Food and Industrial Microbiology

Industrial production of ethanol in India

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1. Introduction

Origin of the word alcohol

Ethanol with a chemical formula C_2H_5OH is a key component of alcoholic beverages. It also has other common names such as alcohol, ethyl alcohol, grain alcohol, cane alcohol, wine spirit and cologne spirit. It is a colourless, transparent, neutral, volatile, flammable, oxygenated liquid hydrocarbon, which has pungent odour and a sharp burning taste. There are various beliefs about the origin of the word alcohol. According to one, it is thought to have been derived from Arabic word 'al kohl' meaning like 'kohl'. The word 'kohl' means powdered antimony which is often used as cosmetic around the eyes in Muslim and Asian countries. Since antimony is made through a process of distillation and alcohol is also recovered by the same process, the origin of the term alcohol becomes clear. The community which opposed the drinking of alcohol during the 19th Century viewed that real root of the word is in another Arabic word 'alghul', which means a Ghost or evil spirit that robs the graves and feeds on dead bodies. Similarly in German, wine spirit or ethanol was known as weingeist – meaning wine ghost . Nevertheless, "alcohol" definitely remains to be a mood altering and pleasure drug. It does reduce stress and anxieties but unfortunately reduces judgments and inhibitions as well.

Properties of Ethanol

Ethanol because of its relatively high affinity for both water and organic compounds has found considerable industrial applications. The composition of other alcohols limit their utility as compared to ethanol. For example, methanol with one carbon (CH_3OH) has reduced solubility in hydrocarbons. A further increase in chain length as in case of pentanol, which has 5 carbons, results in its reduced solubility in water . Thus, the chain length of alcohols determines its solubility in different compounds and in turn its usefulness to various applications. The important physical properties of ethanol are listed in Table 1.

Uses of Ethanol

The compound because of its characteristic properties has been put to a variety of uses. The oldest use had been for potable purposes and dates back to 10000 BC when wine-making was practiced in the households. Wine was a source of both pleasure and relative safety in the ancient world. Water was frequently impure, whereas fermented fruit juices protected from spoilage by their high alcohol content were generally safe to drink. Egyptians produced another alcohol-containing drink called beer, in 5000-6000 BC .Over the years, the art of wine and beer-making had evolved into a full-fledged technology. Today, the alcohol based beverage industry which includes potable spirit such as whiskies and rum also, contributes substantially to the Govt. exchequer.

The most important industrial use of alcohol is that it is a feedstock (starting material) for production of several organic chemicals popularly known as Alco-chemicals (Fig. 1). There are over 200 alcohol-based products manufactured in India. The present day alco-chemical industry is a well dispersed industry established in different states all over India and serves important segments of the national economy. The industry also caters to other important industrial sectors such as drugs and pharmaceuticals, dyestuff & pigments, pesticides & agrochemicals, perfumes and flavors, food processing & preservation, oil field chemicals, plastics & polymers, paints &

coatings, packaging, explosives, adhesives, synthetic fibers & yarns, electroplating, leather chemicals, textile processing, personal care products etc.

Table 1: Physical Properties of Ethanol

Boiling Point (oC, at 760mm Hg)	78.4
Density d20	0. 78510
Refractive index (n20)	1.3633
Viscosity at 20°C (p)	0.0122
Specific heat (cal/g oC)	0.581
Evaporation heat (cal/g)	204
Combustion heat (kcal/mol)	328
Ignition point (oC)	18.3

Ethanol is a known solvent for dyes, nitrocellulose, lacquers and enamels, drugs and chemicals, oils and waxes, purification and crystallization processes, cleaning purposes, preparation of tinctures, gums and resins, soap and essential oils etc. Besides being used as a multipurpose organic compound in the laboratories, it also finds applications in homes especially for cleaning, and in hospitals as an antiseptic.

Over the last few years, shortage of petroleum and the resulting increase in its prices leading to energy crisis has created another way of utilization of alcohol as an energy source. Since the combustion of ethyl alcohol is smokeless and odorless and the net calorific value is 7100 cal/g, it offers a possibility for its use in small stoves in certain situations. It can be used for the lighting purpose also. Bioethanol (fermentation alcohol) is already in use as a fuel in automobiles. It is mixed in different proportions with gasoline or diesel. Brazil has employed 22% ethanol blended petrol since the mid seventies. In the US, a blend of 10% ethanol with petrol called ‘gasohol’ is in routine use in cars.

Synthesis of Ethanol

The alcohol is manufactured from petroleum products or by fermentation. The synthesis of alcohol from the former utilizes either direct or indirect hydration of ethylene. Direct hydration is carried out in the presence of a catalyst like phosphoric acid or tungstic acid impregnated on an inert support such as silica aerogels or celite® diatomite. The reaction is carried out at high pressure and temperature, generally 1000 psig and 300^{o}C. On the other hand, the indirect hydration is a three step process. Initially monoethyl sulfate and diethyl sulfate are produced by absorption of ethylene in concentrated sulphuric acid. Hydrolysis of ethyl sulfates to ethanol then follows with the reconcentration of the dilute sulphuric acid. This method was in practice until

mid- eighties. Later, it was discarded as the direct hydration method produced better yields, less by-products and reduced quantity of pollutants. This synthetic alcohol accounts for only 5% of global production but is hardly produced in India, as the country has plenty of molasses to produce ethanol following the other method representing fermentation.

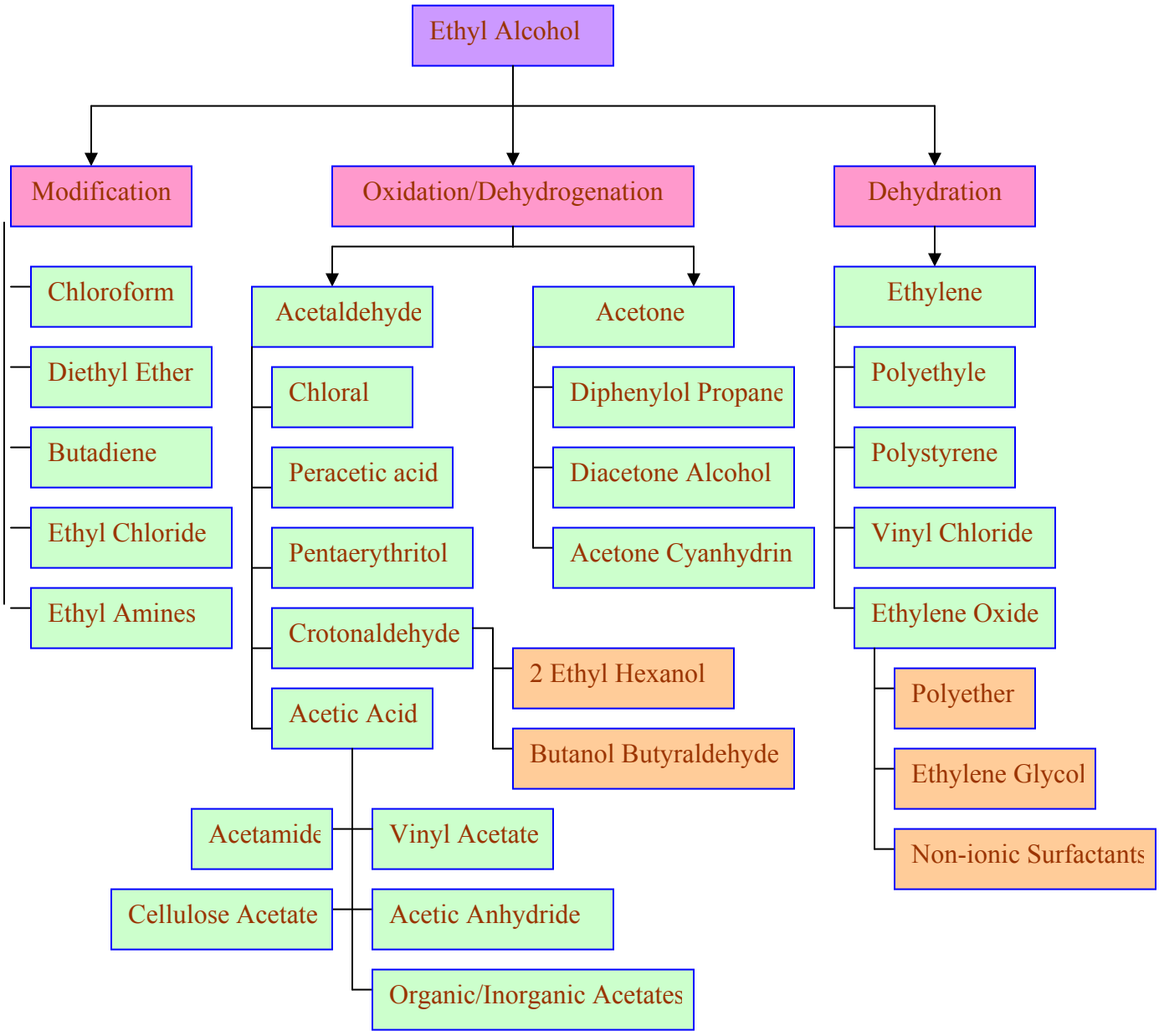

Fig. 1: Major alco-chemicals

Alcohol production by fermentation occurs due to enzyme-catalysed conversion of sugars or sugar-containing polymers, by micro-organisms. *Saccharomyces cerevisiae* is the most commonly used yeast but *Kluyveromyces* have also been employed. Among the bacteria, *Zymomonas mobilis* has been the preferred organism for ethanol production. Sugars in these

organisms are broken down to pyruvic acid by one of the three pathways—the Embden-Mayerhoff-Parnas (EMP) Pathway, the Hexose Mono Phosphate (HMP) pathway and the Entner-Doudoroff (ED) pathway. The pyruvic acid formed, under anaerobic conditions is split by pyruvate decarboxylase into acetaldehyde and CO_2. Ethanol is then produced from the acelaldehyde by reduction due to the enzyme alcohol dehydrogenase. (Fig 2). Yeasts follow the EMP pathway and theoretically from 1g of glucose, 0.511g of ethanol can be obtained. When pure substrates are fermented, the yield is 95% and usually drops down to 91% when industrial raw materials are used. Thus 100g of pure glucose will in practice yield 48.4g of ethanol, 46.6g of CO_2, 3.3g of glycerol, and 1.2g of yeast biomass.

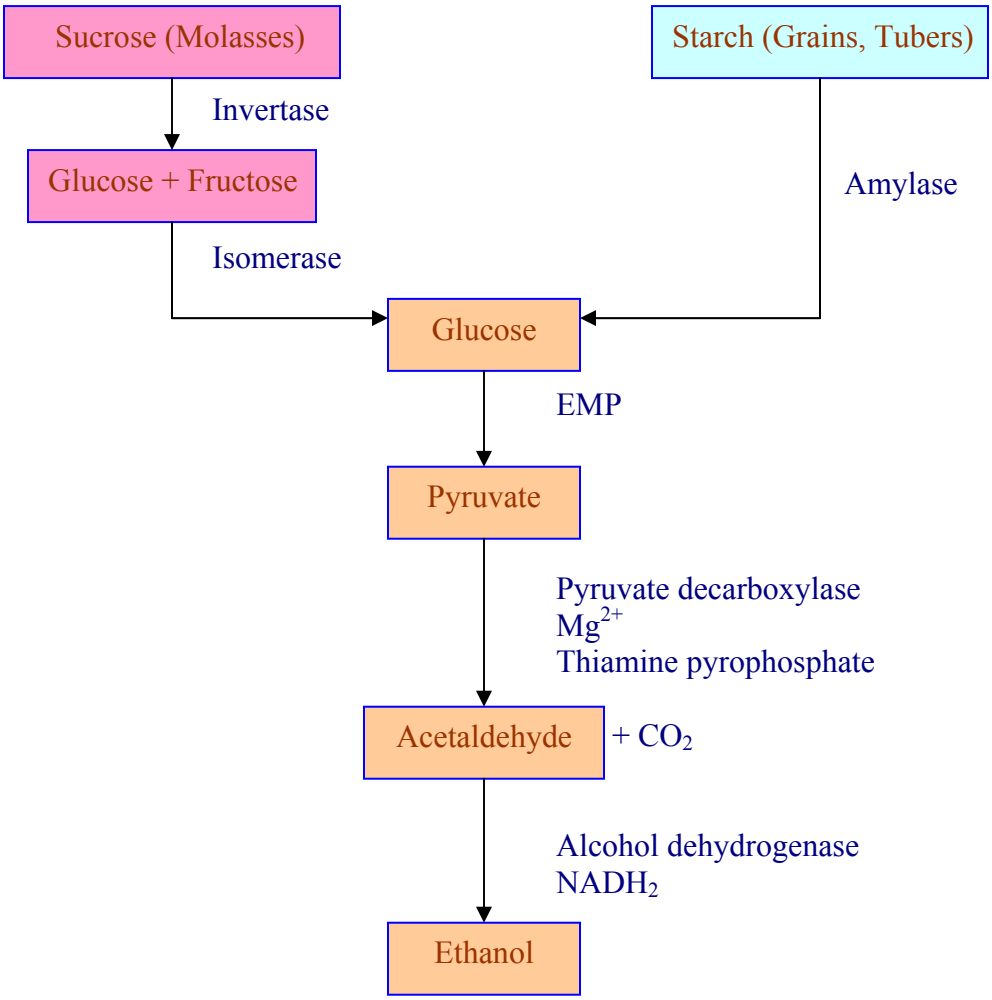

Fig. 2: Biosynthesis of ethanol

2. Industrial Production in India

The bulk of alcohol in our country is produced by fermentation of cane-molasses, which is a byproduct of sugar industry. This molasses-alcohol is generally marketed in hydrated form (95 to 96 % v/v) known as rectified spirit or in anhydrous form (over 99% v/v). It is mainly used for two purposes – a feedstock for chemical industry and for potable use. Out of the total alcohol

available for various end uses, the proportions of industrial and potable use have, over the years, been undergoing significant changes depending upon the Govt. policies and directives. At present about 52% of the total production is used for potable purposes. The fraction used for industrial purposes such as feedstock for chemicals, fuel, solvents etc. is normally denatured to prevent its diversion to potable purposes. Denaturants such as pyridine, methanol and croton aldehyde, depending upon the end use of ethanol, are used. Alcohol may also be coloured for easy recognition, with methyl violet.

