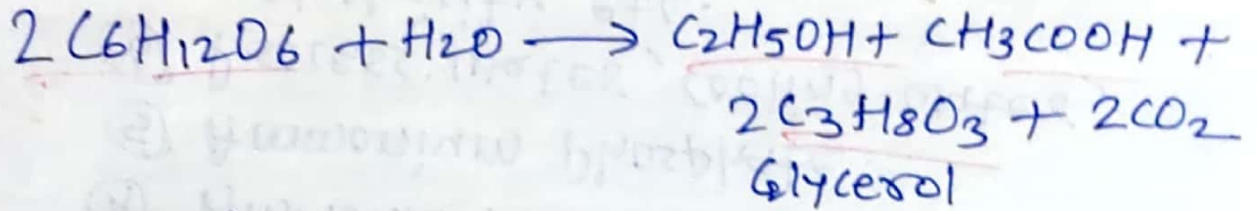
#### \* Bio-chemical reactions involved: →



② Fermentation of monosaccharides :->



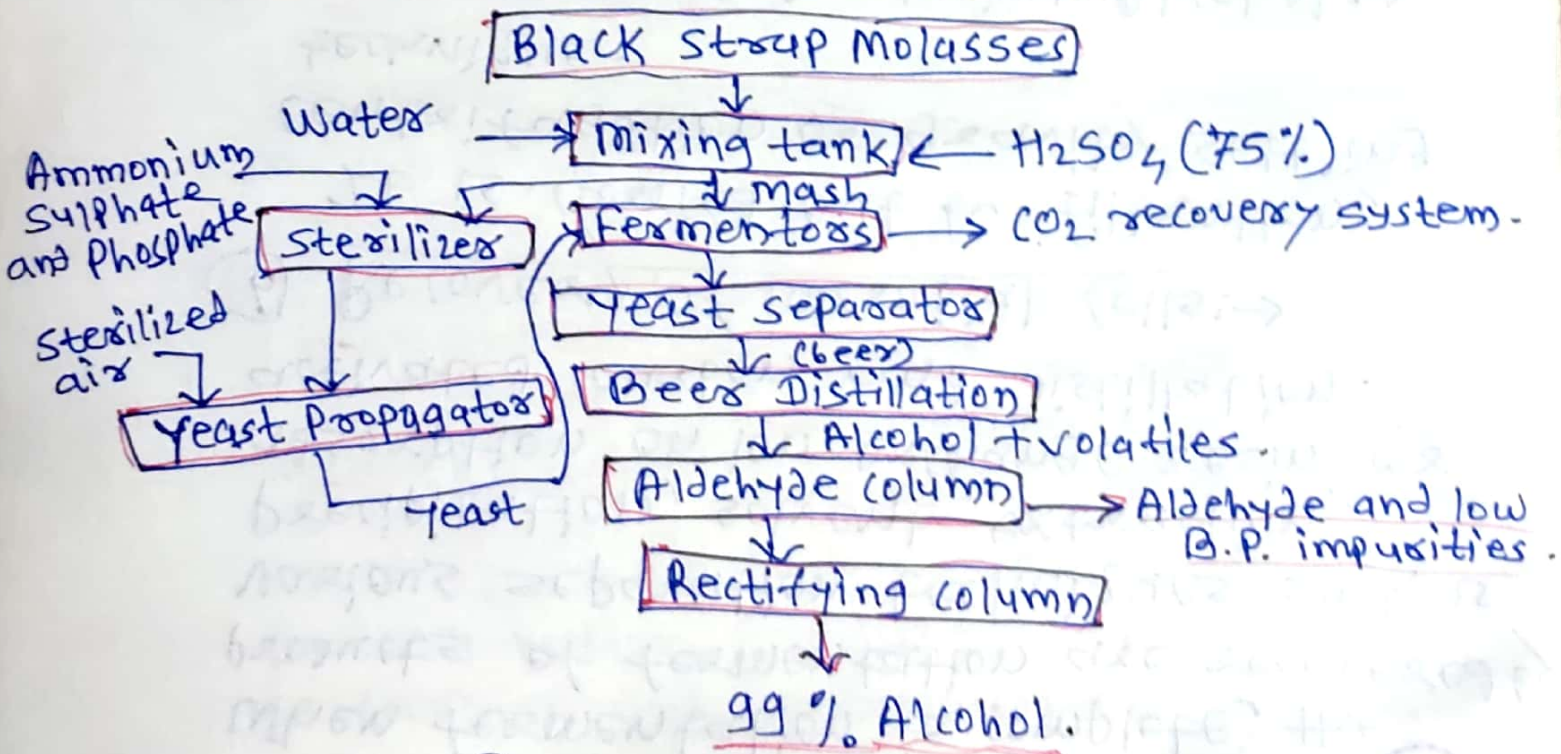
③ Side Reaction :->



\* Steps involved :->

- a) Preparation of medium.
- b) Preparation of inoculum.
- c) Fermentation
- d) Separation of yeast.
- e) Distillation of beer.
- f) Removal of Aldehyde and low b.p. volatiles.
- g) Rectification of alcohols.

\* Flow sheet diagram for manufacture of industrial alcohol from molasses :->



## \* Process :->

### ① Preparation of medium :->

(110)

- Molasses contains about 55% total sugars by weight. i.e. 35 to 40% sucrose and 15-20% invert sugar.
- High concentration of sugar in molasses can not support fermentation. It is diluted to 10-15% sugars. Small quantity of dilute  $H_2SO_4$  is also added to check the growth of undesirable bacteria.
- pH of solution brought down to 4 to 5 with  $H_2SO_4$ .
- The diluted and acidified molasses is called as 'Mash'.
- Nutrients for yeast ammonium sulphate or phosphate are also added.
- It is then pasteurised, cooled and charged into fermentation tank.

### ② Preparation of inoculum :->

- pH maintain between 4-5 and temperature kept around 25 to 30 °C.
- Yeast cells are aerated for rapid multiplication.
- A charge of selected strain is added to fermentation tank.

