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BIOETHANOL PRODUCTION USING LIGNOCELLULOSIC BIOMASS

Besides, the efforts to reduce global reliance on fossil fuels and lower greenhouse gas emissions, an increasing search for renewable biomass resources, which can be used as feedstock for the production of microbiofuels, is becoming the major area of interest. The critical study of biomass feedstocks of first, second, third, and fourth-generation biofuels consider many scenarios of technologies and their associated social, technical, and environmental concerns. The lignocellulosic feedstocks are abundant, cost-effective, and renewable, yet the production cost of second-generation bioethanol is high because of the pretreatment of the complex structure of lignocellulosic raw materials. Biofuels have been studied for many years and are regarded as reliable and cost-effective energy sources. Due to the increasing consumption of biofuel over the world in recent past years, biofuel research and development has significantly moved towards efficient and nonconventional biomass feedstocks. In the view of increasing global biofuel consumption trend, major research attention has been given towards feasible and low‐cost resources of biofuel as reported by the research publications in the last 20 years around the world, especially from Asia, Europe, and the USA. Depending upon feedstocks used for biofuels, they can be classified into four major categories including 1G, 2G, 3G, and 4G generation fuels. The majority of exploration work has been carried out from the last decade on 1G and 2G biofuels. However, due to the major drawbacks of above mentioned two, the focus of research has been shifted towards the 3G and 4G biofuels. Classification of Biofuels Fuels derived from plant biomass can be categorized into primary and secondary biofuels.These fuels has number of advantages and disadvantages (Table 1). Biofuels produced directly from biomass are known as primary biofuels whereas fuels produced from the biomass that is subjected to some kind of transformation are known as secondary biofuels. Secondary biofuels can further be categorized into four different generations namely first, second, third, and fourth-generation biofuels based upon the kind of feedstock used. 1G, 2G, 3G, and 4G biofuels produced from edible food crops, lignocellulosic waste, microalgal biomass, and genetically engineered microorganisms respectively. First-generation biofuels are primarily formed from the edible part of the food and oil crops which have been accomplished a cost-effective level of

commercial production, However, the first-generation biofuels from edibles, including sugar cane, grains, and vegetable oils have been facing the challenge with food-fiber production for human usage which can harm environmental sustainability and profitability. The limitation of first-generation biofuels has caused greater concern and they are required to be replaced by second-generation biofuels. The second-generation biofuels are normally formed from non-edible lignocellulosic biomass, including food crops or whole plant biomass, organic parts of municipal solid wastes, by-products (sugarcane bagasse, cereal straw, forest residues), and feedstock that would include short-rotation forests, vegetative grasses, and further energy crops. Third-generation biofuel, also known as algal fuels or microbiofuels, obtained from microalgae are suitable substitute energy resources that answer the significant disadvantages related to 1G and 2G biofuels. Therefore, microalgal biofuels are the cutting edge biofuels, which are today's intriguing issue in the renewable energy industry and are foreseen to be the substitutes of fossil fuels. Nowadays, algae have been recognized as an unconventional biofuel feedstock due to their higher photosynthetic rate and rapid development as contrasted with any plant. It can be grown on wasteland by using wastewater as a culture medium and therefore, microalgae can sustain the level of water sources. Algae can operate three kinds of mechanisms for their biomass production in a cultivation system i.e. autotrophic, heterotrophic, and mixotrophic thus producing proteins, lipids, and carbohydrates in massive quantities from biomass . Further, these cellular components can be converted into valuable co-products and biofuel leading to the concept of biorefinery. It can yield more biofuel per acre than conventional crops. Furthermore, algal biomass can also be used for the production of biodiesel and bioethanol.

Many algal species including Botryococcusbraunni, Chaetoceros calcitrans, Chlorella spp., Isochrysis galbana, Nanochloropsis sp., Schizochytrium limacinum, and Scenedesmus spp. have been examined as potential sources of biofuel. Fourth-generation biofuels are obtained from genetically engineered microalgae and thus also known as algal fuels or microbiofuels and are considered sustainable and promising substitutes to fossil fuels.

Bioethanol The use of biomass as a feedstock source has great potential for the production of bioethanol. Microalgae are grown mostly anywhere without soil, and also have an extremely short harvesting cycle of approximately 1-10 days, compared with other crop feedstocks which can be harvested only a handful of times per year. An additional benefit is that microalgae require carbon dioxide for growth, creating the potential to absorb some of the greenhouse gas emissions created through the process. Bioethanol production processes can be categorized into the following types: 1. Separate hydrolysis and fermentation (SHF), is a process involving the hydrolysis of the polysaccharides occurs separately from the fermentation. 2. Simultaneous saccharification and fermentation (SSF), is a process involving the hydrolysis of the polysaccharides and fermentation of released sugars occurs together. 3. Consolidated bioprocessing (CBP), is a process involving enzyme production, hydrolysis of polysaccharides, and fermentation of all sugars occurs in one step.

Alcohol production was the first fermentation known to mankind and today it is the largest volume industrial fermentation. Distilleries began to appear in Europe in the middle of the seventeenth century. There is semantic confusion with regard to the term ethanol. Very often the term is used as a synonym for alcoholic beverages. This is misleading, even though ethanol may be used as a raw material for the production of spirits. Ethanol is a clear, colourless, flammable oxygenated hydrocarbon, with the chemical formula C2H5OH. Even though the definition is fairly

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straightforward, there are various categories for describing a particular type of ethyl alcohol which can be classified by feedstock from which it is made, by composition and by end use RAW MATERIALS FOR BIOETHANOL PRODUCTION Feedstock suitable for use in ethanol production via fermentation must contain sugars, starches, or cellulose that may readily be convertible to fermentable sugars. The usual sources of raw material for alcohol production include i) sugar-producing plants such as sugar beets, sugarcane, fruits, and others, ii) starch□containing cereal grains such as corn, wheat, rye, barley, milo (sorghum grains), rice, potatoes of all kinds, Jerusalem artichokes, other high-starch vegetables and iii) cellulose-containing agricultural residues, wood and waste sulphite liquor. Companies presently producing ethanol in the world vary by size, type of feed stock and technology. The substrates that have been classified into various categories and the characteristics of various feedstock are mentioned below:-

Ethanol	Substrate
Directly Fermentable	Sugarcane juice, Fruit juices, Sugar beet juice, Molasses, Sucrose, Sweet sorghum, Honey etc.
Easily hydrolysable substrates	Starchy materials-grains, tubers
Difficult to hydrolyse substrates	Lignoellulosics
Microbial Biomass	Algal biomass

Sugary substrates: These can be directly fermented without any pre-treatment, simply after squeezing from the crops or after dilution with water as in case of molasses. Sugarcane: Sugar cane juice and the important co-products of sugar production, molasses are commonly used for alcohol production in various parts of the world. Molasses is thick brown fluid containing sugary residue which is left behind after primary sugar recovery. Of world's total sugar production of over 110 million tones, more than 65 million tones comes from cane. Cane sugar is produced in South and Central America, Africa, Asia and Oceania. Brazil has an area of 37,000 square kilometer under sugar cane plantation for ethanol production. Total annual sugar cane production in India is about 270 million tones. The juice from the crushed cane is weighed, adjusted for pH and clarified before evaporation and crystallization, then centrifuged to separate the molasses from the sugar crystals. Cane juice contains 15-16% dissolved solids of which 85% is sucrose. The juice is partially evaporated before fermentation is carried out. Molasses: One of the cheapest sources of carbohydrates. In addition to sugar, molasses contains nitrogenous substances, vitamins and trace elements. Composition is variable depending upon the raw material used for sugar production. Quality of molasses varies depending upon the location, climatic conditions and production process of the sugar factory. In addition to conventional molasses, a residue from starch saccharification which accumulates after the crystallization of glucose is also used as a fermentation substrate, known as "Hydrol" molasses . The term 'molasses' is used for the viscous, sugary co-product of sugar refining, deriving from sugar cane, sugar beet and similar products. In refining cane juice for sugar production, 42 kg molasses (50% on sugar basis) are obtained from each tone of cane or 2.2 tones/hectare. Typical molasses compositions are given in Table 4. In addition to its use as a principal raw material for the production of alcohol, as used in various countries including India, molasses is also used in citric acid fermentation. Molasses has the advantage over the cane juice that it can be stored for longer periods, under optimal conditions for long enough to carry over one season to the next. After fermentation, the high ash FA of molasses, which contains a substantial proportion of potassium, is of high value as fertilizer. Molasses is the predominant raw material for alcohol production in the