History

Production of molasses-alcohol in India may be tracked down with the establishment and growth of sugar industry, which today is the second largest of the processing industries in the country, being next only to textiles. It had been largely because of the policy of Govt. of India in 1932, which provided protection to this industry against discrimination. Consequently the same year, rapid growth of sugar mills had begun and their number grew from meagre 32 to 130 by 1934-35. The sugar output which stood at 0.162 million tonnes rose to 0.947 million tonnes by 1935-36. Simultaneously, the byproduct molasses was generated in quantities which led to the beginning of alcohol production industry. The alcohol-producing distillery units were either established in isolation or were annexed to sugar mills. In 1931 about 19 distilleries were set up which produced 3.7 million litres of potable alcohol. A distillery at Mandya (Karnataka) established by Mysore Sugar Company was probably the fist distillery to go into production. The production of alcohol increased to 60 million litres between 1931 and 1947 . By 1960, the number of distilleries increased to 51 and the production reached 81 million litres. The production rose steeply due to the growing demand of alcohol and increased availability of molasses and reached a level of 195 million litres in 1966. In August 1998, with the delicensing of the sugar sector by the Govt.; the number of sugar mills started growing at a faster pace to reach today's 566 with a production capacity of 180-lakh tonnes of sugar . Of these, only 453 are in operation, producing 135.46 lakh tonnes (2004-05) of sugar. Correspondingly, the number of distilleries have also grown to over 300 with an installed capacity of 3198 million litres. The course of production of alcohol over the years is shown in Table 2.

Components of the production process

Raw material

In India, cane molasses is essentially the raw material for ethanol production. The blackstrap molasses, is the final effluent obtained during the preparation of sugar. During sugar manufacture the sugarcane juice is repeatedly evaporated and crystallized to recover the maximum of sugar. The residual syrup thus obtained represents molasses, from which no crystalline sucrose can further be recovered by conventional methods. The yield of molasses per ton of sugarcane is approximately 2.7% but is influenced by a number of factors and may vary within a wide range (2.2 – 3.7%).

The molasses is thick, viscous syrup with a specific gravity varying between 1.39 and 1.49 with 1.43 as an average. It is dark brown or brownish black in colour, acidic in nature, rich in salts and contains sugars which could not be recovered. The sugar content varies from 45 to 55% (w/v) and is largely sucrose followed by invert sugars (glucose and fructose), which are readily fermentable by yeast. Besides, a non-fermentable raffinose is also present. Yet another sugary

compound hydroxymethyl furfural, a reaction product of the sugars and nitrogenous compounds formed due to high temperature during sugar manufacture, is also present. This compound is known to be inhibitory to yeast during fermentation. The presence of yeast nutrients such as biotin, inositol, pyridoxine phosphate and several free amino acids makes the molasses a good substrate for alcohol production.

Table 2: Alcohol Production in India

Alcohol years	Production (Million litres)
1985-86	581
1987-88	637
1989-90	926
1991-92	1016
1993-94	875
1995-96	1221
1996-97	1148
1998-99	1412
1999-00	1654
2000-01	1686
2001-02	1775
2002-03	1870*
2003-04	1969*
2004-05	2074*
2005-06	2187*
2006-07	2300*

*Projected Values (Das, 1999 and www.ethanolindia.net)

Two types of molasses are known: (1) The cane molasses- as the name suggests, is derived from sugarcane and is quite common to countries like India and Brazil , with the common name 'blackstrap molasses' and (2) the beet molasses- is derived from sugar beet, which is rather uncommon in this country. Yet another kind of molasses called high test molasses, which is available normally at the time of surplus crop, is common to USA, Japan and U.K. This

molasses which is manufactured by evaporation of the partly inverted juice, thus, strictly speaking, high test molasses is not a molasses but an inverted sugar syrup and is sometimes also called invert molasses. This, with a total sugar content of 70-75%, is rather rich in invert sugars (40-60%). In contrast, the black strap molasses contains 45-55% total sugars including approximately 30% sucrose and 12-18% invert sugars. It is relatively rich in yeast nutrients. Besides, it contains nitrogenous compounds (2%), ash content (7-18%) and 20-25% of organic non-sugar compounds.

The quality and price of molasses is largely determined by the content of sugars it contains. Decontrol of this commodity by Govt. of India in 1993 coupled with advancement of technology in sugar recovery, not only led to its deterioration in quality in terms of sugar content and yeast nutrients but also shot up its price from Rs. 140 to 4000-5500, a ton. Its price, however, keeps fluctuating from time to time, depending upon the magnitude of sugarcane crop in a particular year and the policies of the state Govts pertaining to movement of molasses from one state to another within the country.

Fermentation

The term 'fermentation' has been derived from a Latin verb 'fervere', - that means, 'to boil' describing the appearance of action of yeast on extracts of fruits or malted grains. The boiling appearance is due to the production of carbon dioxide bubbles caused by the anaerobic catabolism (breakdown) of sugars present in the extract. Basically, it is a process in which microorganisms such as yeasts convert simple sugars to ethanol and carbon dioxide. Technically, the fermentation is a metabolic process which leads to generation of ATP and in which degradation products of an organic compound serve both as hydrogen (electron) donors as well hydrogen acceptors . Oxygen is not a reactant in the fermentation process.

In the context of molasses as raw material for ethanol production, the disaccharide sucrose in molasses is converted to the monosaccharides glucose and fructose by the enzyme invertase, which is secreted by the yeast. The glucose and fructose being fermentable enter the EMP pathway in yeast and are fermented mainly to alcohol and carbon dioxide, with the release of heat energy. Thus, fermentation of sugars to alcohol requires essentially three ingredients: the fermentation medium, an organism generally yeast and the actual fermentation.

Fermentation medium

Fermentation medium generally called production medium is prepared by diluting molasses with water so as to produce a solution (wort) of 12-15% sugars. Most molasses being poor in some of the yeast nutrients, the wort is supplemented with N and P in the form of urea or Ammonium sulfate (0.01 – 0.1 %) and phosphoric acid or disodiumhydrogen phosphate (25-50 ppm) The rate of supplementation is, however, subject to variation depending upon the nutrient status of molasses being used. pH of the wort is adjusted to 4.5 to 5.5 by adding sulfuric acid or sometimes stillage (slope) from the previous round of fermentation, which has an acidic pH. This process is called 'backslopping' and is done at the rate of 20-25% (v/v).

Yeast strain and inoculum build-up

Usually distiller's strains of yeast *Saccharomyces cerevisiae* are used in industry for alcohol production. Besides, other yeasts belonging to *Schizosaccharomyces pombe* and *Kluyveromyces*

have also been used. The strain to be able to produce high yields of alcohol with in the minimum possible time, ought to have high growth rate & productivity, and tolerance to sugar, temperature & low pH. Besides, the strain should be genetically stable and antagonistic to contaminating wild yeasts.

The yeast is generally grown and maintained on yeast extract peptone dextrose agar (YEPDA) slopes. Alternatively, YEPMA in which dextrose has been replaced by Molasses, has also been used. The culture is grown at 25-30 ^{o}C and maintained at 4^{o}C in a refrigerator.

To initiate the fermentation, a substantial number of yeast cells called inoculum is built up by transferring the fresh biomass from a slant culture into the liquid inoculum medium, which is more like the production medium except that the sugar content has been reduced to 4-6 % . The culture is grown at 25-30^{o}C with aeration, to allow a faster multiplication of cells. After 12 h, the contents are transferred to another flask containing the same inoculum medium which is larger in size by 4-5 volumes. After a 12 h growth further transfers are made into the inoculum medium with increasing size in the same fashion. The culture thus obtained is called the inoculum.

Fermentation

In India for ethanol production mainly batch fermentation system is followed. Fermentation vessels (bioreactors) are charged with the production medium (Wort) at appropriate temperature and pH. The nutrients such as N and P are added in the very beginning of the process. Fermentation is initiated by transferring inoculum into production medium in a ratio of 1: 5 to 1:8 so as to a give a final cell concentration of 2X10^{7}/ml of wort. As the fermentation progresses ethanol is formed and also a lot of heat is generated, which raises the temperature of the fermenting wort beyond 35^{o}C . The rising temperature in the presence of ethanol inactivates the yeast cells and in turn reduces ethanol productivities, leaving behind considerable quantities of unfermented sugars. Thus, to maintain the temperature within the ideal range, fermentors' walls are cooled externally by sprinkling water. This besides involving an investment does shorten the life span of the fermentors made of iron (mild steel). To overcome the problem, thermotolerant yeast strains which would be able to ferment equally well at 25^{o}C and over 40^{o}C would be of immense use. A few reports on the development of such strains of yeast have appeared but their industrial use has not been so common, as yet.

The fermentation, depending upon the yeast strain used and temperature of the wort, lasts for 24-48 h . Eventually, the termination of the process is marked by disappearance of bubbling of wort, which at this point of time has produced in it 6-8 % (v/v) ethanol. The fermented wort which is called beer or wash, besides ethanol also contains traces of byproducts such as fusel oils (higher alcohols), aldehydes, organic acids, esters etc. The ethanol is subsequently concentrated and the accompanying products are fractionated through distillation involving a series of distillation columns.

3. Processing of starch/lignocellulosic substrates to fermentable forms

Besides molasses, raw materials containing starch or cellulose can also be used for ethanol production. The two polysaccharides, however, cannot be directly utilized by distiller's yeast, as

it lacks the enzymatic machinery for the purpose. A few yeasts belonging to *Saccharomyces diastaticus, S. fibuligera* and *Schwanniomyces alluvius* are known to grow on starch but are inefficient ethanol producers, as they are sensitive to high concentrations of ethanol. Among these yeasts, the former is the best known and has higher tolerance to ethanol. It, however, has only partial amylolytic activity, as it secretes the saccharifying glucoamylase but not the liquefying enzyme α-amylase. The two polysaccharides, therefore, before fermentation need to be hydrolyzed to their constituent monomer fermentable sugars, by the externally added enzymes or acid/alkali treatments or a combination of the two.

Processing of starchy substrates

The cereal grains such as wheat, rice, corn, barley, sorghum, oats and tubers as potato, cassava and sweat potato are a rich source of starch. These grains on a dry basis, contain around 60-75% of starch, hydrolysable to hexose with a significant weight increase (stoichiometrically the starch to hexose ratio is 9:10) and offers a good resource in many fermentations. Some of these raw materials have been extensively used for many years for production of bio-ethanol for the drinks industry. Corn, wheat and barley have been exploited in USA, Finland, Austria and Sweden for production of bio-ethanol for industrial purposes and fuel use also. Recently, in our country also, an increasing number of distilleries are taking to these raw materials for producing fine-grade ethanol for potable purpose.

The starch is heterogeneous polysaccharide composed of two high-molecular weight components, amylose and amylopectin. The ratio of these two components varies in various raw materials. Generally, starches are a mixture of 10-20% amylose and 80-90% amylopectin. The former is a linear polymer containing 70-2100 glucose units linked to each other via α, 1-4 glucosidic linkage. The latter is more complex, as it is a branched polymer forming branches via α, 1-6 glucosidic linkages to glucose units in the straight chain, which are linked to each other via α, 1-4 bondings. On an average, in a starch molecule, 4-6% of glucose units are branched with a chain length of 20-25 glucose units. Amylose is somewhat soluble in water forming a colloidal solution and the starch molecule due to this fraction, stains blue with iodine. On the other hand, amylopectin is completely insoluble in water and is rather difficult to be hydrolysed due to the presence of α, 1-6 glucosidic linkages. The complete hydrolysis of starch to the constituent glucose units, therefore, requires at least two activities, the one which acts at α, 1-4 glucosidic bonds of amylose and amylopectin and the other, a disbranching activity, working at α, 1-6 linkages of amylopectin.

Until 1960s, starch hydrolysis was mainly achieved by dilute acid treatment at high temperatures. But due to the limitations associated with it, it was replaced by enzymatic hydrolysis, which is more specific and rapid in action. Although malt as a source of enzymes (α and β–amylases) has been and is still being employed for the production of bio-ethanol for finer-brand whiskeys, now, production of industrial, fuel and potable ethanol, invariably utilizes microbial amylases. Commercial formulations of these microbial enzymes (amylases), derived from bacteria and fungi are in common use in distilleries and are active in a specific range of temperature and pH. Novo Nordisk Industries, Denmark, Biocon Pvt. Ltd., Bangalore etc. are a few of the known producers of these enzymes for industrial use. The commonly used α–amylase, an endo-acting liquefying enzyme which acts randomly on α, 1-4 glucosidic linkages but cannot break α, 1-6 bonds, is of bacterial origin from *Bacillus licheniformis* or *Bacillus*

amyloliquefaciens. Similar enzymes from *Aspergilli*, with a temperature optima for activity at 59-60^{0}C are also available. But they are not as useful in starch hydrolysis as the thermostable α–amylase from Bacilli. Alpha amylase from *Bacillus licheniformis* requires a jet cooking at 105-107^{0}C in the presence of Ca^{++} at pH 6.0-6.5 followed by a reduction in temperature to 95^{0}C for a few hours to complete liquefaction. These alpha amylases yield α–dextrins, predominantly G_2, G_3, G_4, and G_6 oligosaccharides. The saccharifying enzyme amyloglucosidase (glucoamylase), on the other hand, is mostly of fungal origin. This is an exo-amylase, catalyzing hydrolysis of α, 1-4 linkages sequentially from non-reducing end and also cleaves α, 1-6 and α, 1-3 linkages, as well. The optimum temperature for this enzyme is 40-60^{0}C and pH 4.5-5.0. This enzyme when acting in succession to α–amylases, yields mostly glucose, fermentable by yeast to ethanol.