### ③ Fermentation:

- It is carried out at 23 to 32 °C. Fermentation reaction is exothermic therefore it is necessary to carry out cooling.

① Fermentation requires 28 to 72 hours to produce an ethanol concn 8 to 10 %.

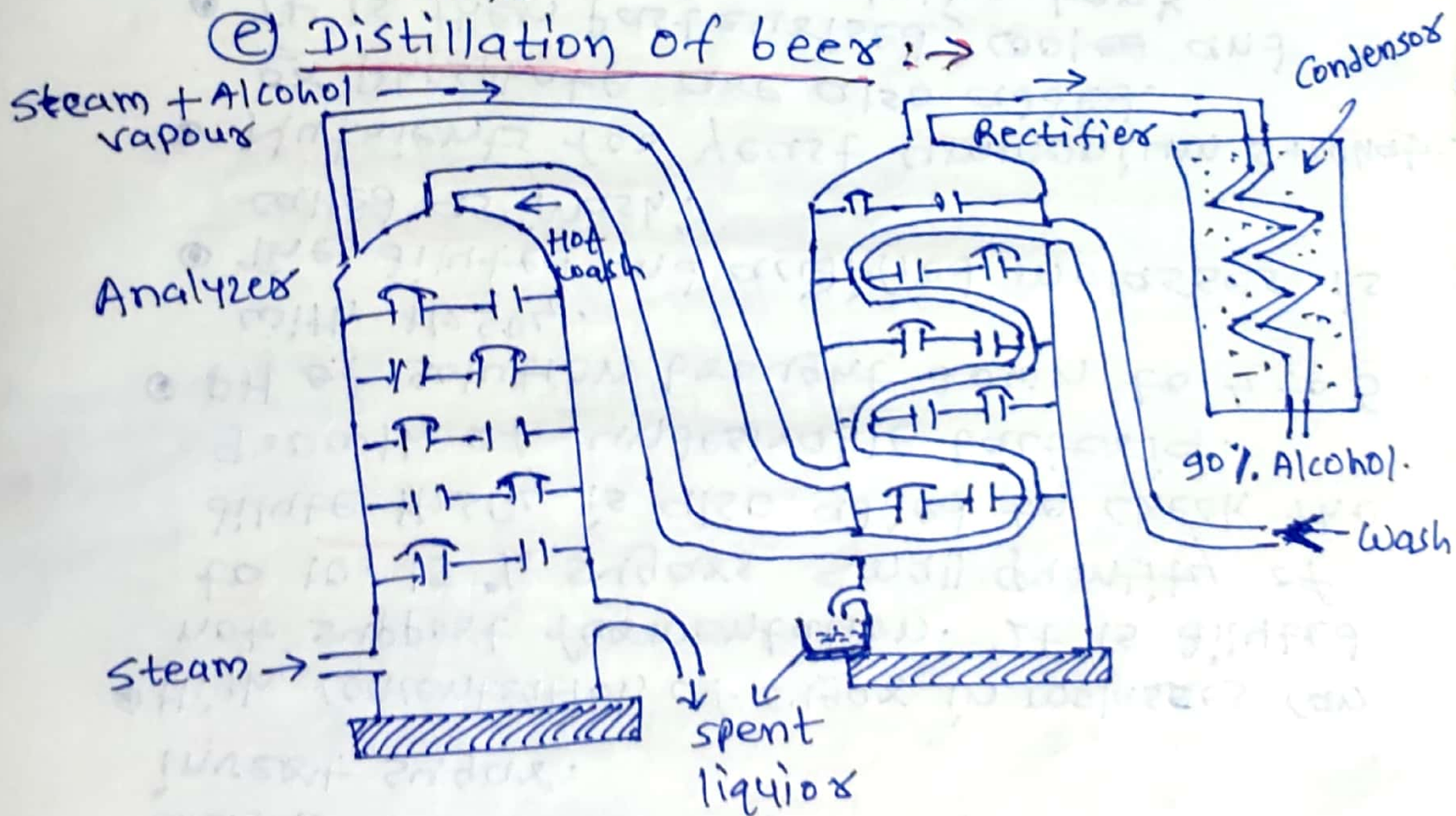
② CO<sub>2</sub> normally vented. The fermented liquor is known as beer.

### ④ Separation of yeast :->

① When the fermentation is completed the yeast in the fermentation broth is separated by gravity settling.

② The separated yeast is recycled or used for making animal feed as it contains residual sugars, proteins and vitamins.