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Asian region with the exception of China and Saudi Arabia, which produce alcohol from grains and synthetic route, respectively. Japan which produces very small quantities of molasses is the largest importer of alcohol ~450 million liters per year. It is apparent that molasses supply becomes a very potent force with the sugar industry which is likely to play it to its advantage. In Philippines, this factor was dealt with head-on by a distillery which faced shortage of molasses supply. They have set up a cassava/cane juice/molasses triple mode distillery to handle the vagaries of molasses supply and price. In India also, this trend has begun. Currently dual route plant operating on molasses as well as grain as a feedstock, are being set up.

The use of molasses is advantageous as it is a cheap source of fermentable sugars and is a byproduct; its prices are not politically controlled. The sugars in molasses can be easily utilized by most of the microorganisms and it also contains a number of other important nutrients such as vitamins, some elements such as N, P, Ca, Mg, K, Si, Al, Fe. However, there is a lack of consistency and batch-to-batch variation is there in the molasses composition. Some batches contain too much metal that pretreatment may be needed for some type of fermentation that are sensitive to specific metals. The quality of molasses is deteriorating, especially in India, due to decreased sugar content and increased solid content due to technological advancements in sugar industry aimed at recovering the last possible sugar crystal. The cost of molasses has also risen many a fold during the last decade due to Indian Government's decontrol policy. Some of the factors influencing the molasses composition and properties include soil composition in which the cane or beet is grown, harvesting Practices, process of sugar manufacture, postproduction treatment to molasses and the storage conditions of molasses. Sugar beet: Sugar Beet is widely used in European countries for the production of sugar and alcohol. It is grown extensively in UK and Europe, however, tropical varieties are being developed for high yield of production in India and other tropical countries. Annual world wide production of sugar beet is 300 million tones. Sugar content of sugar beet varies from 14-19%. The sugar containing juice extracted from the crushed beet is crystallized resulting in the formation of molasses. Beet molasses has similar use as that of cane molasses and generally serving as a feed stock for alcohol fermentation, the yield of which is 90-100 L/tone. The short harvest season limits the use of sugar beet juice and its molasses for alcohol production. Two hundred and fifty kilograms of wet pulp is obtained from each tone of sugar beet. Sugar beet pulp or bagasse is a cellulosic fibrous residue. It is not as yet, commercially processed for alcohol, but it is expected that the hydrolysis and fermentation of pulp can produce 40 L alcohol/tonne of beet. Several attempts have also been made to ferment the beet molasses by some alternative microorganism in place of the conventional distiller's yeast, Saccharomyces cerevisiae. The conversion of sugars present in sugar beet particles to ethanol by bacterium Zymomonas mobilis has also been reported in both conventional submerged and solid state fermentation. Ethanol yield of 0.48 g/g sugar, volumetric productivity of 12 g/L/h and final ethanol concentration of 130 g/L have been reported to be achieved in a solid state fermentation. The bacterium produced fewer by-products during solid state fermentation than in conventional submerged fermentation. An ethanol yield of upto 95% of the theoretical value was obtained. Several studies have been carried out to recycle the spent wash which proved to be beneficial in beet molasses fermentation. The stillage from beet molasses is also recycled for alcohol production to lower the production costs by decreasing the energy requirements for waste water treatment. Some reports have indicated the detrimental effects of recyclings as there can be an in wort osmolarity leading to the inhibition of yeast growth. Moreover, the stillage recycling can lead to the accumulation of inhibitory substances which may be potentially toxic to microorganisms which

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are responsible for fermenting the sugars, although these inhibitory substances may act as a shield against the contaminants. The production of alcohol from sugar beet molasses has also been reported without heat or filter sterilization wherein n-butyl p-hydroxy benzoate has been used as an antimicrobial substance in the sugar beet molasses medium. The yeast cells, immobilized in calcium alginate, completely utilized molasses containing upto 25% of sugar with an ethanol yield of 10.58%, equal to 83% of the theoretical value. The addition of EDTA, potassium ferrocyanide and zeolite-X into sugar beet molasses medium improves ethanol production. The effect is more pronounced when the three substances are added to fermentation medium rather than to the growth medium. Sweet Sorghum: Sweet sorghum is a name given to varieties of a species of sorghum: Sorghum bicolor. This crop has been cultivated on a small scale in the past for production of table syrup, but other varieties can be grown for production of sugar. The most common types of sorghum species are those used for production of grain. There are two advantages of sweet sorghum over sugar cane: its great tolerance to a wide range of climatic and soil conditions, and its relatively high yield of ethanol per acre. In addition, the plant can be harvested in three ways: (1) the whole plant can be harvested and stored in its entirety; (2) it can be cut into short lengths (about 4 inches long) when juice extraction is carried out immediately; and (3) it can be harvested and chopped for ensilage. Since many varieties of sweet sorghum bear significant quantities of grain, the harvesting procedure will have to take this fact into account. The leaves and fibrous residue of sweet sorghum contain large quantities of protein, making the residue from the extraction of juice or from fermentation a valuable livestock feed and can also be used as boiler feed Jerusalem Artichokes: The Jerusalem artichoke has shown excellent potential as an alternative sugar crop. A member of the sunflower family, this crop is native to North America and well-adapted to northern climates. Like the sugar beet, the Jerusalem artichoke produces sugar in the top growth and stores it in the roots and tuber. It can grow in a variety of soils, and it is not demanding of soil fertility. The Jerusalem artichoke is a perennial; small tubers left in the field will produce the next season's crop, so no plowing or seeding is necessary. Although the Jerusalem artichoke traditionally has been grown for the tuber, an alternative to harvesting the tuber does exist. It has been noted that the majority of the sugar produced in the leaves does not enter the tuber until the plant has nearly reached the end of its productive life. Thus, it may be possible to harvest the Jerusalem artichoke when the sugar content in the stalk reaches a maximum, thereby avoiding harvesting the tuber. In this case, the harvesting equipment and procedures are essentially the same as for harvesting sweet sorghum or corn for ensilage. Fodder Beets: Another promising sugar crop which presently is being developed in New Zealand is the fodder beet. The fodder beet is a high yielding forage crop obtained by crossing two other beet species, sugar beets and mangolds. It is similar in most agronomic respects to sugar beets. The attraction of this crop lies in its higher yield.

of fermentable sugars per acre relative to sugar beets and its comparatively high resistance to loss of fermentable sugars during storage. Culture of fodder beets is also less demanding than sugar beets. Fruit Crops: Fruit crops including grapes, apricots, peaches, and pears are another type of feedstock in the sugar crop category. Typically, fruit crops such as grapes are used as the feedstock in wine production. These crops are not likely to be used as feedstocks for production of fuel-grade ethanol because of their high market value for direct human consumption. However, the coproducts of processing fruit crops are likely to be used as feedstocks because fermentation is an economical method for reducing the potential environmental impact of untreated wastes containing fermentable sugars. Starchy residues (Cereal grains): Annual world cereal production amounts to be more than