Manufacturing of ethanol from starchy raw materials thus requires besides fermentation and distillation: (1) milling or grinding the material to expose the starch, to a coarse flour, (2) making a slurry of flour (25-30%) in water to dissolve water soluble starches and cooking to gelatinize the starches which become more susceptible to subsequent enzymatic hydrolysis, (3) conversion of starch to fermentable sugars by α–amylase (liquefaction) and amyloglucosidase (saccharification).

Gelatinization

Gelatinization of the slurry by cooking, results in a colloidal suspension or gel formation in aqueous phase, due to breakage of surface layer of swollen starch granules. It leads to increase in viscosity. Temperature range within which gelatinization occurs varies with the type of starch. While in corn, gelatinization starts at 62^{o}C and is completed at 72^{o}C, in wheat it is initiated at 52^{o}C and completes at 64^{o}C. At higher temperatures the cooking periods are drastically reduced. At 15 pound pressure, for example, grain starches can be cooked in less than 6 minutes. In commercial operations, cooking (gelatinization) is, therefore, done at over 100^{o}C steam pressure. To minimize the problem of handling due to increased viscosity of the cooked slurry, a small fraction of the thermostable liquefying enzyme (α–amylase) is also incorporated. A method that combines milling and cooking into one operation without the use of water, could also be used. This process uses heat generated by friction in the milling process to simultaneously cook the grains. Milling of mash in hot water (wet milling) at 110^{o}C for twenty minutes can be used as an alternative method of gelatinization.

Hydrolysis of starch

The gelatinized slurry is subsequently hydrolyzed (liquefied and saccharified) to sugars by addition of amylases. Prior to liquefaction by α-amylase, calcium ions are added to the mash (pH 5.5-6.5) and the temperature is reduced to 80-90^{o}C. The mash is held at this temperature for 1-2 h and the liquefaction is monitored by starch–iodine reaction. The mash is cooled down to 58-60^{o}C and pH is adjusted to 4.8-5.0. The enzyme amyloglucosidase (glucoamylase) is added and saccharification is allowed to proceed until about 6-8% sugars are produced in the mash. The mash is cooled down to 20^{o}C (low temperature) and inoculated with commercially available wet or active dry yeast to complete fermentation within 48-72 h. During this period, simultaneous saccharification and fermentation (SSF) occurs, as additional sugars are produced and simultaneously fermented to about 8-10% ethanol by volume. The ethanol thus obtained after distillation, is used for potable purpose, as it contains the minimum of by-products such as

fusels etc. due to the lower temperature used for fermentation. Alternatively, to produce ethanol for fuel use, gelatinization and liquefaction could be combined to occur at a temperature of 85-90^{o}C for 1-2 h, followed by saccharification at 58-60^{o}C for a few hours to produce around 14-18% sugars. Subsequent fermentation is carried out within 20-24 h at a higher temperature (35^{o}C) to produce about 8-11% of ethanol by volume. The recovery of ethanol from the fermented wash is done as described before in molasses fermentation.

Processing of lignocellulosic substrates

Lignocelluloses are the renewable and most abundant natural material on the earth. It contains cellulose, hemicellulose and lignin as the major components, where cellulose (β-1,4-linked glucose polymer) is shielded by hemicellulose (complex polymer of pentoses and hexoses) and Lignin (polymer of phenyl-propanoid units). In addition to this small amount of extractives, including fats, waxes, tannins, resins, essential oils, alkaloids, starches, gums etc are also present. All the three components cause hindrance during cellulosic hydrolysis. Although lignin is inert in hydrolysis but it can adsorb a part of active cellulase enzyme. Hemicellulose does not let the cellulose accessible for enzyme by shielding it and extractives cause interference because of their hydrophobic nature. Therefore, to overcome this recalcitrance, two broad approaches can be utilized: one is acid hydrolysis and another is pretreatment followed by enzymatic hydrolysis.

Acid hydrolysis

For acid hydrolysis different acids in concentrated or dilute forms can be used. Sulfuric acid, hydrochloric acid, perchloric acid, dicarboxylic acid, nitric acid etc are the major choice for this purpose. Although concentrated sulfuric acid treatment followed by dilution with water yield significantly higher conversion of substrate to glucose, the mild sulfuric acid, dicarboxylic acid and maleic acid treatments can also be used for hydrolysis of cellulose and cellobiose to glucose.

Pretreatment and enzymatic hydrolysis

Pretreatment

Efficient pretreatment to open up the structure and to make it accessible to enzymatic attack is a pre-requisite for effective utilization of lignocellulosic material. It can be achieved by increasing the surface area for enzymatic activity, solubilisation of hemicelluloses and delignification. The commonly used approaches for pretreatment are:

Physical treatment

It includes either mechanical methods like grinding, milling and extrusion that leads to decreased particle size and increased surface area. Decreased crystallinity of cellulose that can be achieved by the techniques like compression milling, also leads to increased effectiveness of enzymatic saccharification. Another physical treatment is irradiation of lignocellulosic material with gamma radiation, electron beam etc. which leads to depolymerisation of cellulose and thus increasing specific area for enzymatic activity. Radiation causes oxidative degradation and chain cleavage of the cellulose molecule and thus decreases crystalinity and increases digestibility of cellulose molecule.

Thermal treatment

Thermal treatment could be of three types: auto-hydrolysis, steam explosion and hydro-thermolysis. Auto-hydrolysis includes extensive disintegration of lignocellulosic material under high pressure steam for a specific duration followed by sudden release of pressure. It is generally carried out at a temperature range of 170-200^{O}C. Steam explosion is carried out at even higher temperature range around 250^{o}C to extract the highly depolymerised lignin and hemicellulose content, keeping the resident time of high pressure steam further lower than autohydrolysis, to minimize the formation of inhibitory substances. On the contrary, hydrothermolysis make use of water at high temperature and pressure for the pretreatment of lignocellulose. This method is thought to fractionate the lignocellulosic material more effectively leading to the maximum yield and purity of each of the polymeric component.

Chemical treatment

It can be carried out by making use of alkali, acids, oxidizing agents, gases and various solvents individually or in a suitable combination to remove lignin and depolymerise the other components. Alkalies like NaOH, NH_4OH and ammonia cause saponification of intermolecular ester bond leading to swelling of lignocellulosic material. It also decreases the degree of polymerization and crystallinity of the complex lignocellulose substrate enabling effective enzyme accessibility. Acids such as hydrochloric acid, nitric acid, sulphuric acid, perchloric acid, phosphoric acid etc. in dilute forms, at high temperature, could be used as effective pretreatment agents leading to removal of hemicellulose component and decreased degree of polymerization of cellulose. Oxidising agents like hydrogen chloride, peracetic acid, sodium chlorite, sodium hypochlorite etc. carry out chemical oxidation of lignin and produce water soluble compounds librating cellulose for further utilization. Gases can also be used as pretreatment agent. chlorine, ozone, sulphur dioxide, nitrous oxide etc. cause solubilization of lignin. Besides, ozone can attack carbohydrates as well. Some solvents like ethylene glycol, dimethyl sulphoxide, phenol, ethanol, butanol etc. also have delignification abilities and thus could acts as a potent pretreatment agent. Various organic and inorganic compounds like ferric chloride, ferrous sulphate, aluminium chloride, aluminium sulphate, mineral acids etc. can cause hydrolysis of hemicellulose, thereby increasing delignification. Solvents like cadoxen and concentrated sulphuric acid have the ability to disintegrate cellulose also.

Although the chemical methods of pretreatment are quite effective but most of the chemicals are difficult to recover and thus add to the operational cost. Besides, most of the chemicals produce harmful byproducts that can significantly interfere in further utilization of the processed material. Neutralization costs of acid or alkali treatments again decrease cost effectiveness of the process. Moreover specific vessels are required for these processes, as such chemical treatment can corrode or completely damage the vessels. Most importantly, both, the chemical pretreatment as well as the acidic hydrolysis, lead to production of toxic components that will inhibit significantly the successive enzymatic hydrolysis and ethanol production. Weak acids, furan derivatives and phenolic compounds like 4-hydroxybenzoic acid, vanillin and catechol are the common examples. However, this toxicity can be reduced by the precipitation of toxic components with $Ca(OH)_2$, CaO, NaOH, KOH, activated carbon etc. Besides ether extraction and ethyl acetate extraction can remove furfurals and lignin degrading products. Physical treatments like ion exchange chromatography, vacuum evaporation and steam stripping, and enzymatic degradation using laccase and peroxidases can also be employed for detoxification.

Biological treatments

Some saprophytic microorganisms including bacteria as well as fungi, by their collaborative activities, can slowly degrade lignocellulosic biomass. Most of the fungi that are able to produce specific enzymes for degradation of lignocellulosic material belong to the Ascomycetes, Deuteromycetes and Basidiomycetes. Depending on the type of decay these fungi can be classified into soft rot, brown rot and white rot. Soft rot fungi include *Chaetomium cellulolyticum, Aspergillus niger, Trichoderma viride* and *Fusarium oxysporum.* They can efficiently attack wood carbohydrates causing cavities in the secondary wall but modify lignin to a limited extent. Although soft rot fungi can also attack aromatic rings and side chains but mainly they cause demethylation of lignin. Common brown rot fungi are *Poria placenta, Tyromyces balsemeus, Gloeophyllum trabeum* and *Lentinus lepidius*. These fungi can cause rapid and extensive degradation of cellulose and hemicellulose and degrade lignin to a lesser extent but these are unable to degrade pure cellulose. It seems that these fungi get activated in the presence of lignin or lignin like compounds. White rot fungi are wood rotting fungi and can simultaneously attack all the components of lignocellulosic material. The most studied examples of this group of fungi are *Trametes versicolor, Phanerochaete chrysosporium, Dichomitus squalens* etc.

Bacteria generally degrade wood slowly. They generally degrade in moist environment in collaboration with fungi. Rumen bacteria such as *Fibrobacter succinogenes*, *Ruminococcus albus* and *R. flavifaciens,* are the major degraders of lignocellulosic material. Many other bacterial species like *Pseudomonas, Acinetobacter*, *Bacillus* and *Clostridium* also contributes to lignin degradation. Some actinomycetes like *Streptomyces flavovirens* can also actively degrade lignocellulosic material leading to its partial solubilization. These can degrade and remove lignin while *Streptomyces viridosporus* oxidatively depolymerise lignin as it degrades cellulose and hemicellulose.

Enzymatic hydrolysis

The process of conversion of polysaccharides into fermentable sugars is called saccharification. It is a biochemical process in which hemicellulosic and cellulosic portions are converted into monomeric sugars and sugar acids. Cellulose is a structural polysaccharide with a crystalline structure, so it is resistant to saccharification as compared to hemicellulose. Cellulose molecules are made up of a linear chain of β-1,4-linked glucose units. Number of glucose units may reach up to 10,000, which make cellulose a giant molecule that, as such, can not enter in to the microbial cell. Various extracellular enzymes are required for its hydrolysis and the hydrolysed products are transported inside the cell for further catabolism. Cellulose decomposition is initiated by a diverse group of enzymes called cellulases namely β-1,4-endoglucanases, β-1,4-exoglucanases and β-1,4-glucosidases. Endoglucanases carry out cellulose mineralization which involves the loss of crystalinity of the structure, followed by the depolymerization activity by exoglucanases leading to the production of linear chains of 2-3 glucose units called cellobiose and cellotriose. The cellobiose and cellotriose can enter in to the cell where β-1,4-glucosidases can hydrolyse these to monomeric glucose units.

A large number of micro-organisms are known to produce different cellulases but only a few are capable of produing all the necessary enzymes. In case of bacteria, like *Cellulomonas fimi, Pseudomonas fluorescens, Acetovibrio cellulolyticus, Clostridium cellulovorans, Clostridium*

thermocellum and *Ruminococcui albus* etc. the cellulase complex is cell wall bound, while in fungi, such as *Trichoderma viride (reesei), Phanerochaete chrysosporium, Aspergillus niger, Chaetomium cellulolyticum, Fusarium oxysporum* etc. the cellulases are secreted into the growth medium, which act synergistically to hydrolyse the cellulose. Some actinomycetes *Streptomyces lividans, Thermoactinomyces curvata* and *Thermomonospora fusca are potential cellulose producers.* Commercial preparations are available in the market with different trade names. Cellulast of Nova Laboratories are prepared using *Trichoderma viride* and can efficiently hydrolyse cellulose to produce cellobiose and glucose. Cellobiase250L from the same laboratory is prepared to hydrolyse cellobiose to produce fermentable glucose with almost 100% efficiency.

The lignicellulosic substrates despite their abundance in nature, so far, have not been used at industrial scale for ethanol production. Clearly, the major reason remains to be the process difficulties and economical non-viability of the process of hydrolysis. Besides, the low ethanol levels produced also make the production process cost-ineffective.