### ⑤ Distillation of beer :->



• Fig: Distillation of wash in coffee still :->



## (f) Removal of aldehyde and low B.P. volatiles:

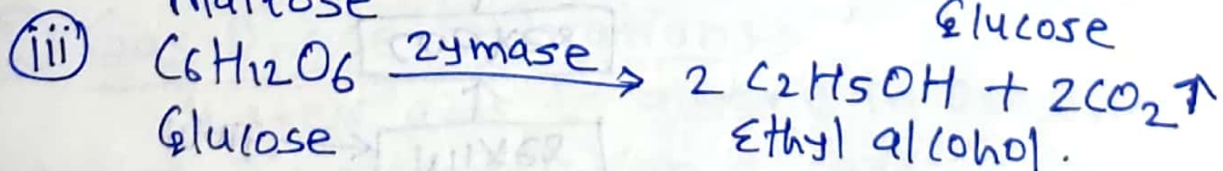
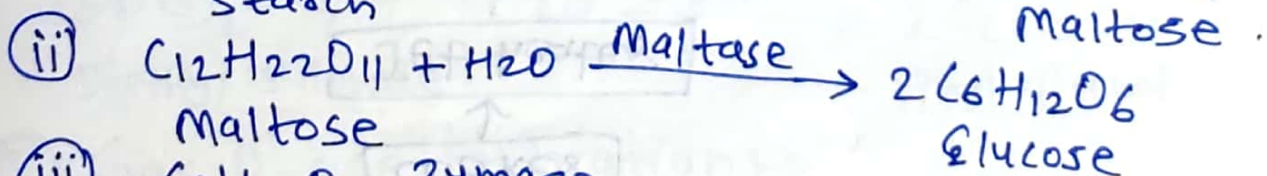
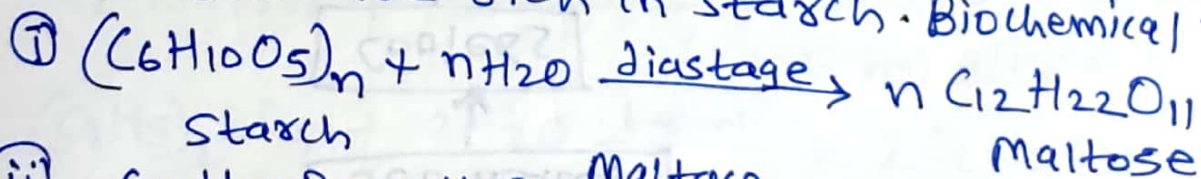
- ⊙ The overhead from beer column is passed through heat exchanger and condensed. The condensate is known as high wine contains about 50 to 60% alcohol.
- ⊙ It is then fed into a purifying column. The overhead from this column are aldehydes and other low boiling impurities.

## (g) Rectification of alcohol: →

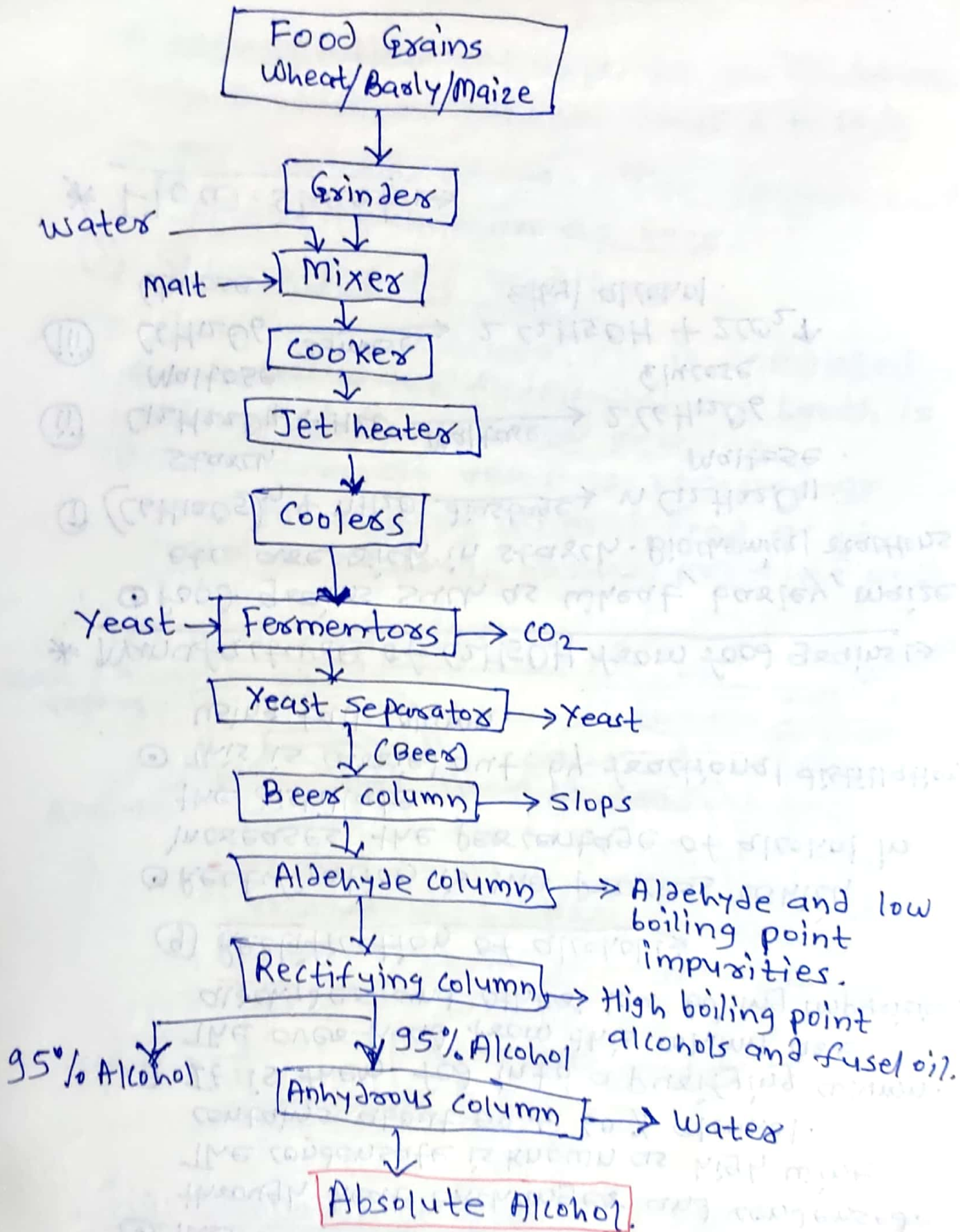
- ⊙ Rectification is the process which increases the percentage of alcohol in the distillate.
- ⊙ This is carried out by fractional distillation using tall column.