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two billion metric tons. Major cereal crops produced worldwide include wheat, rice, maize and barley. Other major cereal crops produced include sorghum, oats, millet and rye. Asia, America, and Europe produce more than 80 percent of the world's cereal grains. Wheat, rice, sorghum, and millet are produced in large quantities in Asia; corn and sorghum are principal crops in America, and barley, oats and rye are major crops in the former USSR and Europe. Apart from human and animal consumption, a major part of these cereal grains are used for the industrial production of starch and its derivatives, or for alcohol. Maize: Corn was originally cultivated in Central America and became the staple of the Incas of Peru, the Mayas and Aztecs of Mexico and early cliff dwellers of the American Southwest. Columbus brought corn back to Europe where it became a popular crop in the south. Different types of maize are classified on the basis of their protein content and the hardness of the kernel. These include pop, flint, flour, Indian and sweet corns. Much of the niacin in corn is in a bound form and this led to pellagra in areas where corn became the food staple. It was not until the late 1920s that pellagra was identified as a vitamin deficiency. The traditional practice by early American natives, of boiling corn in 5 percent lime or ashes, releases bound niacin making it available as a nutrient. Maize is a major cereal crop in Canada and USA and a minor crop in UK. Hybrid corn varieties produce about 210 million tonnes of corn in North America every year which is used for human consumption, manufacture of starch, corn syrup (glucose) and high fructose corn syrup (HFCS). The annual production of maize in India is approximately 10 million tonnes. In a kernel of maize corn (Table 5), the various constituents are starch, (72%); protein, (10%); oil, (4%); and fibre, (14%). For fuel alcohol production, the separation into various components is unnecessary, and is not widely practicised. For most single product plants such as dry ground corn alcohol plants, the kernel corns are first steeped to swell and then ground, saccharified by amylases and the resulting sugar is fermented.

For alcohol production, whole maize corn kernels are steeped in warm water (50oC) for 24 h before grinding. The swollen ground corn is then liquefied by α-amylase at 95oC for 1 h at pH 6.5. The corn is then heated to 105oC by direct steam injection for 10 min followed by heating to 140 oC for 2 min for gelatinization, then cooling to 90oC and held at that temperature for 1 h. The treated corn is then cooled to 50-53oC, pH is lowered to 4.5-5.0 and 0.5ml/L glucoamylase is added for saccharification. The addition of glucoamylase sachharifies the starch in the kernels more or less completely within 1-2 h at 50-53oC. The slurry is then cooled and yeast is added. The alcohol yield from such a process is 450 liter per tonne of dry ground corn. The spent wash recycling in the fermentation of corn mashes increases the overall mashing and fermentation efficiencies and thus the alcohol yield.

Wheat: Wheat is one of the oldest of all cutivated plants. Today, there are more than 50000 cultivars of wheat in existence and as a result it can be grown in a relatively wide range of climatic conditions. Growing best in temperate climates, it is susceptible to disease in warm, humid regions and cannot be grown as far from the equator as can rye and oats. Wheat was brought to America early in the seventeenth century where it came to prominence in the Great Plains by 1855. Different types of wheat are classified based on planting season and endosperm composition. Wheat holds a special place amongst the cereals because upon mixing wheat flour with water, an elastic matrix called "gluten" required for the production of leavened breads is formed. "Hard wheats" tend to contain relatively high levels of starch and relatively low levels of protein, while the reverse is true for "soft wheats". High protein flours are best suited for pastas and breads, while flour from soft wheats is excellent for cakes and pastries, etc. Wheat is the second largest cereal crop of India mainly used as a

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major part human diet with annual productivity level of about 60 million tonnes. Wheat flour usually contains a starch content between 74-81%. About 5-10% of this is recovered as 'B' starch, and the remainder constitutes the 'A' starch or prime starch. The value of the prime starch is very high. For the production of alcohol the feed stock of choice is 'B' starch, which commands a lower price. For cereals, where the gluten does not have high value, dry milling, saccharification and fermentation of the whole grain is the preferable route to alcohol. For the bioalcohol production, wheat starch 'B' is slurried in water at 15% dry solids and pH adjusted to 7.0. Then α-amylase is added and liquefaction is carried out at 85oC for 30 min under constant stirring. After liquefaction the slurry is cooled to 55oC, the pH lowered to 5.0 and glucoamylase is added. After 40 h at this temperature, the sugar solution contains about 130 g/L of glucose. To this solution, nutrients and yeast are added and after 24 h fermentation, alcohol concentration rises to 7.3% (v/v), the yield being 500 L ethanol/tonne of starch 'B'. The wheat mashes contain growth limiting amounts of free amino nitrogen, so the fermentations by active dry yeast (Saccharomyces cerevisiae) is completed in 8 days at 20oC. Supplementing wheat mashes with yeast extract, casamino acids and glutamic acid stimulated the growth of the yeast and reduces the fermentation time. With 0.9% yeast extract as the supplement, the fermentation time is reduced from 8 days to 3 days and a final ethanol yield of 17.1% (v/v) is achieved. However, amino acids such as glycine reduces the cell yield and prolonged the fermentation time. Barley: In the United States, barley is mostly used as feed, for brewing and alcohol production with only about 2 percent used for human food. Barley flour is produced by abrasion dehulling, followed by milling of the "pearled" barley. In the UK, 2 million tones of barley is used each year in brewing and distilling, while another 6 million tones is used as animal fodder. In a dual enzyme process, whole barley grains are steeped in water for 18 h and ground. The ground barley is stirred in water at 20% solids, pH adjusted to 7.0, 0.1% α-amylase is added and the slurry heated to 85oC for 45 min. The liquefied solution is then cooled to 50oC, pH adjusted to 5.0 and 0.1% glucoamylase added. The solution is held at 50oC for 48 h, pH adjusted to 5.5 and then cooled to 30oC and fermentation. Fermentation sugars after saccharification are around 147 g/L and the ethanol produced is 70 g/L or 9% (v/v), yield being 450 L/tone of barley. When saccharification and fermentation are combined, the liquefied starch is treated with 0.1% glucoamylase and fermented using yeast at 37oC, the results on the alcohol yield are the same. When saccharification and fermentation are separate operations, 60 h are required, whereas when the two steps were combined, alcohol production gets completed in 12 h. Malt extract: an aqueous extract of malted barley is an excellent substrate for many fungi and actinomycetes. Dry malt extract contains 90-92% carbohydrates and is composed of hexoses (glucose, fructose), disaccharides (maltose, sucrose), trisaccharides (maltotriose) and dextrins (Table 6). Nitrogenous substances include proteins peptides, amino acids, purines, pyrimidines and vitamins. Amino acid composition varies according to grain used but praline always makes up about 50% of total amino acids. Media containing malt extract must be carefully processed. Overheating in presence of low pH and high proportion of reducing sugars, Maillard reaction occurs in which amino groups of amines, amino acids or proteins react with carbonyl groups of reducing sugars, aldehydes or ketones which lead to the formation of brown condensation products Rice: The second most abundant cereal crop originated in the Indian subcontinent and Africa. Today, 90 percent of the world rice crop is grown in Asia. Its annual productivity level in India is over 80 million tonnes. Alexander the Great is credited with introducing rice to Europe around 300 BC. Growing rice requires more water than other cereal crops, although rice is a highly productive crop. There are several thousand rice cultivars which may differ in color,