4. Recovery of alcohol –- the Distillation

Principle

The fermented wash besides some by-products such as fusels and aldehydes contains only 6-8% ethanol by volume. The ethanol at this concentration can not be put to use for any useful purpose. It, therefore, must be concentrated for various end uses. This is usually achieved by the process of distillation. Further, alcohol distillation separates the alcohol, (BP. 78.3^{o}C) from water (BP. 100^{o}C), from a water-alcohol solution, by heating the solution and collecting the alcohol rich vapours. The process basically is based on the fact that the vapour of a boiling mixture will be richer in the component that has a lower boiling point or in other words is relatively more volatile. When this vapour is cooled and condensed the condensate will contain more concentration of alcohol. Repeated batch distillations of the condensate called refluxing, will thus produce a condensate which will every time be enriched more and more with ethanol until a concentration of 96% (v/v) ethanol is achieved. The condensate of this ethanol concentration makes a constant boiling mixture called an 'azeotrope' and can not be enriched further by the process of conventional distillation. The ethanol as such boils at 78.3^{o}C while water boils at 100^{o}C. A mixture of the two liquids, however, will boil at all temperatures between 78.3^{o}C and 100^{o}C but enrichment of the vapour phase with alcohol will depend upon the ratio of alcohol to water in the mixture. The 'azeotrope' which is a mixture of 96% ethanol and 4% water because of its alcohol to water ratio would thus produces a vapour- phase having equal concentration of ethanol and water, thereby denying further concentration of ethanol.

While distilling large volumes of fermented wash at industrial scale, long vertical distillation columns in series are used. Usually, two columns, a 'Stripper' and a 'Rectifying column' to concentrate the ethanol and exclude the fusel oils impurities etc., respectively, are common. The inner architecture of these columns is designed in such a way that maximum separation of the component fractions, based on differential volatilities, occurs when heat input is provided in the form of steam. The distillation is, therefore, commonly termed as fractional distillation and the alcohol obtained from the rectifying column is named as rectified spirit. The rectified spirit which in strength is 96% (v/v) and is also used for potable purposes. This, however, cannot be used as a fuel extender or fuel supplement.

Production of anhydrous alcohol

Absolute alcohol is nearly 100% pure is an important product required for industrial and fuel use. The rectified spirit being 96% ethanol, which makes an azeotrope, it has to be further dehydrated. This ethanol – water azeotrope can be broken by addition of benzene. The benzene, ethanol and water form a ternary azeotrope with a boiling point of 64.85^{o}C. Since this azeotrope is more volatile than the ethanol-water azeotrope, in the distillation column it will distil first. When all the water is exhausted, a binary system of alcohol-benzene is formed, which will distil at 68.25^{o}C until all the benzene is exhausted. Finally only absolute alcohol will remain, which can be recovered as bottoms from the distillation column. Since such an absolute alcohol does have some traces of benzene, which is a known carcinogen, cannot be used for potable purposes, at all. The benzene, is therefore, being increasingly replaced by cyclohexane. Although most of the ethanol dehydration plants for production of absolute alcohol are based on Azeotropic distillation, its high capital cost, energy consumption and reliance on toxic chemicals like benzene has been eliminated use of this process from modern plants. A few other methods are given below:

Drying

The rectified spirit can be purified by 'drying' it using generally unslacked lime. Industrial alcohol is taken in a reactor and quick lime is added to that and the mixture is left over night for complete reaction. Lime (Calcium Oxide) when mixed with water in ethanol will form calcium hydroxide, which then can be distilled in a fractionating column to get absolute alcohol. The process is used for small scale production of absolute alcohol by batch process.

Distillation at reduced pressure

At pressures less than atmospheric pressure, the composition of the ethanol-water azeotrope shifts to more ethanol-rich mixtures. It is, therefore, possible to distil absolute alcohol directly, as 'azeotrope' does not exist at all, at pressures less than 70 torr (9.333 kPa). This distillation under vacuum, however, is a costly affair.

Molecular sieving

A molecular sieve can be used to selectively absorb the water from the 96% ethanol solution. Besides synthetic zeotile, plant-derived absorbents such as cornmeal, straw and sawdust can be used for the purpose. The zeolite bed can be regenerated an unlimited number of times by drying it with a blast of hot carbon dioxide. Cornmeal and other plant-derived materials may be a cost effective choice only where the ethanol is made from grains.

5. Overview of an Indian distillery

In India, a typical alcohol-producing unit called a distillery utilizes molasses as the raw material. It houses mainly a fermentation house and a section for producing potable liquors or spirits. Production of liqours utilizes essentially the ethanol produced by the fermentation house, as a base. It is more of an art and requires the services of an expert blender. The blends thus produced are usually a closely guarded secret and, therefore, will not be discussed here. The

types of various liquors will, however, are covered in the following sections on distilled beverages. The fermentation house (Fig. 3a, 3b) in a distillery mainly consists of the following:

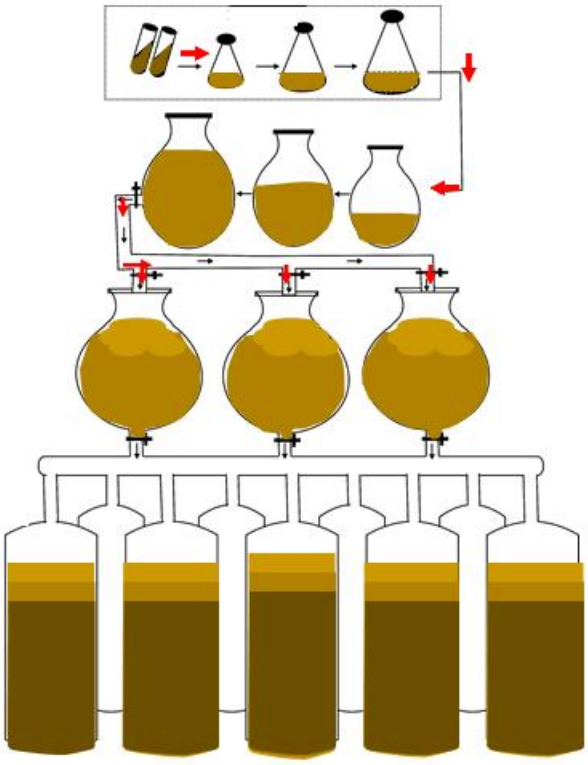

Fig. 3a: Fermentation section

Molasses dilution tanks

These tanks are normally made of iron (MS) having capacities which vary from one distillery to another. They have inlets for molasses, water for dilution and air for thorough mixing of the molasses and water. The sugar content of the diluted molasses is adjusted to 15-16%, using glass hydrometers called Brix or specific gravity hydrometers (Fig. 4). The hydrometer measures the concentration of total soluble solids which in turn can be calibrated with sugar content. Depending upon the batch of molasses, hydrometer when immersed in a molasses solution containing 15-16% sugars, would read somewhere between 22-26^0 B (^{0}Brix).

Yeast vessels

They are used to propagate and produce large volumes of yeast cells. The inoculum build-up, in practice, starts in the laboratory from a fresh slant culture transferred into a flask containing 250 ml sterilized medium (4-6% sugars or 12^{0}B). Every 8-12 h, the growing culture at 30^{0}C is transferred in a sequential manner to another flasks containing increasing volumes of the same medium, in a ratio of 1:5. These forward transfers continue to the yeast vessels interconnected through pipes, in the same fashion. These vessels are made of stainless steel (SS) and have a

built-in system for sterilization and subsequent cooling of the medium. The number of such vessels could always vary. But each of these vessels has invariably increased capacity in an ascending order in almost the same ratio. A 12^0B molasses medium (100 l, 500 l, 2500 l) is charged into the vessels, steam-sterilized in place, inoculated and aerated with microfiltered air to achieve high rate of cell multiplication.

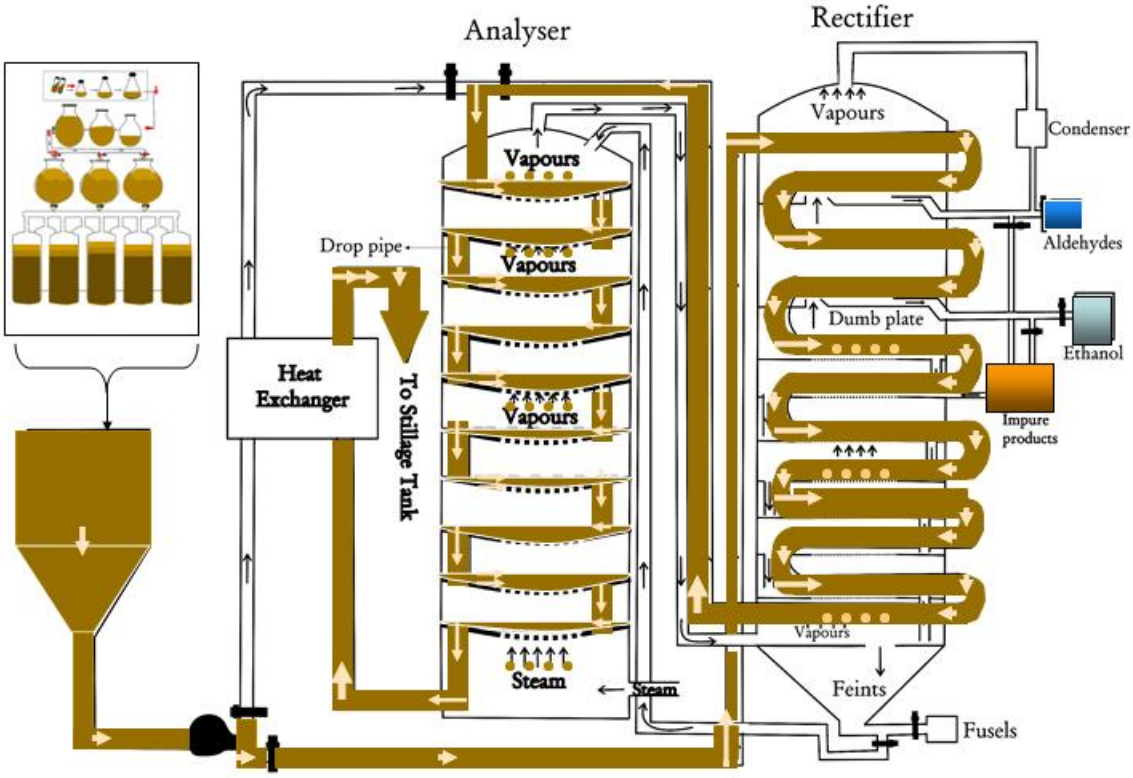

Fig. 3b: Continuous still

Prefermentors

The containers with a capacity of 20000-30000 litres each, that follow the yeast vessels, are placed in the process, just prior to the main fermentors. They are popularly known as 'bubs'and are seeded with inoculum from yeast vessels. The multiplication of yeast cells takes place in an unsterilized but aerated medium (12^0B). The final cell number rises to $250x10^6$/ml and serves as a source of inoculum to the fermentors.

Fermentor

The vat or the bioreactor made of generally iron (MS) or one of its alloys, where the sugars in molasses are fermented by the yeast to and hence it is called a fermentor. Every distillery has a battery of fermentors placed side-by-side under a shed, at ambient temperatures. Each of the fermentors has a capacity of 1.5 to 2.0 lakhs litres and filled with the molasses medium (22-26^0B, pH 5.0) supplemented with nutrients, to 70-80% of the capacity. The fermentation is initiated by transferring inoculum from bub, in a way so as to give a final cell number of $50x10^6$/ml. The fermentation occurs at the ambient temperatures. As the fermentation proceeds, due to the exothermic reactions taking place in the fermentor, the temperature of the wort rises

beyond the ideal temperature range of 25-30^{0}C. This rise in temperature if allowed to go uncontrolled, kills the yeast cells due to the increased inhibitory effect, the ethanol. As a result, fermentation stops prematurely leaving behind a lot of unfermented sugars. The temperature is, therefore, maintained by sprinkling cold water drawn from a cooling tower, on the external walls of the fermentors. This, however, makes the fermentation house quite wet and reduces the life span of fermentors due to corrosion. Another technique involving the use of energy saving device such as 'heat exchangers', has come to be used recently in many of distilleries. The fermenting wort of each fermentor is continuously circulated using a motor, through a plate heat exchanger (PHC), which also has cooling water in constant counter-circulation through another chamber encapsulating the wash chamber. Consequently, the wash cools down by transferring its heat to the cooling water, which is returned to the cooling tower.

During fermentation, the fall in 'Brix' or specific gravity is monitored using the hydrometers. The fermentation usually lasts for 20-24 h and its completion is marked by disappearance of bubbling in the wash and also becomes evident from the requisite fall in specific gravity of the wash. The wash which at this point contains 6-8% (v/v) ethanol, is given a 4-h settling time before being pumped into the still for ethanol recovery.

Distillation section

To recover the ethanol out of fermented wash, usually two kinds of distillation stills are in use. The 'pot still', which distils off the ethanol from the wash concentrates the ethanol to only 50-60% (v/v) in the first run. Further, concentration to 95-96% requires repeated distillations, which is not economical. The 'continuous still' on the other hand, can operate continuously for 24 h a day, handle large volumes of wash achieving 96% ethanol in the very first run and do not have to be shut down periodically. These stills, therefore, are in common use in the distilleries and one of the kinds is described below.

Continuous Still

Principle

A continuous stream of fermented wash is made to trickle down against a counter current of steam. The difference in temperature of these two streams deprives the wash of its alcohol, which goes with the steam. The steam thus mixed with the alcohol passes into two columns, the 'analyser' and the 'rectifier' and is further concentrated. The left-over material called spent wash exits as bottoms from the still (analyser).