## \* Manufacture of $C_2H_5OH$ from food grains: →

⊙ Food grains such as wheat, barley, maize etc are rich in starch. Biochemical reactions



## \* Flow sheet: →



\* Manufacture of Industrial C<sub>2</sub>H<sub>5</sub>OH from HC :->

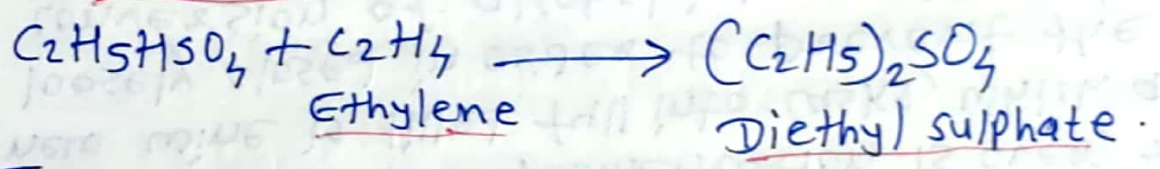
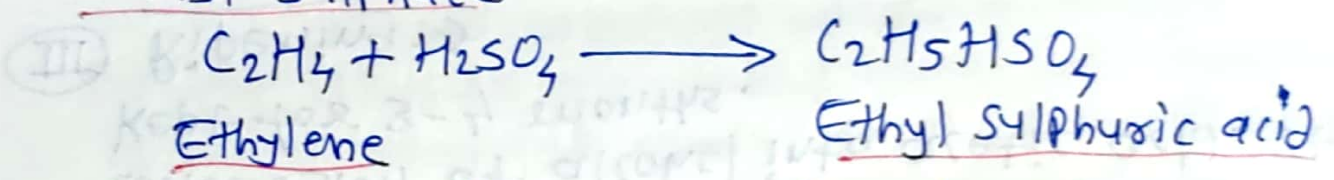
The process involve two routes:

(I) Liquid Phase hydration

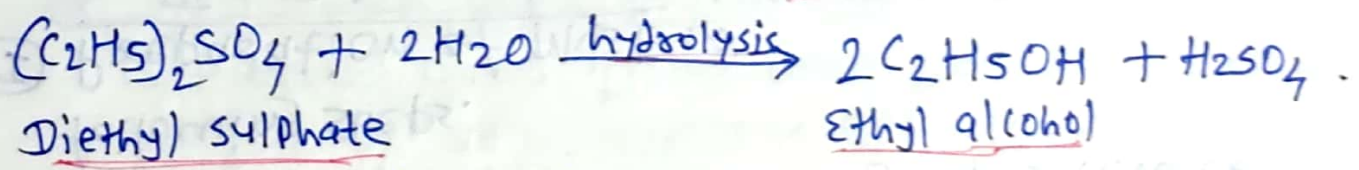
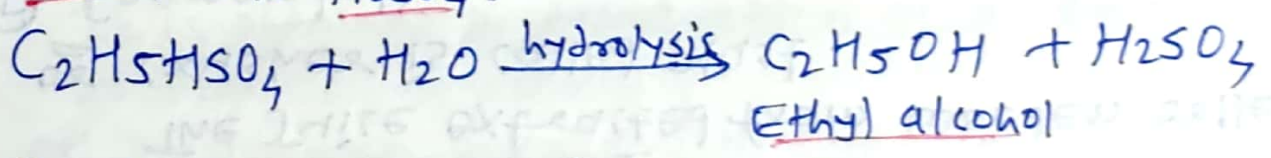
(II) Gas Phase hydration

(I) Liquid Phase hydration :->

In this process ethylene is absorbed in conc. H<sub>2</sub>SO<sub>4</sub> forming ethyl sulphuric acid and diethyl sulphate.

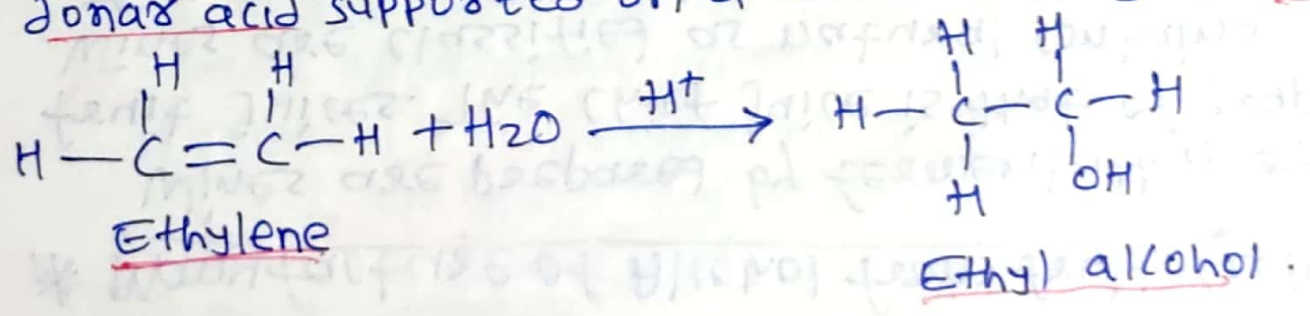


These ethyl sulphuric acid and diethyl sulphate on treatment with water hydrolyse to ethyl alcohol and H<sub>2</sub>SO<sub>4</sub>.



(II) Gas Phase hydration :->

Water adds to alkene in presence of proton donor acid supported on a carrier to yield alcohol.



## \* Manufacture of Alcohol from fruit (wine)

Wines are prepared by fermentation of fruit juices. The chief juice is that of grapes. Wines are classified as natural contains 7-14% alcohol and fortified contain 14-30% alcohol.

To obtain wine from grapes involve following steps.

### (I) Preparation of Must :->

The Juice extracted from wooden roller or crusher is called Must.

### (II) Fermentation :->

The must is first fermented into ethyl alcohol. After first active fermentation is over, the new wine is filled full into casks, which are loosely closed in order to prevent the conversion of alcohol into acetic acid. It is kept for 3-4 months.

### (III) Ripening :->

The clear wine is then allowed to ripe for 2-4 years. During ripening tanning and some other impurities are precipitated. In this period alcohol and fusel oil react with acids present to form esters. These esters give characteristic flavour to the wine.

### (IV) Bottling :->

After ripening the wine is bottled. The quality of wine varies from place to place and varieties of groups.