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aroma and grain size. The main commercial distinction between rice types is the grain size, i.e. long, medium and short. Long grain rice, also called "Indian", tends to separate relatively easily on cooking and is dry and flakey. Short grain rice, also called "Japanese" is sticky, moist and firm when cooked. Unlike wheat, rice is most often consumed as grain rather than as a flour. Different grades of milling include brown rice (hull removed), unpolished rice (hull, bran and most of germ removed), and polished rice (aleurone layer removed from unpolished rice). Since polishing removes most of the lipid, the latter product is relatively stable during storage. The discovery that rice bran can alleviate beriberi led to the discovery of the vitamin thiamine. The traditional technique of parboiling rice in India and Pakistan (also called "converted rice") prior to milling improves the nutritional quality of the grain by allowing the B vitamins in the bran and germ to diffuse into the endosperm. Rye and mixed grains: Rye is an impotant bread grain in Eastern Europe and Scandinavia. World production of rye is over 25 million tonnes. The main uses of rye is for bread and other foods, for animal feed, and/or rye whisky. The use of rye in not reported for industrial or fuel alcohol production. It can, however, be used as a component of mixed grains. While its starch content is lower than that of corn or barley, it is less expensive. Millet and Sorghum: Millet and sorghum are often grouped together because their growing conditions, processing and uses are similar. Millets are native to Africa or Asia and have been cultivated for more than 6 000 years. Millets grow well in regions with poor soils and are valued for their relatively high protein content among the cereals. Sorghum originated in East Africa and today is an important food crop in Africa, Asia, India and China where it is made into porridge, unleavened bread ("roti" in India) and beer. India is second largest producer of sorghum in the world with production of about 10–11 million tonne from a total area of 12 million ha. This crop is ideally suited for semi-arid agroclimatic regions of the country and, it gives reasonably good yield with minimal requirement of irrigation and fertilizers. On the other hand, cereals such as wheat and rice cannot withstand the harsh semi-arid climates. These crops also require fair amount of water and other inputs such as fertilizers and pesticides. Therefore, sorghum is one of the few cereals, which can be grown in semi-arid regions. However, demand for sorghum for human consumption is decreasing with enhanced socioeconomic status of population in general and easy availability of preferred cereals in sufficient quantities at affordable prices. Since sorghum must be cultivated in the semi-arid regions for fodder to feed the large cattle population of the country, industrial applications for this grain are needed so that sorghum cultivation becomes economically viable for farmers. Whole grain sorghum flour has a relatively short shelf-life. The production of low-fat sorghum flour requires removal of about 20 percent of the grain weight by abrasion . In North America, millet and sorghum are used primarily as livestock feed. Sorghum vulgare or S. bicolor is a member of grass family. It bears starchy seeds upon its stalks and has ability to withstand adverse conditions of draught and water logging. The annual production of sorghum is about 75 million tones, grown on 48 million hectare. The leading producers are USA (31%), India (16%), China (10%), Argentina (10%), Mexico (9%) and Nigeria (5%). Over 95% of the total food use of sorghum occurs in Africa and Asia, where it serves as a staple food and constitutes a major source of energy and protein. About 97% of the total use of sorghum in developed countries is for animal feed. Nevertheless, sorghum is also a substrate for producing industrial alcohol, distilled spirits, starch, dextrose, syrups and edible oil. Traditional alcohol and non-alcoholic beverages are brewed using sorghum in parts of Africa and Asia. The use of sorghum, as an adjunct or as a substitute for barley in beer brewing has also been reported by several workers. The stalks of sorghum contain 14% sugars. It is very fast growing and two crops per year are cultivated in the most favourable areas. Yields of 35 tonnes/hectare, in

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addition to about 3 tonnes of seedsare recorded. The sorghum crop of 1 hectare allows production of 2800 L ethanol. Sweet sorghum, similar to the normal sorghum varieties, is also used in various parts of the world for alcohol production. However, in addition to the grains, the sweet sorghum variety contains sweet juice in the stalk materials. This juice is very similar to cane juice in characteristics and has between 12 to 16 % (w/w) sugar (Sucrose). Sweet sorghum also contains a large amount of fibrous material in the stalks (Similar to sugarcane), which generates equivalent bagasse for energy generation. Direct conversion of sorghum carbohydrates to ethanol by a mixed microbial culture has been reported by several workers who produced ethanol from sweet sorghum in submerged as well as solid state fermentation. One of the study involved simultaneous saccharification and fermentation of sweet sorghum carbohydrates to ethanol by Fusarium oxysporum alone or in mixed culture with Saccharomyces cerevisiae or Zymomonas mobilis. The process achieved its maximum value by the mixed culture of the fungus and the yeast. Under optimum conditions, ethanol yields and concentrations as high as 29.7 g of ethanol per 100 g of dry sorghum stalk and 7.5% (w/v), respectively, were obtained. Another study involved the production of bioethanol from sweet sorghum carbohydrates which were simultaneously saccharified and fermented to ethanol by a mixed culture of Fusarium oxysporum and Saccharomyces cerevisiae. The optimum yield of bioconversion and ethanol concentration was 5.2-8.4 g ethanol/100 g of fresh sorghum and 3.5-4.9 % (w/v) respectively. In all experiments, the ethanol yield exceeded the theoretical level, based on soluble sugars, by 20.0-32.1% due to the bioconversion of polysaccharides to ethanol. Raw cassava: Cassava, perennial woody shrub is an annual crop. This is a low cost carbohydrate-producing crop, which finds applications in food as well as alcohol production. Brazil is the largest producer of cassava followed by, Thailand, Nigeria, Zaire and Indonesia. However, Thailand is a major exporter of cassava. The enlarged cassava roots contain maximum concentration of starch on dry weight basis among all food crops. The cassava is processed in different ways in different countries for food and for ethanol production. Cellulosic materials (Crop residuses): The "backbone" of sugar and starch crops, the stalks and leaves, is composed mainly of cellulose. The individual six-carbon sugar units in cellulose are linked together in extremely long chains by a stronger chemical bond than exists in starch. As with starch, cellulose must be broken down into sugar units before it can be used by yeast to make ethanol. However, the breaking of the cellulose bonds is much more complex and costly than the breaking of the starch bonds. Breaking the cellulose into individual sugar units is complicated by the presence of lignin, a complex compound surrounding cellulose, which is even more resistant than cellulose to enzymatic or acidic pretreatment. Because of the high cost of converting liquefied cellulose into fermentable sugars, agricultural residues (as well as other crops having a high percentage of cellulose) are not yet a practical feedstock source for small ethanol plants. Current research may result in feasible cellulosic conversion processes in the future. Bagasse: Bagasse is the fibrous residue of extraction of sugar from cane. About 270 Kg raw, wet bagasses or 130 Kg dry matter is produced from each tone of cane. Bagasse is a lignocellulosic residue with substantial carbohydrate content. It typically consists of cellulose, 35%; pentosans, 25%; lignin, 20%; other organics and ash. In most sugar mills, bagasse is used largely for its fuel value. India produces 50 million MT of bagasse a year. Whilst bagasse based paper production was considered to be a significant revenue-earner over two decades ago, the importance of bagasse as fuel has overridden this importance. In fact, co-generation of energy is the new wave which is being promoted actively in certain states to counter the deficit of electrical power as well as to improve financial health of the industry. Forage crops: Forage crops (forage sorghum, Sudan grass) hold promise for ethanol