Construction

A typical continuous still illustrated in Fig. 3b consists of two columns, one of which is called the 'analyser' (stiripper) and the other 'rectifier'. The two are connected to each other for the passage of vapours from the top of the former to the bottom of the latter, and for the condensate just in a reverse manner. The analyzer on the other side has inlets at the top and the bottom, for feed (wash to be distilled) and steam, respectively. To preheat the feed, the feed pipe may be routed through an energy saving device called heat exchanger, which has the hot spent wash released from the analyzer, running through it. Alternatively, the feed pipe could be routed in a (coffey's still also called patent still) coiled manner through the rectifier. This besides raising temperature of the feed due to the ascending steam and vapours, would also act as a condenser to

cool the latter. Thus, it results in saving of fuel and condensing water. The rectifier is further connected to a condenser, which condenses the vapours of alcohol and other associated byproducts, arising from the top of the rectifier. Further, to recycle (reflux) part of the condensate to the rectifier, a connection between the condensate- receiver and the rectifier may also exist.

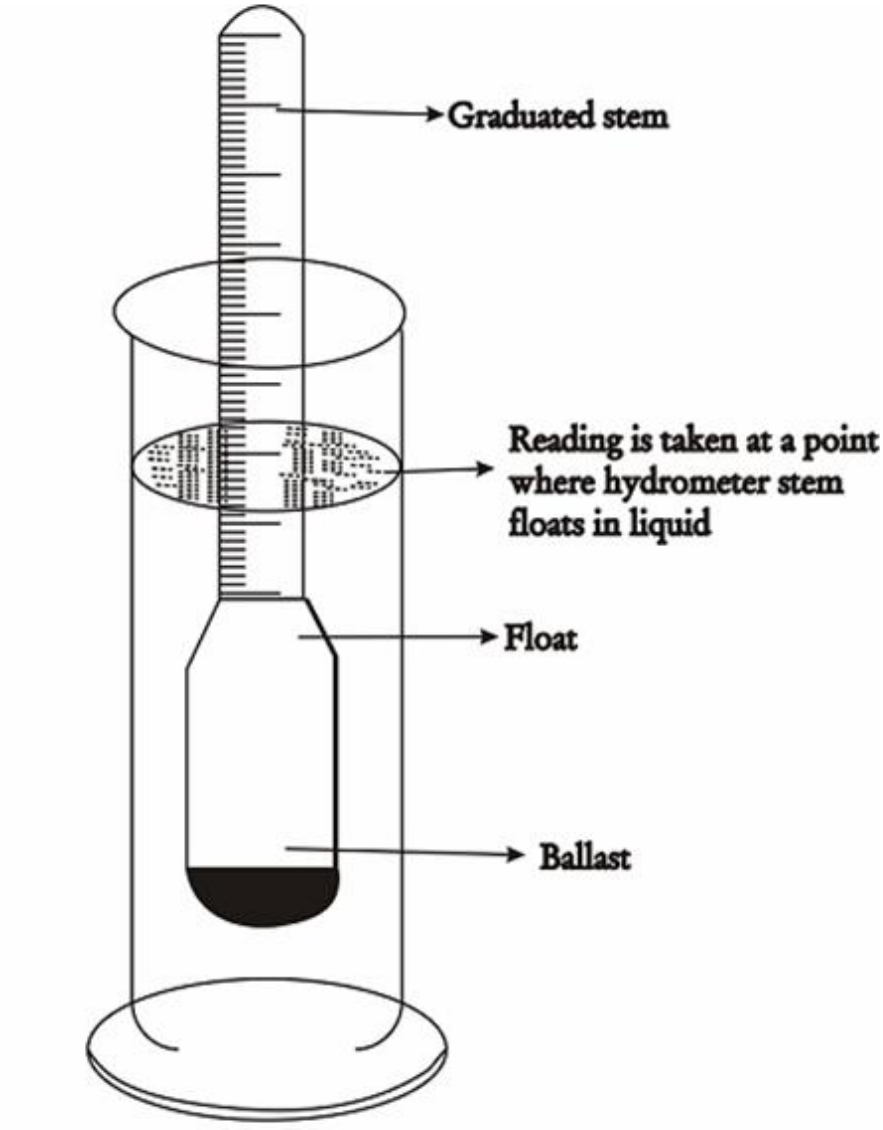

Fig. 4: Glass hydrometer

Both the columns throughout their height, incorporate appropriately placed metal plates or trays to hold the liquid, from which ethanol have to be recovered. Each tray represents a stage and accordingly a column having 10-trays is called a 10-stage column. Normally, higher the number of stages, better is the separation of ethanol achieved. Each of the metallic plates or trays is provided with a large number of perforations (called sieve trays) and a drop pipe (downcomer). The latter stands up about an inch above the plate making a weir (dam) and passes through it to hang over the next tray below. These drop pipes are arranged at alternate ends of the succeeding trays (Fig. 3b). Alternatively, bubble cap trays are also in common use. A bubble cap tray has riser or chimney fitted over each hole of the tray, and a cap that covers the riser. The cap is mounted in such a way that there is space between the riser and the cap to allow passage of vapour through the hole into the liquid on the tray. Vapours rise through the chimney and due to the cap above is directed downward, finally bubbling through the liquid on the tray.

Working of the continuous still

To start with, the two columns are flushed with super-heated steam entering at the bottom of the analyzer. The wash (feed) is pumped into the analyzer through a coiled pipe running from top to bottom of the rectifier or alternatively through a heat exchanger. The pre-heated wash drips down the analyzer and is discharged on the perforated plate or tray forming the top stage. The wash cannot pass to the next plate below until it has filled the top plate above to the level of drop pipe (downcomer). It then overflows and passes to the plates below in the same fashion.

The wash on its way down, meets the super-heated steam rising up the column. The volatile components in the wash, including the ethanol, are evaporated by the steam; the rest falls down to the bottom of the column and finds its way out as spent wash. The liquid feed thus right from the bottom plate gets deprived of the alcohol and other volatiles by ascending vapours of the steam. As the vapour mixture of steam and volatile components rises further up into the column, it vaporizes the liquid on successive plates, carrying its volatile vapours along. If compared with a pot still, which has to be run in batches, each plate of the column acts as a separate distillation unit (batch), because the rising vapours get enriched (concentrated) in volatiles including alcohol, as they bubble through the successive plates above, along the height of the column. Consequently the vapours leaving the top of the analyzer are free from most water and enter the bottom of the rectifier, where the remaining water is removed.

In the rectifier, the vapours again, begin to rise but are cooled by the fresh wash descending through the coiled pipe. The high-boiling point components such as fusels along with water and some ethanol (called feints) condense quickly at the bottom of the column. They are recycled to the analyser for redistillation and eventually fusels are drawn off. The rising ethanol vapors condense into a liquid at about two-thirds of the way up the column, as the temperature which prevails at this point corresponds to the condensation temperature of ethanol. Ethanol is drawn off through a dumb plate (non-perforated) provided at this height. If required, the foreshots (initial runs with low concentration of ethanol) may be recycled back to the rectifier or analyser. The highly volatile components such as aldehydes etc., are drawn off either at the top of the column or later out of a condenser and if need be may be recycled back to the rectifier or analyzer for redistillation.

The ethanol obtained from the rectifying column is about 96% and is called rectified spirit (RS). On an average, about 200-220 liters of ethanol is produced from one tonne of molasses as against 370-400 liters from grains. The RS is used as a base material for most purposes including for potable purpose, such as country liquor. However, for production of fine liquors called IMFL (India made foreign liquors), it needs to be further purified to ENA (Extra-neutral alcohol). The ENA is made out of RS by its redistillation again through a set of columns, a 'purifier' and a 'rectifier'. Besides, if need be, additional distillations using various columns for removal of aldehydes and methanol are also employed. The end product, ENA, obtained is similar to RS in ethanol concentration but is relatively free of flavouring impurities. For fuel use, as described earlier, the RS has to be dehydrated.

Unit of strength of spirits

In the assessment of duty on spirit and in commercial transactions relating thereto, the unit to measure the strength of spirits is termed as 'proof'. The term 'proof' originated in the early days of distillations, when the strength of a spirit was tested or proofed, by mixing equal amounts of the spirit and gunpowder together. The mixture was ignited by applying a light. If the mixture did not catch fire, the alcohol was too weak (under proof). If it burned too quickly, the alcohol was too strong (over proof). If the alcohol burned with a steady blue flame, the alcohol was of proper strength and said to be proven. This crude method was later improved upon by Bartholomew Sikes in the early 19^{th} century. He, through the use of a hydrometer invented by himself, determined that the steady blue flame is produced when the alcohol by volume is 57.1%. In the Sikes system, this is called '100^0 proof' or simply 'proof spirit'. Thus, a proof spirit also called 'London Proof Spirit', which is a mixture of alcohol and water contains precisely 57.06% of alcohol at 60^0F. At 51^0F, this mixture shall weigh exactly 12/13 part of an equal quantity of distilled water. In other words, 13 volumes of proof spirit shall weigh the same as 12 volumes of water at that temperature. The 'proof' spirit thus has a density of 0.91984 at 60^0F.

Spirits weaker than proof spirit are called 'under proof'. For instance, '20^0 under proof (20^oUP)' spirit would otherwise mean 80^0 proof (80^0 P). Similarly, spirits stronger than proof spirit are called 'over proof' spirits. A '20^0 over proof' (20^0OP) would be otherwise 120^0 proof spirit. Pure water is 100^0 UP or 0^oP. A spirit of 75.25^0 OP (175.25^0 P) is equal to nearly 100% (v/v) alcohol. On an average, each per cent of alcohol in the British system, which is followed in Canada and India as well, would equal to 1.75 P. In the American system, on the other hand, a simpler relationship prevails. The proof of the spirit is precisely twice the per cent of alcohol content. Yet another French standard called Gay-Lussac system, which gives directly the exact amount of alcohol. This system uses a scale of 0-100%. A spirit labeled 60 would contain 60% alcohol.

Distillers determine the proof of a spirit by using a hydrometer. This instrument consists of a calibrated scale attached to weighted bulb. When dropped into a sample of spirit, the level to which it sinks within the container indicates the density or alcohol by volume, of the spirit. Correction tables (Sikes Tables) are available for variation of temperature and also to take into consideration the contraction which takes place when ethyl alcohol is mixed with water.

6. Ethanol as biofuel

What is fuel alcohol?

Fuel alcohol is a clean and renewable product that contributes to the reduction of the greenhouse effect and lowers air pollution substantially, minimizing its impact on public health. Alcohols derived from the non-renewable petroleum are chemically very much the same as their counterparts from renewable biomass. Although they also can be potentially used as fuel, but by definition, they ought not to fall in the category of fuel alcohol, as they would rather add to the greenhouse effect. On the other hand, ethanol, one of the important alcohols, derived from renewable biomass such as sugar crops, starchy crops and cellulosic wastes, does make an excellent automotive fuel. This fuel alcohol is either used in blends, for example in gasohol (gasoline + alcohol) or diesohol (diesel + alcohol), or in its pure form. However, at present Brazil is the only country that uses 96% (v/v) ethanol as a 100% substitute for gasoline.

History of ethanol as automobile fuel

Its first use as a fuel dates back to the early days of the automobile. In 1872, when Nikolaus Otto invented the internal combustion engine, gasoline was not yet discovered. Ethanol at 180-190 proof was the recommended fuel. The model 'T' Ford was subsequently designed to run on the available crude gasoline, alcohol or a combination of the two. Anhydrous alcohol has been used since the 1930's as an additive to gasoline in Brazil. Several other countries also used it as a fuel during World War-II. Ethanol fuel suffered a setback during the post-war period, as the gasoline became not only readily available but at much cheaper prices, also. The renewed interest in ethanol as fuel began in 1970's when the world faced crisis in crude oil supplies due to the oil embargo by the middle east countries. The prices of crude oil shot up phenomenally. The ethanol, as fuel, was intensively researched as against the existing fuel, gasoline. As a result, programmes on fuel alcohol were launched in the US and Brazil. In the United States, ethanol derived from corn is most commonly blended with gasoline as a blend of up to 10% ethanol, known as E10 and nicknamed "gasohol". Brazil has been employing E22G (blend of 22% bio-ethanol with gasoline) successfully since the mid-seventies. High ethanol – gasoline blends such as E85G are also common in vehicles in which engines have been suitably modified. Besides, in Brazil 96% ethanol is also being used as a 100% gasoline substitute in cars with dedicated engines. The European Union had also recommended a 5% admixture of ethanol in gasoline for internal combustion engines before 2010.

Other countries such as Thailand, India, China and Japan have also launched their national gasohol policies. Thailand started blending 10% ethanol in 1985. General Motors of Canada are also preparing the launch of E85 flex-fuel vehicles, and will be sold at the same price as their gasoline-only versions. The Govt. of India also, had resolved that with effect from 1st Jan., 2003, 5% ethanol-doped petrol would be supplied in nine states and four union territories. The project, however, did not make substantial headway due to various reasons. Recently, it has been revealed by the Petroleum Ministry that the ethanol-blending-programme (EBP) will be effective from Nov.1, 2006 in all States except the North-East Region, Lakshadweep and Andaman and Nikobar Islands. In the second phase, ethanol in the blend is likely to be increased to 10%.