## \* Grades of Alcohols :->

### (I) Silence spirit :->

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It is well purified or clean spirit with no flavour. It contains about 94-96%  $C_2H_5OH$ . It is neutral spirit. It is used in manufacture of rectified spirit and absolute alcohol. It is also used for therapeutic neurolysis of nerves.

### (II) Rectified spirit :->

The alcohol water mixture containing 95.6% alcohol obtained after intensive rectification of the spirit using tall rectifiers is called rectified spirit. Its b.p. is slightly lower than the b.p. of pure alcohol. It is used in many organic reactions as a solvent. It is used in manufacture of absolute alcohol.

### (III) Absolute Alcohol :->

It is 100% pure alcohol. It boils at 78.3 °C. It is obtained by dehydration of rectified spirit. It obtained as follows.

(a) Distillation over quick lime: The 95% alcohol is passed over quick lime ( $CaO$ ) which absorbs water by reacting with it and then subjecting it to distillation.

(b) Azeotropic distillation :-> The rectified spirit is mixed with benzene and a ternary azeo- trope of benzene, alcohol and water is formed (74.1% benzene + 18.5% Alcohol + 7.4% water). This mixture when distilled removes water from rectified spirit.

### (IV) Proof spirit :->

It is legally defined as alcohol - water

mixture which contains 57.1% ethanol by volume and is having a specific gravity 0.91976 at 60 F°. The strength of alcohol is determined by density measurements and excise duty is imposed on proof spirit.

A sample is called under proof if it is weaker than proof spirit and it is called over proof if it is stronger than proof spirit.

#### (V) Denatured spirit :->

The process that makes the alcohol undrinkable is called denaturing of alcohol. The various agents such as wood naphtha consisting of methyl alcohol, acetaldehyde, acetone and other ketones and also small portions of methyl acetates are used for denaturing alcohol.

#### (VI) Duty and Duty free alcohols :->

Alcoholic beverage is taxable. The tax is called duty. The duty levied is very high in order that people may not be able to drink alcohol in large amount.

#### \* Importance of Power Alcohol as fuel :->

Power alcohol is used as fuel when mixed with petrol in internal combustion engines as a motor spirit. Blends containing upto 25% of alcohol with petrol have been used as motor spirit.

Industrial alcohol containing 95% alcohol

and 5% water can also be blended with petrol using another blending agents such as benzene, ether, tetraolin etc. Industrial alcohol is not soluble in petrol without blending agent.

Gasohol is a fuel mixture of 10% alcohol and 90% gasoline. It is most important use of fermentation alcohol.



### 3. Sugar and Fermentation Industry:

a) Sugar: Introduction, Manufacture of cane sugar, Extraction of juice, Purification of juice, sulphitation and carbonation, Evaporation, Crystallization, Separations of crystals, Drying, Refining, Grades, Recovery of Sugar from Molasses. By-products of sugar Industry.

b) Fermentation Industry: Introduction, Importance, conditions favourable for fermentation, characteristics of enzymes. Short account of some fermentation processes. Alcohol beverages, Manufacture of Beer, Manufacture of spirit, manufacture of wines, manufacture of vinegar, manufacture of power alcohol, Ethyl alcohol from molasses.

#### a) Sugar Industry:

##### \* Introduction:

Carbohydrates of simple structures are called sugars. Sugar and starch are among those chemicals found so abundantly in nature. The plants containing sugar and starch are like sugar cane, sugar beet, potato etc. Cane sugar belongs to this class called as sucrose having the formula  $C_{12}H_{22}O_{11}$ .

Sucrose is obtained commercially in substantial amounts from sugar cane and sugar beet plants only. Sugar cane supplies about 56% of the world total sugar and beet provide 44% sugar. Sugar in the cane and beet has same chemical composition as sucrose. Sugar is formed in the stalk of the sugar cane by a process of photosynthesis.

##### \* Importance of Sugar Industry:

- ① Sugar is used all over the world as a sweetening agent which is refined, purified and white.
- ② Sugar supplies about 13% of the energy required to existence to man.



- ③ It supplies energy required for the growth of living cells and their reproduction and movement.
- ④ Sugar cane contains 70-75% water, 10-15% crystalline sugar, 0.5-1% reducing sugar, 10-18% fibre, 1% ash and 1% organic acids.
- ⑤ The molasses obtained as a byproduct in sugar industry is used for the manufacture of alcohol by fermentation technique.
- ⑥ The byproduct bagasse is one of the important raw materials required for manufacture of pulp, celotex, paper, wall board, etc.

### \* Cultivation and Harvesting of Sugar cane :->

Sugar cane is a large perennial tropical grass. It has a bamboo like stalk which grows to a height of 8 to 15 feet and contains 10 to 15% sucrose by weight. The cane is usually planted with cuttings from the mature stalk, in rows separated by 3-6 feet distance. Each cutting should contain two or more buds which sprout and produce a number of new stalks. Approximately 12 to 15 months are required for first crop while the subsequent crops require about 12 months. The cane stalk consists of a series of joints or internodes separated by nodes. The nodes are woody nature while the internodes are soft pith containing sucrose. Generally, after cultivation 2 to 5 harvests are obtained from the original planting.

The workers cut off the sugar cane close to the ground level as well as they remove leaves and tops of the stalk.

Transportation of sugar cane from field to mill is mainly done by the use of trucks, trailers, cart tandems etc. After cutting there can be no delay in transporting the freshly cut cane to the factory because failure to process it ~~within~~ within less

than 24 hours after cutting causes loss by inversion to glucose and fructose.

### \* Processes or steps involved in cane sugar Manufacture:

The cane sugar manufacturing involves numbers of physical and chemical processes or steps as follows.

- (i) Preliminary treatment to sugar cane.
- (ii) Extraction of juice.
- (iii) Purification of juice.
- (iv) Evaporation or concentration of cane juice.
- (v) Crystallization of sucrose.
- (vi) Centrifugation.
- (vii) Drying.
- (viii) Screening and bagging.