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production because, in their early stage of growth, there is very little lignin and the conversion of the cellulose to sugars is more efficient. In addition, the proportion of carbohydrates in the form of cellulose is less than in the mature plant. Since forage crops achieve maximum growth in a relatively short period, they can be harvested as many as four times in one growing season. For this reason, forage crops cut as green chop may have the highest yield of dry material of any storage crop. In addition to cellulose, forage crops contain significant quantities of starch and fermentable sugars which can also be converted to ethanol. The residues from fermentation containing nonfermentable sugars, protein, and other components may be used for livestock feed. Straw and Chaff: The annual production of straw and chaff is enormous. In addiiton to 1.5-4.0 tonnes of straw/tonne of grain, the chaff adds 100-150 kg/tonne. Wheat straw contains 73% polysaccharides, with about equal quantities of cellulose and hemicellulose. Wheat chaff contains 68% polysaccharides of which 45% is cellulose. If half of all straw and chaff were hydrolysed and fermented, alcohol production would amount to 480,000 million L/year equivalent to 3000 million barrels of oil, about 15% of all crude oil production. Hemicellulose is made up of the 5 carbon sugar xylose arranged in chains with other minor 5 carbon sugars interspersed as side chains. Just as with cellulose, the hemicellulose can be extracted from the plant material and treated to release xylose which, in turn, can be fermented to produce ethanolm (Figure 4). Xylose fermentation is not straight forward and depending upon the microorganism and conditions, a number of fermentations are possible. The array of products can include ethanol, carbon dioxide, and water. The bioconversion of straw to fuel alcohol has been the subject of great deal of research in the past several years. There is a large pilot plant at Soustons in South Western France owned by “Institue Francais de Petrole, IFP” which is capable of processing 2-4 tones/h of straw to ethanol. After a steam pretreatment the fibres are enzymatically saccharified and the reducing sugars fermented. The fact that straws and chaffs being bulky materials, difficult to collect, transport and process. The use of straw and chaff as animal feed and farm yard manure has effectively hindered the development of industrial conversion of these to ethanol, though several reports have appeared indicating the production of alcohol from these, at laboratory scale. One study employed the fungus Trichoderma viride and Pachysolen tannophilus for the single batch bioconversion of wheat straw to ethanol. Another study reported production of ethanol from barley straw in a solid state fermentation using Kluyveromyces marxianus. After fermentation, the ethanol concentration increased to a maximum of 20 g ethanol/100 g of straw.

According to a recent report issued by the Energy Information Administration (EIA), USA, “Outlook for Biomass Ethanol Production and Demand,” the advances in cellulose-ethanol technology over the next twenty years can reduce the cost of producing cellulose-ethanol MICROORGANISMS FOR ALCOHOL PRODUCTION Efficient ethanol production depends upon the choice of microbes, which can adapt to the production procedures employed, and the composition of the feedstock. The microorganism selected should have i) large scale easy cultivation on cheap and simple substrates, ii) high fermentation rate, iii) high yield of product per unit substrate assimilated, iv) substantial tolerance for alcohol, v) stability under adverse environment conditions, including low and high pH, temperature etc, vi) inability to produce undesirable products Although ethanolic fermentation is common to a wide variety of microorganisms (Figure 2), with a few exceptions. Only yeasts have traditionally been used for industrial scale production of alcohol or alcoholic beverages. The near-exclusive application of yeasts is due, in part, to their homofermentative mode (EMP pathway) which yields mostly a single product, ethanol, often in relatively high concentrations. Saccharomyces cerevisiae and S. uvarum (S. carlbergensis), for example, produce 12% ethanol, whereas in a slow

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fermentation S. sake produce up to 20% ethanol. Under anaerobic conditions, these yeasts normally produce small amounts of byproducts such as glycerol, methanol and fusel oil, which require that specific measures be taken for their separation during distillation of alcohol. One of the advantages of using yeasts has been their ability to ferment a wide spectrum of substrates. For example, when whey is used as substrate for ethanol production, the yeasts Torula cremoris, Kluyveromyces fragilis, K. lactis and Candida pseudotropicalis may be employed. Substrates containing low molecular weight oligomers of various sugars, dextrins, can also be fermented directly after partial hydrolysis. K. marxianus and K. fragilis can ferment inulin, a polyfructan directly, whereas S. diastaticus can produce ethanol from a partial digest of liquefied starch (maltodextrins) and Torulopsis wickerhamii has been shown to give good yields of ethanol from depolymerized cellulose (cellodextrins) while Pichia stipitis has been shown to be useful for the production of ethanol from sugars derived from the hydrolysis of lignocellulosic biomass.

One disadvantage of ethanol-producing yeasts has been their inability to tolerate high temperatures as temperature above 40oC usually inhibits growth and fermentation. This means that during large-scale fermentation a cooling system is an absolute requirement. It has been reported that certain thermotolerant yeasts may overcome this handicap. The strains of Pichia stipitis have been reported to carry out fermentation well at 41oC, which results in reducing the cooling costs. Some bacteria are capable of producing ethanol under both aerobic and anaerobic conditions as efficiently as yeasts, and therefore have attracted attention for their possible application as an alternative organism for alcohol production. However, in contrast to yeast, bacteria characteristically generate multiple end products in addition to ethanol. These include other alcohols (butanol, isopropanol), glycols (2-3-butanediol), organic acids (acetic, propionic, butyric, formic, lactic), polyols (arabitol, glycerol, xylitol), ketones (acetone), and gases (methane, CO_2, H_2). Of a number of bacteria capable of producing good yields of ethanol, only a few may be considered for a alcohol production at industrial scale. Within the mesophilic group only the Gram-negative facultative anaerobic bacterium Zymomonas mobilis can be regarded as an efficient ethanol producer. It offers advantageous that makes it a potential organism for industrial scale production of alcohol. This bacterium is used in tropical regions of America, Africa and Asia in the fermentation of plant juices and production of native alcoholic beverages such as pulque, palm wine and cactus wine and there are reports of its adaptation for fuel alcohol manufacturers in the United State because of its high fermentation efficiency. In contrast to the yeasts, which use the classical glycolysis (EMP) pathway, Z. mobilis uses the less common Enter-Doudoroff (ED) pathway for anaerobic production of ethanol from glucose. In this pathway glucose is phosphorylated and then oxidized or dehydrogenated to 6-phosphogluconate which loses H_2O to form 2-keto-3-deoxy-6-phosphogluconate (KDPG). This is then cleaved by KDPG aldolase. The net yield is 2 mol of pyruvate from 1 mol of glucose and the generation of 1 mol of ATP. Zymomonas mobilis achieves 92.5- 97.5% of theoretical yield, which is better than most yeast fermentation yields, typically 88-90%. However, fermentation with Z. mobilis also generates acetylmethyl carbinol, acetic acid and glycerol. The biology, taxonomy, physiology and genetics of this bacterium have been very well reviewed. Comparision of ethanol production by bacteria and yeasts Bacteria Zymomonas mobilis and Thermoanaerobacter ethanolicus, have been shown to offer some advantages over traditional yeast for ethanol fermentation and are finding attention as alternative organisms to yeast for commercial scale production of ethanol. Zymomonas mobilis grows rapidly under anaerobic conditions and, unlike yeasts, does not require the presence of oxygen to maintain viability at high cell concentration. In contrast, yeast fermentation requires addition of a controlled