Characteristics that make ethanol a natural fuel

Heat value

One of the most important properties of a fuel is the amount of energy we can get from it on a per unit basis, when it is burned. The hydrocarbon octane (C_8H_{18}), which represents an 'ideal' gasoline, contains no oxygen. As against this, all of the alcohols contain an oxygen atom bonded to a hydrogen atom in the hydroxyl radical. When alcohol is burned, the hydroxyl combines with a hydrogen atom to form a molecule of water. Thus, the oxygen present in the alcohol structure does not contribute to the fuel value. As a result, ethanol contains only about 60% (11550 Btu/lb) of the heat value of gasoline (19,000 Btu/lb). If only the heating value of ethanol were considered it would appear to be one of the poor fuels. But since ethanol as a fuel undergoes different changes as it is vaporized and compressed in an engine, the outright heating value of the ethanol is not as important, when it is used as a motor fuel. In other words, other important qualities/characteristics such as volatility (vaporization), latent heat of vaporization, octane rating etc. must be taken into consideration.

Volatility

Volatility of a fuel refers to its ability to be vaporized. This, in fact, is an essential requirement for a substance to be used as a fuel. If vaporization does not occur readily, the fuel cannot be evenly mixed with air and is of little value in an engine. Some substances that are highly volatile cannot easily be used as a motor fuel, while others such as waxes and tars, which have excellent heating value are not volatile enough to be used in an engine. Simultaneously, it ought not to be ignored that very volatile fuel is also potentially dangerous, as it could simply explode just at the site of heat or a spark. This is why ethanol with a higher flash point than gasoline is a much safer automotive fuel.

Latent heat of vaporisation

Closely related to volatility of a fuel is a characteristic called latent heat of vaporization. When a liquid is at its boiling point, a certain amount of additional heat is required to change the liquid to a gas. This additional heat, which is 361 Btu/lb and 140 Btu/lb in case of ethanol and gasoline, respectively, is called the latent heat of vaporization. As a result of absorption of latent heat from the engine chamber where vaporization of the gasoline-air mixture takes place, a temperature drop of about 4.44^0C occurs. In comparison a mixture of ethanol-air, ethanol's latent heat being 2.5 times as much as of gasoline, will thus result in a temperature drop of over 11.0^0C. Clearly, due to this phenomenon, an ethanol-powered engine will run cooler than its gasoline-fueled version, thereby prolonging its own life span.

Octane rating

Gasoline is a mixture of hydrocarbons including the component 'Octane' which in its pure form has been assigned a numerical value of 100. This number has been used for fuel-rating purpose. However, as such, it is not applicable directly to alcohol-fuels. The octane number given automotive fuel is an indication of the ability of the fuel to resist premature detonation within the combustion chamber of the engine. The premature detonation or engine knock comes about when the fuel-air mixture ignites spontaneously towards the end of compression stroke because

of intense heat and pressure within the combustion chamber. Since gasoline engines are designed so that the mixture is detonated by the spark plug at a slightly later point in the engine cycle, pre-ignition is undesirable, and can actually damage or even ruin the engine.

Because a high compression ratio in an engine results in more power per stroke, greater efficiency and better economy, a fuel that resists pre-ignition even under high compression conditions is extraordinarily good. And alcohol is, on the average, about 16 points higher on the octane scale than premium gasoline. In other words, ethanol has a relatively high anti-knock or octane rating, and thus have the ability to raise considerably the octane ratings of gasolines with which they are blended. The effect is greatest on the poorer grades of gasoline. A 25% blend of ethanol and 40 octane gasoline will have a net increase of almost 30 points. This increment is one of the major advantages of 'gasohol'. The role of ethanol as an octane enhancer can thus enable the use of even poor quality gasoline and also provides for the elimination of traditional pollution producing anti-knock additives such as tetraethyl lead.

Exhaust emissions

When gasoline burns in an engine, a large amount of poisonous carbon monoxide (CO) and other noxious emissions arising from impurities like sulfur and additives such as lead or phosphorus, are produced. In comparison, since the ethanol has oxygen in its structure, it burns more clean and produces much lower amounts of CO. Also the absence of above impurities and additives makes ethanol a better fuel than gasoline as undesirable combustion by-products are not produced. The ethanol when used as blends with gasoline up to 20% or less, results in the proportionate emission improvements in terms of CO and other by-products. Besides, the added advantage the ethanol blends offer, is that the ethanol replaces the lead and other undesirable compounds used as octane enhancer in gasoline.

Engine performance

An alcohol powered engine if modified correctly, will have performance equivalent, if not better than, the same gasoline powered version. This is simply because that ethanol has a higher 'octane' rating (hence the timing can be slightly advanced), and can stand much greater compression ratios.

Keeping economics aside, a major advantage of blends is that up to a certain concentration (between 10-20%) they can be used with absolutely no modification of the engine. While some studies claim a slightly better fuel economy, others signal to a slight decrease. Some tests claim improved emissions, others claim no significant change. As regards power output, the tests are rather ambiguous. However, when all data is put together, the overall conclusion is that in terms of fuel economy, emissions and performance, there is just not any difference.

Ethanol as biofuel offers added advantages

The ethanol as mentioned before has in it the traits such as the heat value, volatility, latent heat of vaporization, octane enhancing ability, improved tail-pipe emissions etc., of a natural automotive fuel. The use of biofuel ethanol which gives a net positive energy balance (energy

contained in a tonne of ethanol is greater than the energy required to produce a tonne), over and above, gives some additional advantages. The biggest advantage it offers, is that the carbon dioxide released to the atmosphere during its production process is entirely recaptured by crops, thus producing no net greenhouse effect. Secondly, as the bioethanol production is biomass based and is indigenous, and the fossil fuel or the ethanol derived from it is mostly imported, it would save quite a foreign currency. From a macroeconomic point of view it may also be good for the disadvantaged rural areas by promoting ethanol industry which creates jobs. And finally promotion of the biofuel ethanol is likely to promote the advances in research in biotechnology to make bioethanol more cost-effective.

Weak points of ethanol as fuel

Bioethanol has also some weak points or disadvantages in comparison with conventional fossil fuels. First of all, the current gasoline powered engines unless modified cannot run on pure bioethanol. Modifications such as replacement of components made of zinc, brass, lead, aluminium or other softer metals, are a must as ethanol could cause leaching from soft metals. Similarly, all rubber elements, because ethanol is a good solvent, need to be replaced. Other disadvantages of pure ethanol are: lower octane number; low vapour pressure and high latent heat of vaporization, which cause start problem in winters or cooler climates; increased formation of acetaldehyde, etc. Because of these disadvantages it is generally believed that large-scale application of ethanol will cost very high.

On the other hand with ethanol blends, most of the operational disadvantages encountered with pure ethanol, are generally not observed. It is generally accepted that the low blends of ethanol are more promising from practical point of view than high concentration blends. Although, current modern gasoline engines may be able to run smoothely without any modification with up to 22% bioethanol blends, but in practice, blends of gasoline with not more than 10% content of bioethanol, work the best. And since the blends require anhydrous ethanol, it adds to the cost of blended fuel, as production of this ethanol is a further energy requiring process.

Another important disadvantage of ethanol is that neither pure ethanol fuel nor blends may be shipped via conventional pipelines, which is the cheapest mode of transportation of liquid fuels. The reason is that ethanol absorbs water and other residues. Hence, the handling process of bioethanol fuels is more complicated in comparison to gasoline or diesel. Therefore, blending needs to be done only at supply terminals, with all the associated costs for extra storage capacity, blending equipment etc.

The most important disadvantage of bioethanol is its lower energy content in comparison with gasoline. It has got about 60% of the volumetric energy content of gasoline and about 59% of diesel. Due to this fact the fuel economy of ethanol is reduced by 33% (4o%) and 41% in comparison with gasoline and diesel, respectively. In other words, it should take about 1.5 litres of ethanol to replace a litre of gasoline and 1.7 litres to replace a litre of diesel. However, once ethanol is used in low concentration blends (E5G, E10G), the fuel economy penalty is reduced. Also the strong points of bioethanol may partly compensate the impact of its lower energy content in volumetric terms.

Another major disadvantage pertains to the cost of bioethanol production which remains to be rather non-competitive with fossil fuels. This is mainly because of the high cost of raw biomass and the operational cost such as the fractional distillation to produce anhydrous ethanol. The ability to produce bioethanol from low-cost biomass and improvement in downstream processing will, therefore, be the key factors to make bioethanol competitive with the gasoline. Although cellulose materials which have lower costs than other feedstock, could be the best bet, but they are presently more costly for conversion into bioethanol, due to the extensive processing required.

Clearly, the bioethanol as fuel remains non-competitive under current economic conditions. However, it may increase its competitiveness to fossil fuels, if the low cost biomass is useable, the production process improves its effectiveness and the positive externalities of bioethanol such as environmental benefits including decreased greenhouse emissions, promotion of the industry based on renewable raw materials thereby providing more jobs to the disadvantaged rural populace and non-reliance on imports of fossil fuel, are taken in the price mechanism.

7. Common alcoholic beverages

All alcoholic beverage industries are based on the production of ethanol and a wide range of other quantitatively minor but organoleptically very important volatile and non-volatile compounds, by fermentation of molasses or sugarcane juice or an extract of cereal or fruit (grapes) by strains of yeast. The major organoleptically active compounds which impart flavour and aroma to the beverages are organic acids, esters, aldehydes, fusel oils (higher alcohols) and terpenes. Their type and concentration is determined by the yeast strain, the raw material and conditions used such as temperature of fermentation, nutrient status, inoculum size etc. The yeasts involved are invariably strains of *Saccharomyces* particularly *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. Strains of *Saccharomyces* are endowed with a capacity to effect an efficient alcoholic fermentation of sugars. The fermentable sugars may be in the aqueous solution/extract of the raw material (such as molasses and sugarcane juice or grapes and other fruits) used to make the alcoholic beverage. In other processes such as brewing of beer (also whiskies) and sake, the sugars are furnished by hydrolytic breakdown of the polysaccharide, starch.

A further subdivision of the alcoholic beverages arises from the fact that some products of the fermentation process are consumed more or less as such (wines and beers), whereas in others the fermented product is distilled to form the consumable product (distilled beverages or liquors). In the latter, technique of distillation used such as pot still or continuous still, would also determine the characteristic taste, flavour and aroma of the final product. Because the fermentation associated volatile products mentioned above, which are loosely called as congeners, tend to co-distil during ethanol recovery from the fermented wash. Thus, an ingenious distiller/still man could achieve a desired concentration of the congeners in the distillate and in turn produce a liquor of organoleptic specifications, he or she is interested in.

The fermentation and recovery of ethanol from molasses and starchy raw materials have already been discussed in the foregoing sections and the same for each of the distilled beverages will,

therefore, not be repeated here. The types of liquors with their important characteristics and non-distilled beverages such as wines and beers with production operations are described.

Distilled beverages (liquors)

Distilled beverages (spirits) having 35-50% alcohol represent a substantial proportion of the market for alcoholic beverages. Owing to their high ethanol content they are resistant to microbial spoilage. They have been categorized into two broad groups. One group is represented by those beverages where the raw material has an important influence on the sensory quality of the final product. This group includes whisky, which is derived from cereals; rum, produced from sugarcane juice or molasses and brandies, distilled from fermented juice of grapes or other fruits. The second group includes products such as gin and vodka, which consist of distilled alcohol–base that has been processed to give specific flavour characteristics. Though highly intoxicating, the liquors do not carry health promoting components present in fermented brew, as vitamins, amino acids, sugars etc. are left behind during distillation.

Country liquor

Commonly called as 'Desi Sharaab/Daroo', it is molasses based rectified spirit diluted to 30-33% ethanol, by volume. The product is coloured generally with caramel (burnt sugar or glucose) and bottled. Depending upon the State Excise Policies, the concentration of ethanol in these liquors may vary from one State to another. As opposed to this, the other fine liquors (IMFL) described below, uniformly contain 42.8% ethanol by volume (75^{o}P or 25^{o}UP). In the State of Haryana, although many variants of this liquor with different additives under various brand names are available, yet they are popularly called as 'Jagadhari Brand'. It is perhaps because of the oldest brand 'Jagadhari', a product of the distillery located at Jagadhari in the State. This brand has, over the years, been extremely popular in the state.

Rum

A key product from the West Indies is made by distilling fermented sugarcane juice or molasses. The raw material used largely determines the type of rum produced. Cane juice is the best suited for the production of rum with a light aroma. Diluted raw material containing 12-20% sugars is fermented by *Saccharomyces cerevisiae* at 20-30^{0}C for 2 days for light-flavored rum and longer times (12 days) for heavy flavored products. Fission yeast *Schizosaccharomyces pombe* strains are, however, best suited for production of rums with heavy aroma. The ferment is distilled using pot still for heavy and continuous still for lighter rums. As a result, the latter contain only 10% of aroma compounds of that of the former. They are matured in oak barrels, if desired. Caramel may also be used to colour the rums, if required.

Whisky

Whiskies are distilled alcoholic beverages that are produced from fermented cereal extract and are usually aged in oak barrels before bottling. They are produced principally in Scotland, Ireland, North America and Japan. The most commonly used cereals are maize and wheat, but barley, rye, oats and rice are also used depending upon prevailing economics. For full malt whisky for example, prepared in Scotland, specially kilned malt by burning peat is used. Such

malt carries certain organoleptically active volatile compounds particularly of phenolic nature to the final product called Scotch malt whisky. In addition to yeasts, lactic acid bacteria as a natural contaminant may contribute to the fermentation of malted barley mashes. Method of distillation using small sized pot stills is another important feature of scotch production. Scotch malt whisky is marketed both as malt-whisky and also as a blend with another type of whisky produced in Scotland namely scotch grain whisky. Most scotch whiskies available on the international market consist of blends with 30-40% malt whisky. Within the blend, there may be as many as 20-30 individual malt whiskies and grain whiskies. These blends are by law, matured for at least 3 years and in practice, this period is much longer. Unblended scotch malt whiskies are usually matured for minimum of 8 years. The word 'Scotch' is of geographical and not generic significance.