(i) Preliminary treatment to sugar cane: → Before milling sugar canes are given cleaning treatments such as washing in order to remove mud and dirt. e.g. soil, rock, clay etc. Washing systems can range from a simple wash with warm water on the carrier or table. In modern plants the washing system consists of conveyors with water jets, baths for removal of adhered dirt.

(ii) Extraction of juice: → (a) By chopping or cutting the canes into smaller pieces with one or two sets of rotating knives. The speed of rotating knives is about 400-600 rpm (revolution per minute).

(b) By disintegrating the canes into finer pieces by a crusher. The crusher consists of two roller mills.

The crushed sugar cane passes through a series of three horizontal rollers. The rollers are fixed at the corners of a triangle. The top roller from each mill rotates in

anticlockwise direction while the bottom two rollers rotate clockwise. The heavy pressure on the top roller is exerted by hydraulic rams. Below each mill a juice pan is placed for collection of extracted juice. The crusher and first mill extract 60-70% cane juice while the remaining mills extract 22-24% cane juice. To reduce sucrose in the fibre compound imbibition process is used.

**\* Compound Imbibition process:** In this process the juice from second mill is sprayed on the fibre i.e. bagasse as it passes from first mill. The juice from the third mill is sprayed on first as well as second mills while the juice from the fourth mill is sprayed with hot water and the ~~expressed~~ juice from that mill is brought back to the second mill. It removes nearly 95-98% juice from bagasse by extraction.

The juice from milling station is pumped into a weighing tank. The weighed juice is screened with metallic cloth to remove suspended impurities. This juice is acidic, opaque greenish liquid containing soluble impurities like soils, fats, proteins, waxes, gums and colouring matters.

**(iii) Purification or clarification of juice:** → After weighing the juice is sent for purification process to remove soluble impurities. The purification or clarification is done as follows.

**(a) Purification or clarification by lime defecation process:** → The screened juice is acidic having pH 4.9 to 5.5. In acidic solution the inversion of sucrose may take place, so to avoid inversion of sucrose it should be neutralised by adding milk of lime i.e.  $\text{CaO}$  to bring the pH to the range 7.5 to 8.5. Then this solution is heated to a temp. between  $90^\circ\text{C}$  to  $115^\circ\text{C}$ . The combined action of lime and heat on the juice leads to precipitate tricalcium phosphate. Along with this remaining

colloidal impurities such as proteins, gums etc are coagulated and precipitated. The precipitate is removed by sedimentation or settling in clarifiers. Sugar is recovered from the muds by using rotary vacuum filters with perforated metallic screen cloth. The turbid filtrate is returned to the purification system and then ~~press~~ press cake is discarded or used as a fertilizer.

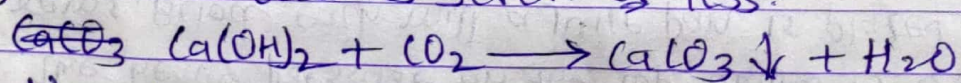
**(b) Sulphitation process:** The juice can be treated with calcium phosphate and  $\text{SO}_2$  is bubbled through the juice until pH becomes 7 to 7.1 which is heated to about  $60-70^\circ\text{C}$ . The hot juice is sent to the settling tank where suspended matter and impurities get precipitated out and clear juice is obtained. Then impurities are removed by filtration.  $\text{SO}_2$  acts on lime for forming a precipitate of calcium sulphite. It can be removed by filtration.



Sulphurous acid is formed by passing  $\text{SO}_2$  gas in the juice, it is a strong bleaching agent. The  $\text{SO}_2$  in purification serves three purposes

- (i) It neutralises excess quantity of lime added.
- (ii) It bleaches juice by acting on the colouring matters.
- (iii) It decreases the viscosity of juice.

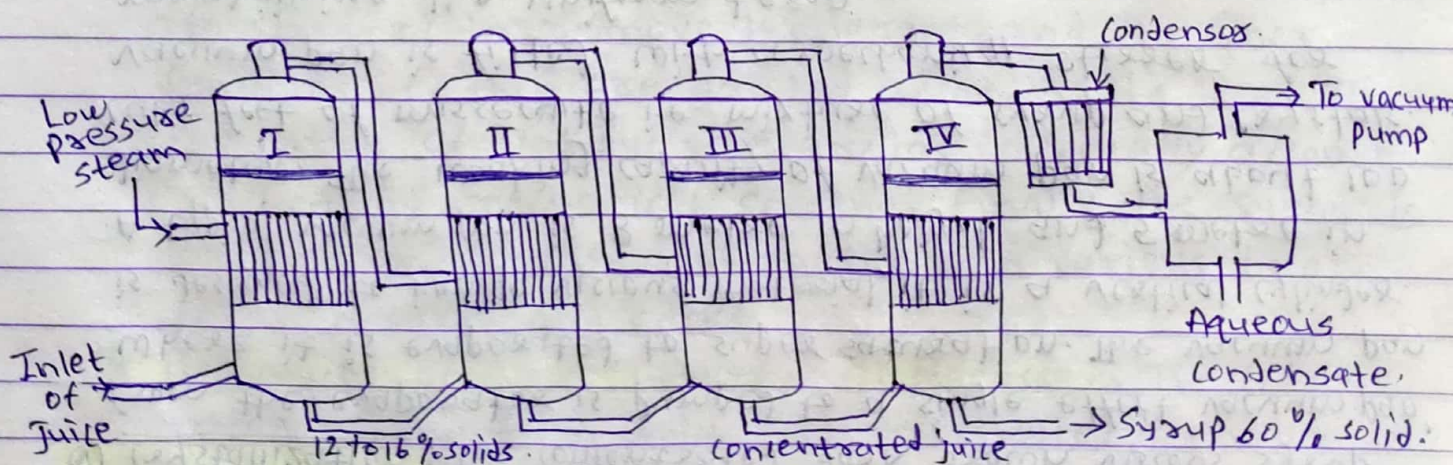
**(c) Carbonation process:** The purification of cane juice is also carried out by passing  $\text{CO}_2$  gas and which acts as a neutralising agent while the lime is used as a clarifying agent. Sugar cane juice is heated at about  $60^\circ\text{C}$  and milk of lime is added to it. The  $\text{CO}_2$  gas is obtained by burning limestone in the kiln. Then  $\text{CO}_2$  gas is passed through limited cane juice till the pH of the resulting solution becomes seven or less.