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amount of oxygen for cell wall, lipid synthesis and general maintenance of cellular health . However, oxygen also leads to an increase in biomass concentration (aerobic growth) and a decrease in ethanol production due to the Pasteur effect. With Z. mobilis, under strictly anaerobic conditions, higher ethanol and lower biomass production are obtained. Lower cell production is also a consequence of the reduced energy available for growth (1 mol of ATP/mol of glucose consumed via the ED pathway versus 2 mol of ATP/mole of glucose via the EMP pathway with yeasts). Also Z. mobilis takes advantage of uncoupled growth metabolism in which glucose fermentation is continuous in the absence of cell growth. The fermentation rate of Z. mobilis is higher than that of yeasts. The key fermentation kinetic parameters indicating the superiority of this bacterium include higher specific growth rate, μ (2.4 times high than yeast), high specific ethanol producitivity Qp, (g/g/1) 2.9 times greater and high specific glucose uptake rate Qs, (g/g/h) 2.6 times greater than for S. uvarum. Zymomonas mobilis is also reasonably ethanol (over 7%, v/v ethanol), glucose and thermotolerant. Another advantage of Z. mobilis is that it being a prokaryote, genetic manipulation can be carried out with greater ease than in yeasts. Z. mobilis was discovered in 1936 and despite its several favourable attributes and high production effectiveness, it has not yet worked its way into an industrial alcohol process for several reasons. Zymomonas mobilis being a temperamental, it requires some skill to work with; unless fermentation is closely controlled acetaldehyde tends to accumulate in the beer. Z. mobilis converts ethanol to acetate, in the presence of even traces of oxygen, through the Krebs respiration cycle, thus resulting in reduction in ethanol yield. Moreover, beer containing acetate can cause technical separation problems during distillation. Z. mobilis grows optimally at pH values above 5.0, which is more than the pH of most yeast fermentations. Higher pH increases the chances of contamination by other organisms, especially at the beginning of the fermentation when the ethanol concentration is low. Z. mobilis ferments a very limited range of substrates: glucose and fructose and only a few strains decompose sucrose. It cannot be utilized to ferment some classical substrates of the alcohol industry such as lactose, maltose, mannose, galactose or starch, or lignocellulosics. The thermophilic bacteria, Themonoanerobacter ethanolicus and similar thermophiles have several advantages over yeast for ethanol production. These grow at high temperatures, exhibit rapid metabolic activity and a high fermentation efficiency with high product output. The ethanol yield by T. ethanolicus approaches that of yeasts. Fermentation at a high temperature would favour continuous operation and at the same time assist in ethanol recovery directly from the fermenter. Use of thermophiles minimize the chances of contamination and need for cooling. Viscosity of growth medium decreases at high temperature, thereby the energy requirement to maintain agitation is reduced. Reduced solubility of oxygen and other gases at high temperature assists maintenance of anaerobic conditions. However, the thermophilic bacteria have the following disadvantages. Majority of thermophilic bacteria follow the EMP pathway, as do yeasts, but they also synthesize large amounts of acetic and lactic acids, thereby lowering yield of ethanol. The energy relation is 2.5-3.0 mol ATP/mol of glucose. High yields of organic acids and ATP diminish the amount of substrate available for the formation of ethanol. e.g. T. ethanolicus produces 1.8 mol of ethanol, 0.1 mol of acetic acid and 0.1 mol of lactic acid per mol of glucose. Clostridium thermohydrosulfuricum produces only 0.5-0.6 mol of ethanol and 0.5 mol of each acid. For Cl. thermocellum and Thermoanaerobium Brockii, the rates of ethanol production are below 1 mol alcohol/mol glucose. Technical problems are experienced in the separation of the acids from the alcohol. Thermophilic bacteria require specific substrates and growth conditions, for instance Cl. thermohydrosulfuricum produces large amounts of ethanol at a starting pH of 9.5 which decreases

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during the course of fermentation to 6.9 or even less. Also, the medium needs a significant amount of yeast extract as a growth factor for ethanol production. Thermophilic bacteria have low ethanol tolerance. Yeasts are known to tolerate 6-12% ethanol without a significant decrease in the growth rate, but, in contrast the thermophilic bacteria are able to tolerate only up to a maximum of 3-7% ethanol. Strains of Cl. thermocellum and Cl. thermnohydrosulfuricum are reported to be inhibited by only 1% ethanol in the fermentation medium. Bacterial strains are susceptiblie to viral infection (bacteriophages), while the yeasts are not amenable to viral contamination. It has become evident that the bacteria do not at present meet the criteria for industrial alcoholic fermentation as an alterative to yeasts. In order to use them as alternative to yeast for industrial alcohol production an improvement through genetic transformation is necessary. It is most unlikely that yeast will be replaced by other microbes for industrial scale production of ethanol in the near future, however, Z. mobilis may be adopted in some specific situations, especially where glucose is present as main sugar. INDUSTRIAL PROCESSES FOR ETHANOL PRODUCTION The present industrial fuel ethanol production is based on the use of yeast species of the genera Saccharomyces. Under anaerobic conditions, each gram of glucose can theoretically give 0.51 g ethanol. In practise, however, the ethanol yield does not exceed 90-95 percent and on industrial scale 86-90 percent of the theoretical value. Some parts of the nutrients are required for biomass synthesis and cell maintenance reactions. About 4-5 percent of the total substrate usually is utilised for glycerol and succinate formation. The medium for ethanol production requires small quantities of O2, P, S, K, Mg, Mn, Co, Cu, Zn as well as organic growth factors such as amino acids, nucleic acids and vitamins which are generally present in the feedstock used in the fermentations. Yeasts are very susceptible to ethanol inhibition. For example, 1-2 percent [w/v] ethanol decreases cell growth and 10 percent [w/v] stops ethanol synthesis. During the fermentation process, fusel oils are formed from a -keto acids, derived from deaminations of amino acids. The major fusel oil component is isoamyl alcohol [40-60 percent] with optically active amylalcohol and iso-butylalcohol taking 15-20 percent each of the total. In general, the yield of fusel oil is 20 L m-3 of ethanol produced. Fusel oils can remain in the final product as a component of fuel ethanol. Medium Preparation from sugar cane When sugarcane is used as a raw material, the raw cane is crushed and the juice extracted. After clarification by precipitating the inorganic fraction with milk of lime and H2SO4, the resulting cane juice is a green, sticky liquid more viscous than water and contains an average of 15% dissolved solids of which 85% (13% of the juice) is sucrose and less than 1% of the invert sugars glucose and fructose. The sugarcane juice forms the major raw material for the Brazilian ethanol industry and is directly used as a medium for fermentation after adjusting the pH to 4.5-5.0 and supplementing some nitrogenous compound in the form of urea, diammonium phosphate or ammonium sulphate at a concentration of 0.05% (w/v). The fermentation is highly productive, producing 75 liters of alcohol per tone of cane or 4800 liters/ hectare of land. This is six times the amount of alcohol obtainable from molasses based on the same land area. Several ethanol tolerant yeast strains have been isolated which are able to produce and tolerate above 20% (v/v) alcohol during the fermentation of sugar cane syrup. Because of low solid and salt contents the cane juice offers many advantages: i) higher efficiency of fermentation (up to 90%) , ii) savings in sugar due to yeast recycling, iii) reduction in water consumption by stillage recycling, iv) massive reduction in the spent wash generation to the tune of 3 liters per liter of ethanol produced, without using any additional energy, v) distillation efficiency of 98.5 % is achieved. In sucrose producing industries, the sugarcane juice is concentrated to 80-82 percent sucrose content to be able to crystallise raw sugar. The residual juice is referred to as molasses. Depending on the