Brandies and Wine Spirits

These products are the distillates of fermented fruit juices normally of grapes. The well known products Cognac and Armagnac are produced from grapes in specific regions of France. Yeasts are responsible for alcoholic fermentation of the fruit juice to produce a wine-base for distillation. Malolactic fermentation during wine making may or may not be desired before distillation using pot stills. The wine-base for distillation may or may not include yeast lees, which impacts on product sensory quality. Finally, a period of maturation in oak barrels may be required, as for most high-quality brandies. The brandies are also produced from other fruits and are accordingly named after the fruit.

Gin and Vodka

Neutral alcohol (flavourless) is the base for producing these flavored beverages, which are colourless in appearance. They are generally produced by alcoholic fermentation of extracts from cereals (maize etc.) or potatoes, with emphasis on efficiency and economy of production. The final distillate (base) contains greater than 96% (v/v) of ethanol with very little flavour contribution from congeners. Various flavours are added to this alcohol-base to produce quite distinct products. The flavours may be directly added as essences or the product may be redistilled from a mixture of the base and flavour contributing plant and diluted to give an appropriate strength of alcohol. Gin derives its flavour from addition of juniper berries etc. called botanicals. Vodka is pure, unaged spirit that has been filtered through charcoal. It may be flavored with a variety of materials such as orange and lemon peels, ginger, cloves and sugar.

Liqueurs

The liqueurs are strong and sweet alcoholic drinks consumed in small quantities usually after meals and are often called 'digestif'. They consist essentially of spirit, flavouring and sugars. The sugar content may vary from 2.5 to 10.0 to 35-40%. Liqueurs with large concentration of sugar are often called 'cremes'. The base may be brandy, whisky, gin or other forms of potable spirit. Flavouring may be added before distillation or less desirably by the addition of essences. Like wines and other spirits, good liqueurs are matured in oak casks and colouring is often added before filtering and bottling. Honey or malt rather than sucrose as sweetening agents are used.

Flavouring substances include: Apricot, cherry, peach, chocolate, peppermint, coffee, almond etc.

Undistilled beverages

Wines

Wine refers to a fermented alcoholic beverage produced usually from grapes and contains vitamins, amino acids, esters, sugars and tartaric acid and hundreds of chemical compounds derived from grapes and yeast. The science which deals with the study of wines is named as enology. In principle, any fruit having sufficient sugars would ferment to make a wine. The common examples are cider (apple), perry (pears), cherry wine (cherry), mead(honey), sake & sonti(rice) and pulque from cacti. Most wines are generally made from one species of grapes, namely *Vitis vinifera.* This is because of the presence of high sugar content in grape berries. Besides, high natural acidity of its juice inhibits growth of undesirable micro-organisms during and after fermentation. However, it is not so high as to be unpalatable. Also, aroma and flavours of the grapes, which are carried into the final product, wine, are organoleptically pleasing. Further, selection of various varieties of this grape species for wine production would, however, be important from the viewpoint of varietal flavour and aroma, as they are wholly or in part carried over to the wine. Naturally fermented wine would contain from 8-14% alcohol, as yeast metabolism would be inhibited by more than 14% alcohol. These wines are called 'Table Wines', as they are consumed along with the food. In contrast, 'fortified wines' are strengthened in ethanol content with the addition of wine spirits (brandy). Wines may be fermented by the naturally occurring yeasts in the vineyards as in several regions of France or may be inoculated with pure cultures of wine yeast, *Saccharomyces cerevisiae* var. *ellipsoideus*.

Our country does not, at present, produce any significant quantity of wines worth a mention, and to most of us even the term 'wine' is misunderstood. To most Indians, wine is synonymous with such other distilled hard liquors as whisky, gin, brandy and rum. Many shops displaying sign boards that read "English wine and beer shop", as a matter of fact, sell everything but wine.
The distinctive character of wine originates from the grapes as raw material and subsequent processing operations. The grapes contribute trace amounts of volatile components (terpenes) that give the wine its varietal, fruity character. In addition, they contribute non-volatile acids (principally tartaric and malic acids), which impact on flavour, and tannins (flavonoid phenol), which give bitterness and astringency. The fermentation steps, especially alcoholic fermentation, increases the chemical and flavour complexity by assisting extraction of compounds from the grapes, modifying some grape-derived substances and producing a vast variety of volatile and non-volatile metabolic end products. Further chemical alteration occurs during aging and storage, where enzymes from the grapes and those excreted by microorganisms may still be active.

Production operations

A typical wine-production process (fig.5) is initiated with the crushing of grapes (or other fruits) to extract juice of a winemaking variety and addition of sulphur dioxide usually in the form of potassium metabisulphite (KMS) @ 100-150 ppm. The KMS kills the resident wild flora of grapes and prevents oxidation of juice. In the case of red wines, the grape must (crushed grapes

including juice, skin and seeds etc.) is inoculated with the wine yeast for fermentation to take place for 7 days at 20-30^{o}C. The higher temperature is necessary to extract colour from the grape skin. After 2-3 days when the requisite colour has been extracted into the fermented juice, the skin is removed. Alternatively, the juice may be heated (thermovinification) in the presence of skins to 43^{o}C for 8-16 h, then cooled, pressed and fermented. Once the fermentation is complete, the wine is racked off the yeast lees and goes in for aging in oak barrels, followed by clarification and packaging. However, prior to clarification and packaging, a malolactic fermentation of the wine could be desirable. It is quite common with premium red wines during storage, where some lactic acid bacteria (*Leuconostoc, Lactobacillus, Pediococcus)* are 'at home' in the wine. These bacteria besides reducing the acidity of wine through decarboxylation of malic acid into lactic acid, also bring about a positive change in the flavour of wine. After the malolactic fermentation, wine needs to be sulfited using KMS and filtered. Clarification, if required, is achieved by treating the wine with bentonite, gelatin etc., followed by bottling up to the brim, leaving no air space at the top. White wines essentially follow a similar scheme of operations except that the skin is removed prior to yeast inoculation and the juice is allowed to ferment at 10-18^{o}C for 7-14 days or more to favour the extraction and retention of volatile flavour compounds. Malolactic fermentation may or may not be desired.

Type of Wines

Wines may be classified on the basis of variety of grape used, colour, flavour added, added herbals, content of alcohol, carbon dioxide, sugar and other constituents. Wines have been divided into two broad groups (1) with added herbal and/or other flavours (vermouths); and (2) without added herbs or flavours. Further classification is then based on other characters listed above, but in any case to produce various classes of wines the base invariably remains either the white or red table wine.

Vermouths are prepared by addition of different spice mixtures and can be white or red in colour, sweet or dry. They contain about 15-20% alcohol and when sweet, contain 14-16% sugar as well. Vermouths in general are made from average or poor quality wines but addition of herbs and spices raises their acceptability and value owing to enhanced flavours and aroma.

Wines without any added herbs, spices or flavouring agents constitute the majority of wines of the world. These wines can be either sparkling (effervescent due to excess carbon dioxide sparged therein) or the normal still variety. Sparkling wines were originally produced in Champagne region of France. These wines have a special tongue taste due to the presence of carbon dioxide produced as a result of secondary fermentation. They contain 2-6% sugar and 2-3 atmosphere carbon dioxide pressure and are usually reserved for ceremonial occasions like marriages and celebration of birthdays. The still wines mostly contain 8-14 per cent alcohol and may or may not contain sugar (sweet or dry) but can be judged on the basis of varietal aroma, flavour and taste. Rhine and Reisling wines of Germany, Chainti of Itly, Burgundy of France are some of the many wines having varietal characters.

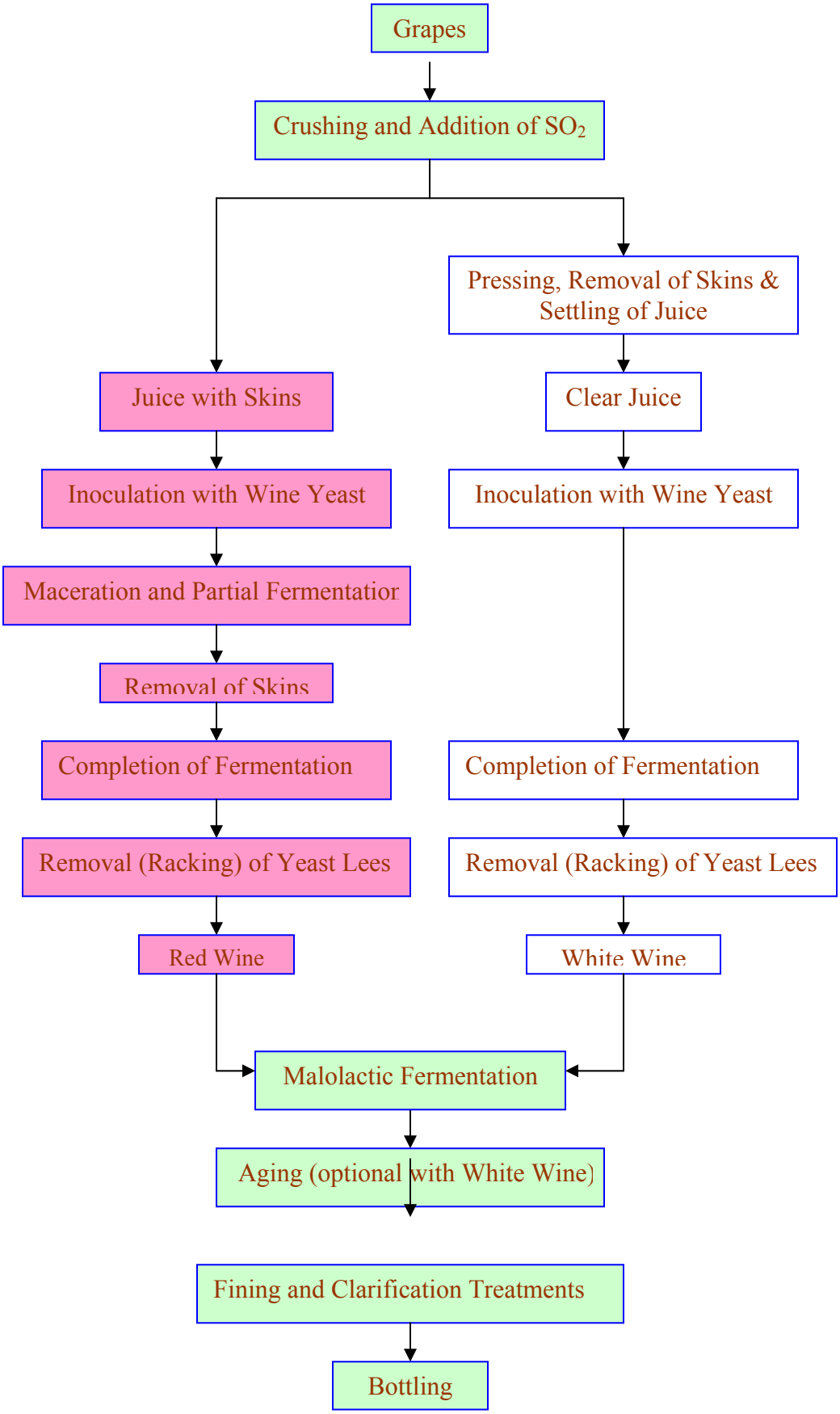
Grapes
Crushing and Addition of SO_2
Pressing, Removal of Skins & Settling of Juice
Juice with Skins
Clear Juice
Inoculation with Wine Yeast
Inoculation with Wine Yeast
Maceration and Partial Fermentation
Removal of Skins
Completion of Fermentation
Completion of Fermentation
Removal (Racking) of Yeast Lees
Removal (Racking) of Yeast Lees
Red Wine
White Wine
Malolactic Fermentation
Aging (optional with White Wine)
Fining and Clarification Treatments
Bottling

Fig.5 Outline of Wine Production

Fortified wines, such as sherry and port have ethanol concentrations of 15-22%. Sweet fortified wines are also called dessert wines as they are normally consumed after the meals. Higher levels of ethanol in these wines are obtained by the addition of wine spirits at certain stages during the process. In addition to high ethanol content, their distinctive character is due to specialized processes of maturation in oak casks. Sherries utilize white wine base, whereas port, a red wine base. These wines are characterized by the presence of higher concentration of aldehydes formed either due to the oxidative metabolism of 'Flor yeast' *Saccharomyces beticus* (*S. bayanus*), which makes a scum on the surface of wine in casks, or by baking and oxidation by air.

Beer

Beer is the fermented extract of malted cereal grains principally barley. It has an ethanol content of 2-6% or even higher and a distinctive flavour, which arises from constituents of the malt, extract of hops and products of yeast metabolism. The final product is generally clear, with a colour that ranges from golden amber to black. Another distinctive property of beer is the formation of a head (foam) when it is poured into the glass. By varying the malt ingredients and process of fermentation several types of beers can be produced. Most beers produced throughout the world are of lager type. This is a clear amber coloured beer produced by 'bottom' yeast S. *uvarum* (previously known as *S. carlsbergensis)* at a fermentation temperature of 5-12^{o}C. As opposed to this 'Ale', another kind of beer is fermented at 18-25^{o}C with strains of 'top' yeast *S .cerevisiae* and is darker in colour, because it is produced using darker malt. Stout beers are very dark because the malt has been roasted.

Production Process

A typical beer production (brewing) process includes (i) malting (ii) actual brewing (Fig 6).