Carbonation process removes large amount of non-sugars as compared to sulphitation process.

Good quality sugar produced is also superior when carbonation process is used instead of sulphitation process.

(iv) **Evaporation or concentration:** The clarified juice is pumped to evaporator where it is concentrated to a clear heavy syrup containing about 65% solids. Evaporation is carried out in multiple effect evaporator in order to achieve maximum steam economy. Each evaporator is made up of steel plate having cylindrical shape. All evaporators are placed vertically and are heated by means of steam. This arrangement allows the juice to be drawn from one vessel to the next and permits the juice to boil at low temperature. The steam is produced under vacuum and it is circulated through 'multiple evaporators' system. When the steam enters in second evaporator which have less temp. still the evaporation becomes rapid. This is because higher vacuum is maintained in the second evaporator. The surfactants are added to the juice to improve the rate of evaporation. The water is removed at comparatively lower temp. which does not allow sugar loss due to evaporation. From the fourth evaporator the pale-yellow syrup is obtained containing about 60% solid matter.



**Fig: Multiple effect evaporator system:**

④ Crystallization: The concentrated dark brown viscous syrup from the evaporator is pumped to a single effect 'vacuum pan' where it is evaporated to super saturation. The vacuum pan is designed to handle viscous material, it is a vertical cylinder. A typical vacuum pan is 8 metre in height and 5 metre in diameter. The working capacity of vacuum pan is about 100 cubic feet of massecuite i.e. mixture of syrup and crystals. Vacuum pan is fitted with a mechanical stirrer for maintaining the uniform temp.

The Crystallization is carried out in four different stages

- ① Seeding or grainin
- ② Establishing the seeds
- ③ Growth of crystals and
- ④ concentration.

The 'seed grain' is added in the first compartment which serves as a nucleus for the sugar crystals. Additional syrup is added to control the fluidity of the massecuite. Approximately 20% of the syrup is introduced at the concentration and 80% of the syrup is fed to the various compartments.

The crystallization of sugar in vacuum pan is called sugar boiling and each boiling is termed as strike. A single crystallization is not sufficient to recover all the sugar from the syrup and hence it is necessary to follow three or four boiling. The product of first boiling would be given the letter A and its products after centrifugation are A-sugar and A molasses. Similarly products of second and third boiling would be assigned the letters B and C respectively. The products of first and second boilings are termed as A and B massecuites and are sent to centrifugal for separation of crystals. While the product of third boiling is a low purity, highly viscous material containing a large amount

of recoverable sucrose remained in the solution. Centrifugation of 'c' massecuites yields final molasses and 'C' sugar is used for seeding the sugar A and B.

(vi) **Centrifugation**: Massecuites A and B from vacuum pan are sent to the centrifugal machines where the crystals are separated from the mother liquor. A centrifugal machine consists of a cylindrical perforated basket. It is lined with a perforated metal sheet. The basket is placed on a shaft which can rotate the massecuite from a vertical layer on the screen lining. The basket rotates with the speed 1000-1800 revolutions per minute. The perforated lining helps to separate the sugar crystals which wash with a spray of water. Due to this the molasses adhering on the crystals are removed. The basket continues rotating until the sugar is dry.

(vii) **Drying**: The wet sugar is fed to drying equipment called granulators which are nothing but rotating horizontal drums. Heated air is passed through the dryer counter current with the flow of sugar. The temp. of the hot air must be controlled otherwise high temp. removes the luster of the sugar crystals.

(viii) **Screening and bagging**: The resulted dried crystals are screened to remove the larger and the finer fractions i.e. they are graded according to size. The separation of various size grains is usually accomplished by mechanical screens. Finally the sugar is weighed and packed automatically in various bags, boxes, barrels and sacks.

\* **Byproducts of Sugar Industry**: (i) **Bagasse**: The fibrous portion of cane from which juice is extracted is known as bagasse. It contains about 46-52% moisture, 43-52% fibre

and 2-6 % soluble solids.

### \* Uses of bagasse: →

- (i) It is used as a fuel for the generation of steam which is utilised in electricity production.
- (ii) It is utilized as a raw material for the pulp, paper, paper board and wall board industries.
- (iii) Bagasse charcoal is manufactured from bagasse which on destructive distillation gives charcoal, combustible gases, methyl alcohol, acetic acid and tar.
- (iv) Pit is separated by gravity separators from bagasse and used in the manufacture of explosives.
- (v) Bagasse is used in the manufacture of  $\alpha$ -cellulose, plastic, furfural and bagasse concrete.
- (vi) Bagasse mixture with molasses and ammoniated bagasse are used as animal feed.
- (vii) The compost of bagasse contains potassium 0.3 %,  $P_2O_5$  0.005 %, nitrogen 0.4 % and hence used as a neutral fertilizer.
- (viii) A high quality wax has also been extracted from bagasse.

(2) **Molasses:** → It is a dark coloured viscous liquid left after crystallization of cane sugar from cane juice. A residual mother liquor left in the crystallization of sugar from which no additional sugars can be recovered economically is a by-product, commonly called black strap molasses. It is concentrated cane juice from which no cane sugar has been extracted. It consists a significant quantity of both sucrose and reducing sugars.