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sugarmill efficiency, a maximum of three crystallisation steps can be carried out leading to an A-molasses [70-71 percent sucrose], B molasses [60-65 percent sucrose] and/or C- or blackstrap molasses with a total carbohydrate [sucrose, glucose, fructose] concentration of 40-55 percent [w/v]. Molasses may be stored for long periods of time and diluted before fermentation, as either the high sugar content [A- and B-molasses] or the salt concentration [C-molasses] act as preservative. Molasses with 60% fermentable sugars or 1.32 tones/ha, upon fermentation, yields 11.5 liters of alcohol/tone of cane or 730 L/hectare. Before fermentation, molasses may be clarified, refined and diluted with water to a solid concentration of about 20-25%. This may then be treated with H2SO4 (to precipitate calcium salts) and ferrocyanide (to remove excess levels of metals). After adjusting the pH to 4.5-5.0, some nitrogenous compound in the form of urea, diammonium phosphate or ammonium sulphate at a concentration of 0.05% (w/v). A similar technology can be applied for the medium preparation from sugar beet roots and sweet sorghum. Medium preparation from cereal grains In the case of grains such as corn [maize], wheat, rice, sorghum and barley as raw materials, the starch must first be enzymatically hydrolysed before fermentation can proceed. Amylase preparations are applied for liquefaction and saccharification of starchy substrates. The enzyme α-amylase [α -1,4-glucan 4-glucanohydrolase, EC 3.2.1.1] is an endoenzyme, which causes random hydrolysis of α -[1,4] linkages in the starch molecule resulting in the liquefaction of the medium thereby reducing the viscosity of starch. The β-amylase [α -1,4-glucan maltohydrolase, EC 3.2.1.2] is an exo-enzyme generating maltose and dextrins and is only available in plants. Finally, the enzyme glucoamylase [amyloglucosidase, α -1,4-glucane glucohydrolase, EC 3.2.1.3] is an exo enzyme being able to hydrolyse α -[1,4] as well as α-[1,6] linkages and is able to produce glucose from the starch molecule. Two different grain processing technologies exist, dry- and wet-milling. In wet-milling, the major objective is to divide and convert the corn into a number of products, such as starch flour, fibre, gluten, germ, oil, meal residue, dextrose [glucose], fructose and other products. In this case, ethanol is produced from the lower grades of starch or from all of the starch. The dry-milling technology is much simpler as the whole grain is ground by hammer mill and slurred. The liquid starch slurry containing the liquefying enzyme a -amylase is cooked and thus liquefied. Saccharification using the glucoamylase can either be performed prior of or simultaneously during fermentation. Milling: All grains must be ground before mashing to expose the starch granules and help them remain in suspension in a water solution. The grain should be ground into a meal, but not a flour, that will pass a 20-mesh screen. Potatoes and similar high-moisture starch crops should be sliced or finely chopped. Since potato starch granules are large and easily ruptured, it isn't necessary to maintain the hard rapid boil which is required of the tougher, dryer "flinty" starches found in grains. Cooking: Grain must be cooked to rupture the starch granules and to make the starch accessible to the hydrolysis agent. This process which increases the viscosity of the mash and converts it into a gel is also called gelatinization. Cooking time and temperature are related in an inverse ratio: high temperatures shorten cooking time. Industry practice is to heat the meal-water mixture by injecting steam directly rather than by heat transfer through the wall of the vessel. The latter procedure runs the risk of causing the meal to stick to the wall; the subsequent scorching or burning would necessitate a shutdown to clean the surface. The meal is added with water in the cooker slowly, to prevent lumps from forming. When, cooking with steam, or at higher temperatures, it is possible to save energy by using less water at the beginning. But for the "small batcher" with an ordinary cooking apparatus, the most complete conversion is obtained by using the full amount of water right from the start to encourage a rapid rolling boil. The high temperature bacterial α-amylase

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is added next to the mixture and the temperature of the mash is raised to 170oF (77oC), the optimum working environment for the enzyme. The solution is then held at that temperature for 15 minutes while agitating it vigorously. At this point all the starch available at 170oF gets converted to dextrins. The temperature of the mash is then raised to the boiling point for cooking at which it is then held for 30 minutes to complete the liquefaction stage during which all the starches come in solution. Now the temperature is reduced back to 170oF, using the cooling coil, and more bacterial α-amylase is added. After 30 minutes of agitation at this temperature, all the previously released starches gets reduced to dextrins, thereby completing primary conversion. During secondary conversion the dextrins are further reduced to simple sugars (maltose and glucose) by the β-amylase or glucoamylase and the yeast necessary to carry out secondary conversion and proper fermentation simultaneously as soon as the temperature is brought down to 85oF (29oC) using the cooling coils. Instead of using commercial enzymes, it is possible to affect conversion by employing barley malt, at the ratio of 15% by weight, in both the pre- and post-cooking. However, such a technique requires a more acidic medium (about pH 4-5) and lower temperatures, about 145oF (63oC). Starch can also be hydrolysed by acids which is accomplished by directly contacting starch with dilute acid to break the polymer bonds. This process hydrolyzes the starch very rapidly at cooking temperatures and reduces the time needed for cooking. Since the resulting pH is lower than desired for fermentation, it may be increased after fermentation is complete by neutralizing some of the acid with either powdered limestone or ammonium hydroxide. It also may be desirable to add a small amount of glucoamylase enzyme after pH correction in order to convert the remaining dextrins Cooking can be accomplished with continuous or batch processes. Batch cooking can be done in the fermenter itself or in a separate vessel. When cooking is done in the fermenter, less pumping is needed and the fermenter is automatically sterilized before fermenting each batch. There is one less vessel, but the fermenters are slightly larger than those used when cooking is done in a separate vessel. It is necessary to have cooling coils and an agitator in each fermenter. If cooking is done in a separate vessel, there are advantages to selecting a continuous cooker. The continuous cooker is smaller than the fermenter, and continuous cooking and hydrolysis lend themselves very well to automatic, unattended operation. Energy consumption is less because it is easier to use counterflow heat exchangers to heat the water for mixing the meal while cooling the cooked meal. The load on the boiler with a continuous cooker is constant. Constant boiler load can be achieved with a batch cooker by having a separate vessel for preheating the water, but this increases the cost when using enzymes. Continuous cooking offers a high-speed, high-yield choice that does not require constant attention. Cooking at atmospheric pressure with a temperature a little over 200 deg F (93 deg C) yields a good conversion ratio of starch to sugar, and no high-pressure piping or pumps are required.

The hydrolyzed mash contains solids and dissolved proteins as well as sugar. There are some advantages to separating the solids before fermenting the mash, and such a step is necessary for continuous fermentation. Batch fermentation requires separation of the solids if the yeast is to be recycled. If the solids are separated at this point, the beer column will require cleaning much less frequently, thus increasing the feasibility of a packed beer column rather than plates. The sugars that cling to the solids are removed with the solids. If not recovered, the sugar contained on the solids would represent a loss of 20% of the ethanol. Washing the solids with the mash water is a way of recovering most of the sugar. Medium preparation from lignocellulosics Lignocellulosic materials are pretreated by milling and then chemically [by acid hydrolysis] or enzymatically hydrolysed before fermentation. The dilute sulfuric acid method for wood hydrolyses is basically a semicontinuous