Malting

The malting which represents conversion of cereal grains particularly barley to malt goes as follows:

Steeping

Steeping is a process of soaking of barley grains in water for a period of 40-48 h, so as to raise the moisture content of kernels to around 45%, to facilitate germination.

Germination

The water is drained off and the grains are germinated for 5-7 days, until the desired growth of embryo has been achieved. The process is monitored by the length of acrospire. Usually a length which is 2/3rd to 3/4th of the length of kernel is considered optimum, as the level of enzymes such as α and β–amylases and proteases developed at this point is believed to be at its maximum. As a result, some of the barley starch gets converted to maltose, dextrins etc. It is because of this reason that the final malt which looks like the normal barley grains, tastes sweet. The product obtained at the end of germination is called green malt.

Kilning

The green malt being difficult to preserve is dried in ovens at various temperatures. While the light malts are normally dried at 80^{o}C, the dark malts (Munich malt) at over 100^{o}C. Besides fixing in the grains the desirable properties that have arisen during germination, kilning imparts flavour, aroma and colour to the malt. The malt thus obtained serves as a source of carbohydrates and enzymes for the subsequent mashing process.

Actual brewing

It consists of (1) mashing to extract the contents of malt to produce a wort containing fermentable sugars, (2) Boiling of 'Sweet wort' with hops and (3) fermentation by brewer's yeast to produce alcohol.

Mashing

The purpose of mashing is to digest and dissolve all the valuable portions of malt into the suspension. The starch present in malt (also malt adjunct) is hydrolyzed by malt enzymes to simpler sugars, which are fermented to ethanol and CO_2. Proteins are converted to peptides, peptones, amides etc. The sweet wort thus produced is a complex mixture of sugars such as maltose, glucose, dextrins etc. and organic nitrogenous compounds and inorganic constituents. The wort, therefore, makes an excellent medium for yeast growth.

The mashing is achieved by holding the infusion of ground malt in water at 45-50^{o}C for about 1 h to let the proteases convert the proteins of malt into its degradation products as already mentioned above. This period is called 'protein rest' period. The temperature of the mash is subsequently raised to 65-70^{o}C for 90 min. During this period α and β–amylases, which act in quick succession convert the starch to fermentable sugars and dextrins.

Malt adjuncts, which represent additives to the malt in the form of unmalted cereals such as barley, wheat, corn or rice or non-starchy malt derived sugar syrups, have also been in use for economic reasons. The unmalted cereal adjuncts after gelatinization in a cooker, are added to the high-amylase malt derived from six row barley varieties with high protein content, and mashed The wort produced in this way has a diluted (lower) protein content as against the one produced from pure malt. The corresponding beer in turn would be resistant to a beer defect called 'chill haze'. This defect causes turbidity in beers at low temperatures due to the presence of a protein–tannin complex, which is otherwise soluble at room temperature. The malt made from real brewing quality two row barley, because of its low protein content may, therefore, not require the addition of unmalted cereal adjuncts. The syrupy adjuncts on the other hand, do not need any prior mashing and may be added directly to the sweet wort, to produce a beer of higher ethanol content.

As a final 'mashing off' step, the temperature is raised to 75-80^{o}C, thereby resulting in inactivation of enzymes and a wort of constant composition. The particulate matter is removed by use of a filter press or a lauter tun. The clarified wort after being transferred to a brewing kettle is hopped and boiled.

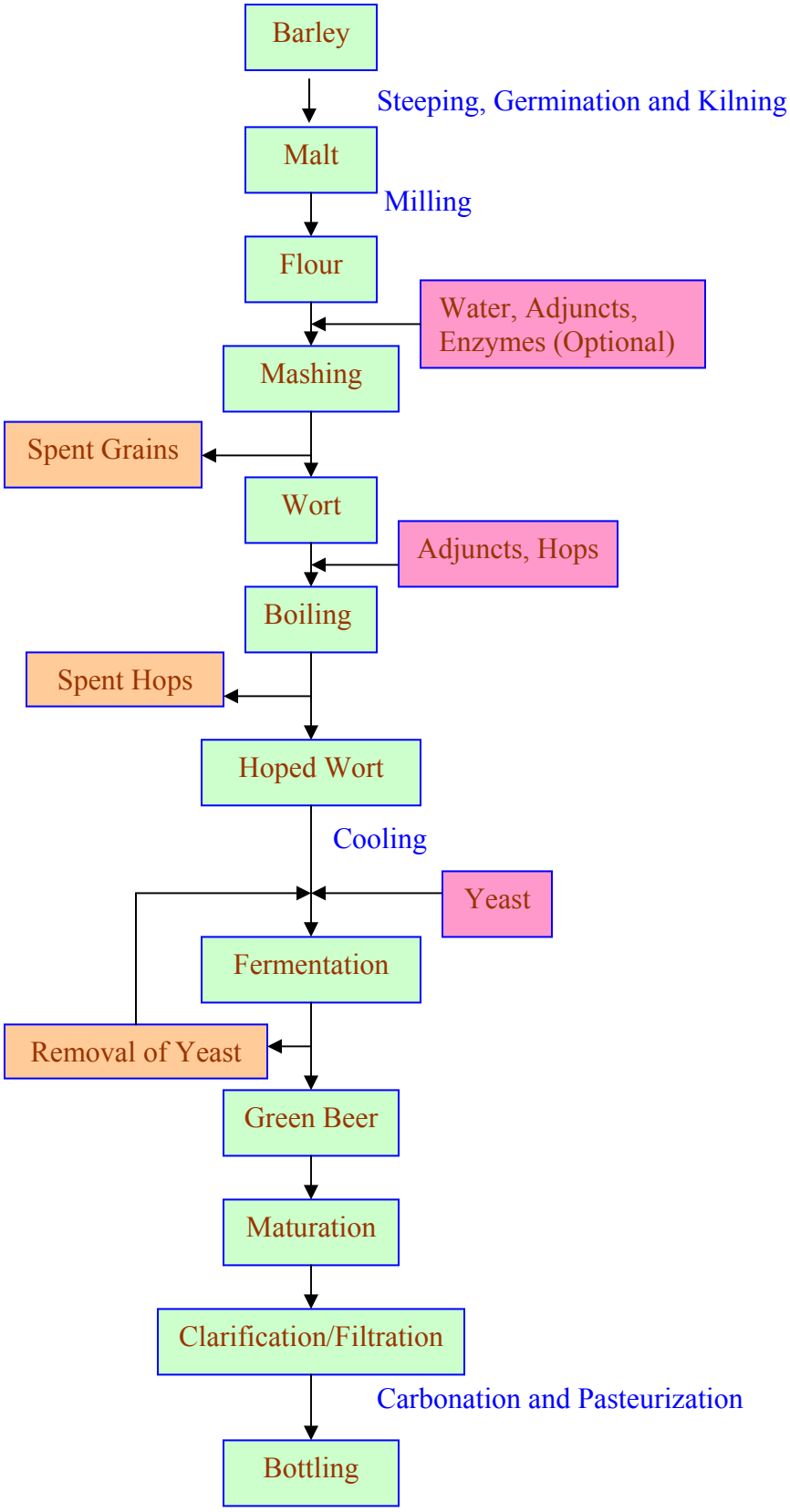

Fig. 6 : Flow chart of beer production

Boiling of Wort with Hops

The sweet wort is boiled under pressure at 105^{o}C for 1 h with petals of the female flower of 'hops' plant. It besides extracting the soluble contents of the hops, also sterilizes the wort and slightly caramelizes the sugars in it. The characteristic bitterness and flavour of the beer is in fact because of the bitter acids, resins and essential oils, present in the hops. The spent hops are removed. The proteins which get coagulated during boiling, are called 'hot trub' (hot break) and are also removed. The hopped wort is cooled down in order to give a 'cold break' which produces a 'cold trub' due to precipitation of proteins and tannins at low temperature. The cold trub is separated and the wort goes for fermentation.

Fermentation

The hopped wort is subjected to fermentation by the brewers' strain of either a bottom or a top fermenting yeast. While the former produces a lager beer, the latter produces an ale. Further, selection of the strain is important as the quantitatively minor metabolites produced by it, as well, determine the flavouring characteristics of beer. The strain should, in particular, produce lower concentrations of diacetyl, a compound responsible for imparting undesirable flavour to beers. Once the fermentation is complete, the yeast cells are removed and after screening for contaminants, if required, may be recycled for next round of wort fermentation. The beer obtained is called 'green beer'. During maturation for 1-3 weeks at low temperature, the 'green beer' may be left with a small number of yeast cells to degrade undesirable compounds such as diacetyl, aldehydes etc. Besides, particularly in Europe, this 'after fermentation' (secondary fermentation) by yeast in closed tanks, is used to partially carbonate the beers. This process is called 'krausening' and the product thus produced is called a 'krausen beer'. The green beer after filtration or a fining treatment may be carbonated and put into kegs (draught beer) or after pasteurization is bottled up.

Types of Beer

Most beers basically fall into two categories, i) the lager beers and ii) the ales. As already described, the former utilizes bottom fermenting yeast, whereas the latter, a strain of top fermenting yeast. However, deviations from this rule may also happen. Further typing of beers occurs on basis of certain characters such as the colour of the beer, name of the city, alcohol and hops content of beer etc. The lagers which mean stored beers, usually have a lower alcohol and hops content than the ales. Important types are as follows:

Bock Beer

It is a darker lager beer from caramelized malt. Bock is a German word for goat which in turn signifies 'Aries' sign of zodiac for March. It, therefore, is a traditional spring beer sold normally in the festive month of March.

Pilsner Beer

Is a beer which was first produced in Pilsen, Czechoslovakia. It is almost dry with light colour and has a good hop aroma.

Münchner Beer

It is the beer which is slightly sweeter due to less hops and is produced in Munich, Germany.

Stout and Porter

They represent ales which have been highly hopped and also contain higher concentration of ethanol.

Krausen Beer

It is a beer which has achieved its CO_2 partly from secondary fermentation by yeast.

No Carbohydrate Beer

It is a beer brewed from wort, where all the dextrins have been converted to ethanol using externally added amylases and yeast.

8. Vinegar

Vinegar is a sour tasting liquid made from oxidation of ethanol, where the source of ethanol could be wine, cider, beer, fermented fruit juices or any other liquid containing alcohol. Depending upon the raw material, vinegars are differentiated as wine vinegar, cider vinegar, malt vinegar, beer vinegar, fruit vinegar etc. Commercially available vinegar is about 4-8% acetic acid and usually has a pH of about 2.4. The term vinegar comes from the French word "vin aigre" that means sour wine. This spoilage of wine is caused by certain acetic acid bacteria (*Acetobacter*) which are obligate aerobic, gram negative, motile, rods generally having the tendency to form a film on the top of the liquid layer. *Acetobacter xylinoides, A. orleanse, A. acetigenum, A. aceti, A. pasteurianum, A. oxidance* etc are some of the commercially used bacteria for vinegar production. Under highly aerobic conditions, the action of such bacteria, on a liquid containing around 10-13% alcohol, leads to the production of vinegar at a suitable temperature, ranging from 15-34^{o}C. Modern vinegar fermentations require uninterrupted aeration to produce high concentrations of vinegar. The Frings acetator is the first commercial equipment, modified time to time, to meet the upcoming requirements of vinegar fermentation. It is a baffled fermenter containing a bottom driven hollow-body turbine which rotates at 1450-1750 rpm and enables air to be sucked through the hollow rotor and distributed radially over the whole cross-section of the fermenter. In another approach 12-15% acetic acid is produced in a semi-continuous manner. The fermentation is carried out in different cycles of equal duration ranging from 24-48 h, starting with an alcohol and acetic acid concentrations of 7.5% and 5.5% respectively. At the end of each cycle when alcohol concentration drops to 0.1-0.3%, approximately one-third quantity of fermenting mash is discharged and replaced with new mash containing 1-2% acetic acid and 12-15% ethanol. The discharge and refilling operations are carried out automatically. The metabolic process involves conversion of ethanol to acetaldehyde in the presence of the enzyme alcohol dehydrogenase, followed by the conversion of hydrated acetaldehyde to acetic acid by acetaldehyde dehydrogenase:

$$2CH_3CH_2OH + O_2 \longrightarrow 2CH_3CHO + 2H_2O$$

$$2CH_3CHO + O_2 \longrightarrow 2CH_3COOH$$

Natural vinegar also contains smaller amounts of tartaric acid and citric acid etc. The finished vinegar is stored and kept for aging in well filled wooden barrels followed by filtration with the aid of diatomaceous earth. For preservation, vinegar is usually pasteurized or sterile filtered. Such preservation will also eliminate vinegar eels (nematodes). The selection of the proper organism is important for vinegar production. The organism must not be slime former. It should be ethanol and acidity tolerant. Most importantly, its oxygen requiring tendency should not be so high that over-exposure to oxygen leads to oxidation of acetic acid, as shown in the reaction:

$$CH_3COOH + 2O_2 \longrightarrow 2CO_2 + 2H_2O$$

Uses of Vinegar

Because of its acidic properties vinegar has variety of uses:

- Acidic nature inhibits growth of bacteria so vinegar acts as a food preservative.
- It acts as mild disinfectant in cleaning.
- It is a popular flavouring agent in cooking and salad dressings.
- It is potent, inexpensive and environment friendly cleaning agent e.g. in removal of mineral deposits left when hard water evaporates.
- Vinegar is a folk medicine used in China to prevent the spread of viruses such as SARS (Severe Acute Respiratory Syndrome) and other pneumonia outbreaks.
- Pure acetic acid can be isolated by the distillation of vinegar.

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