### \* Uses of molasses: →

- (i) It is extensively used for cattle feed, although it provides



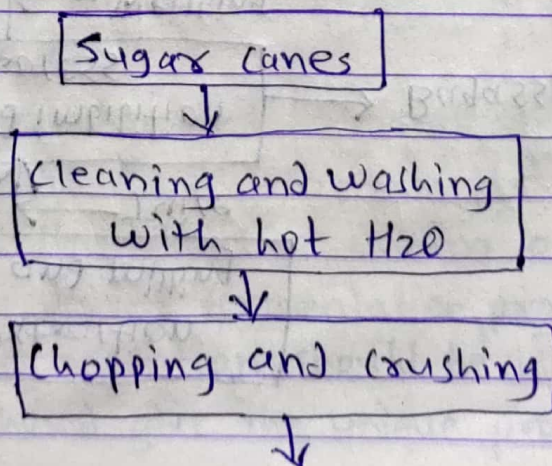
carbohydrates.

- ② molasses is used in the manufacture of organic chemicals like acetone, citric acid, glycerol, acetic acid and vinegar.
- ③ Edible syrup is manufactured from molasses.
- ④ Molasses is utilized to produce compressed yeast which contains enzymes invertase and zymase responsible for fermentation.
- ⑤ It is used in the distilleries for the production of rum and ethyl alcohol by the process of fermentation.
- ⑥ It is also used as a neutral fertilizer as for every one metric tons of molasses spread in the field the land receives on the average 5.2 Kg nitrogen, 2.5 Kg  $P_2O_5$  and 51.3 Kg  $K_2O$ .

③ **Furnace ash:** The bagasse as contains silica ( $SiO_2$ ) and oxides of other metals like Ca, Mg, K, Na, Al, Fe and P. The quantity of ash produced averages 0.3 to 0.5 % of the weight of the cane.

\* **Uses of furnace ash:** →

- ① It is mostly used as a neutral fertilizer because it contains  $K_2O = 10\%$  and  $P_2O_5 = 3\%$ .
- ② It is also used in the glass industry, as it is easy to grind, it resist to water below  $100^\circ C$ .



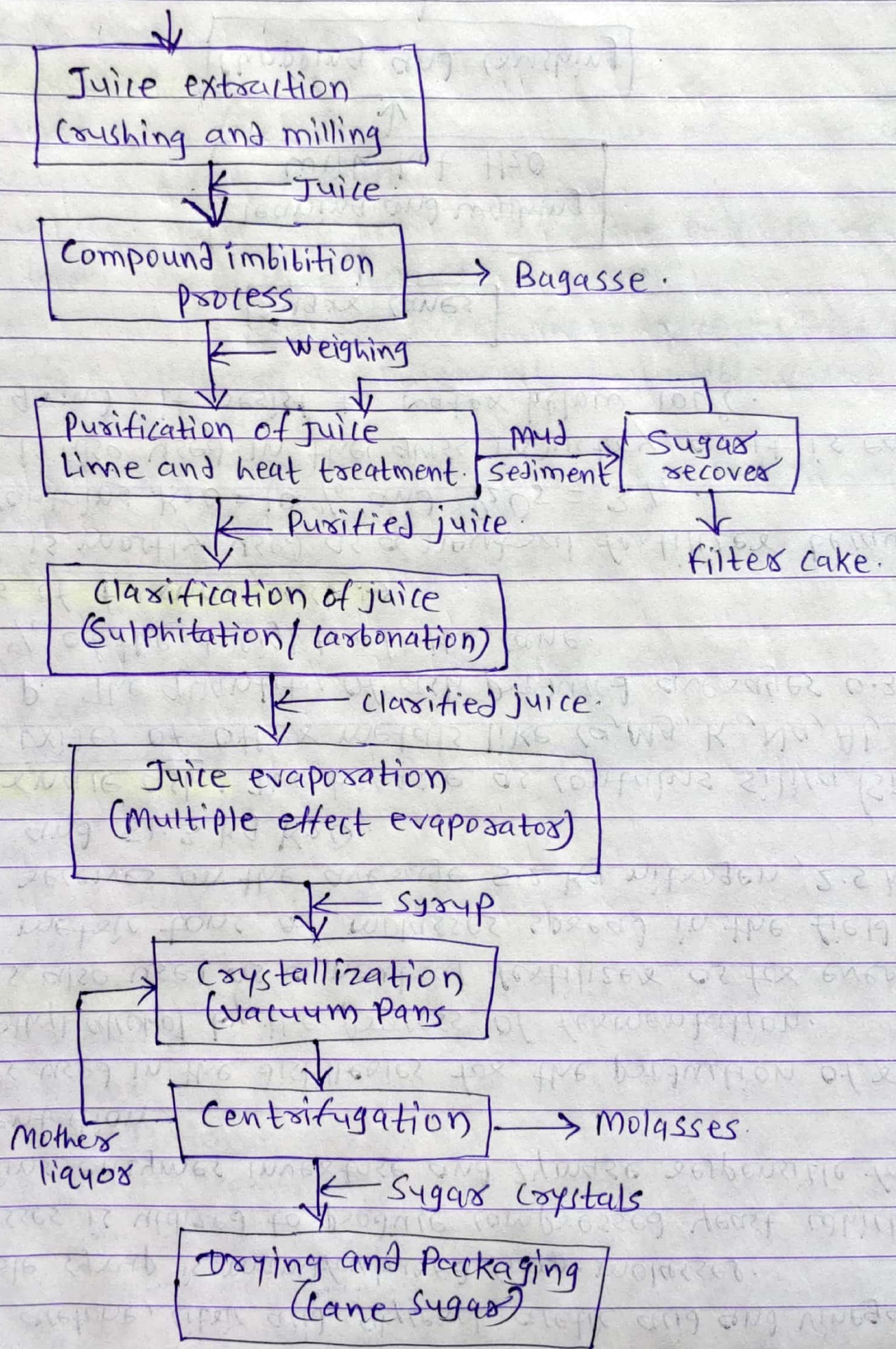


Fig. Flow-sheet for the Manufacture of Cane Sugar.