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process with the following parameters: acid concentration 0.53 percent, temperature of percolation 196°C, percolation time 145-190 min, percolation rate 8.69-14.44 L min-1 m-3 and a total water-oven□dried wood ratio of 10. At the end of the percolation cycle the lignin-rich residues are discharged, recovered and used as fuel. Furfural is recovered by distillation. The hot acid hydrolysate is neutralised with a lime slurry and the precipitated calcium sulfate is separated. The neutralised hydrolysate is blended with yeast and fermented. Genetically modified producers are necessary to obtain ethanol from both hexoses and pentoses Fermentation Yeast propagates in a solution containing free fermentable sugar feedstocks including sucrose from sugarcane, sugar beet and molasses as well as hydrolysed starch or lignocellulosics , with or without air. If the medium is continually agitated, the yeast will reproduce faster and make less carbon dioxide and alcohol. But if the solution becomes anaerobic (without air) the yeast slows down reproduction and makes more alcohol and carbon dioxide. so the wort is agitated only enough to saturate the wort with air and then let it stand still. Yeast also produces enzymes of its own to convert complex sugars. Since sugar conversion and alcohol conversion can take place simultaneously in case of starchy and cellulosic mashes, the enzymes and the yeast work in cooperation to convert the dextrins to glucose and fructose and then to alcohol and CO2. Fermentation is a biochemical process and produces heat. In concentrated or particularly large mashes, the temperature can actually rise to levels dangerous to yeast. Since the ideal temperature for yeast is around 85oF, it's best to maintain that temperature by either utilizing cooling coils. Conversion of sugars to alcohol and CO2 gets completed in three to five days, depending on the temperature of the mixture and the type of yeast used. During fermentation, the rising CO2 keeps the solids in constant motion, but when the bubbling stops, the solids fall to the bottom. At the end of the fermentation the solids get separated from the liquids and settle at the bottom. The final ethanol concentration is between 10-16 percent [w/v] with a carbohydrate conversion efficiency of 90-95 percent and a system productivity of 1.8-2.5 kg ethanol m-3 h -1 . Product yields during fermentation Ethanol: The yield of ethanol from agricultural crops can be estimated if the amount of fermentable components, sugar, starch, and cellulose, is known prior to fermentation. If the yield is predicted based on percentages at the time of harvest, then the loss of fermentable solids during storage must be taken into account. This factor can be significant in the case of sugar crops, as discussed earlier. The potential yield of ethanol is roughly one-half pound of ethanol for each pound of sugar. However, not all of the carbohydrate is made available to the yeasts as fermentable sugars, nor do the yeasts convert all of the fermentable sugars to ethanol. Thus, for estimating purposes, the yield of ethanol is roughly one gallon foreach 15 pounds of sugar or starch in the crop at the time the material is actually fermented. Because of the many variables in the conversion of liquefied cellulose to fermentable sugar, it is difficult to estimate active ethanol yields from cellulose. Carbon Dioxide: The fermentation of six-carbon sugars by yeast results in the formation of carbon dioxide as well as ethanol. For every gram of ethanol produced, 0.957 grams of carbon dioxide is formed; stated another way, for every 1 liter of ethanol produced, 0.758 kg of carbon dioxide are formed. This ratio is fixed; it is derived from the chemical equation: $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ + Heat Glucose Ethanol Other Co products: The conversion and fermentation of agricultural crops yield products in addition to ethanol and carbon dioxide. For example, even if pure glucose is fermented, some yeast will be grown, and they would represent a coproduct. These coproducts have considerable economic value, but, since they are excellent cultures for microbial contaminants, they may represent a pollutant if dumped onto the land. Therefore, it becomes doubly important that these coproducts be put to good use. Alcohol recovery: Alcohol product recovery is

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energy intensive, typically accounting for more than 50% of the total fermentative ethanol plant energy consumption. When heat from burning of raw material residues (such as bagasse) is not available, this constitutes a significant operating cost. Depending on recovery system design, recovery equipment cost generally makes up 6-12% of the plant total capital investment. Industrial alcohol is produced in various grades. The majority is 165 proof (95 vol% or 92.4 wt%, minimum) alcohol used for solvent, pharmaceutical, cosmetic and chemical applications. Technical grade alcohol (containing up to 5% volatile organic aldehyde, esters and sometimes methanol) is used for industrial solvents and some chemical syntheses. A high-purity 175 proof anhydrous alcohol product (99.85 wt%) is produced for specialized chemical applications. For fuel use in mixtures with gasoline (gasohol), a nearly anhydrous (99.2 wt%) alcohol, but with higher allowable levels of organic impurities, is used. Today, distillation is used almost exclusively as the means for ethanol recovery and purification and various designs are used to produce the different product grades. Ethanol distillation technology was highly refined during the 1940s to reduce energy consumption to approximately 2.5 kg of steam per liter of anhydrous ethanol produced. Recent further refinements in distillation technique make possible marginal improvements with increased capital investment. Alternatives to distillation are under study to further reduce costs. Factors influencing ethanol production Various process variables and conditions that influence the growth and efficiency of industrial ethanol production by yeasts are discussed below: pH: Hydrogen ion concentration has a significant influece on industrial fermentation due to its importance in controlling bacterial contamination and has effect on yeast growth, fermentation rates, and by product formation. In an uncontrolled batch fermentation of a highly buffered medium, the best ethanol yields are generally obtained at pH 4.5-4.7. At higher pH more glycerol and organic acids are formed at the expense of ethanol. In lightly buffered media, the optimum starting value is pH 5.5. At the completion of fermentation pH falls to about 3.5. If low pH is used initially in a lightly buffered medium, the final pH tends to fall enough to slow the fermentation rate. This difficulty can be overcome to a certain extent by the use of larger inocula. Yeasts survive in a pH range of pH 2.0-8.6. Choice of organism: The ability to ferment rapidly and efficiently the sugars present in the fermentation medium containing high concentrations and sometimes unusual sugars, ethanol tolerance and ability to remain stable and viable under prevailing fermentation conditions determines the yeast to be used for industrial scale ethanol production. Presently, large-scale ethanol producing industry use Saccharomyces strains. The yeasts, however, have limitatios in fermenting lignocellulosic biomass, due to the presence of cellobiose, xylose, arabinose and other wood sugars. Moreover, the yeasts are not able to ferment inulin form Jerusalem artichoke, an attractive renewable resource. However, a xylose-fermenting yeast like P. stipitis or an inulin-fermenting yeast such as K. marxianus can be used to ferment such feedstocks. Sugars fermented by yeasts: Yeasts can ferment a wide variety of oligosaccharides and sugars in addition to glucose. Different yeasts have different capabilities in this regard due to the presence or absence of enzymes which are capable of converting these different sugars to substaces which appear in the metabolic pathways of alcoholic fermentation. With very few exceptions, L sugars, including the common L-arabinose, are not fermented by yeasts, Some yeasts are known to ferment other pentoses but not methyl pentoses although the latter may be utilized in the respiration of aerobic yeast. The substrates for alcoholic fermentation are, thus, primarily hexoses, xylose and oligosaccharides. D-hexoses and oligosaccharides fermented most often by yeasts are glucose, mannose, fructose, galactose, maltose, sucrose, lactose, melibiose, trehalose, and raffinose. The last, a trisaccharide, is partially fermented by some yeasts, but is assimilated

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completely by many more species during aerobic growth. This behaviour toward raffinose differentiates the bottom and top fermenting brewers' yeasts S. cerevisiae and S. uvarum. Certain generalizations can be made regarding the fermentation characteristics of yeast on the basis of following rules or guidelines: • Yeast unable to ferment D-glucose will not ferment other sugars. • If D-glucose is fermented, so is D- mannose. • If maltose is fermented, lactose is not, and vice versa (with a few exceptions, such as Brettanomyces clausenii, an ale yeast that ferments both sugars). • If sucrose is fermented, so is raffionse. • All sugars fermented, are also utilized aerobically. • No L sugars are fermented. S. cerevisiae and P. sipitis The advantage of using yeast strains P. stipitis is that in addition to sugars utilized by S. cerevisiae, this yeast can also ferment xylose and cellobiose. These sugars are normally present in lignocellulosic. Also the ability to ferment cellobiose and perhaps cellodextrins offers advantages in using partially hydrolysed lignocellulosic substrates. Sugar concentration: The concentraion of sugar which can be fermented most efficiently depends to some extent on other components of the medium. Most industrial processes utilize a fermentation solution containing 12-20% sugars by weight. The advantages of a high concentration feed solution include reduced water requirement, suppression of osmosensitive contaminants and reduced distillation cost if ethanol inhibition can be limited. Temperature: The rate of alcohoic fermentation increases with temperature and is optimum between 30 and 40oC. Both optimum and maximum temperature tolerance for growth and fermentation are strongly strain□dependent. However, for most ordinary yeasts, if the ethanol concentration reaches 8-9% by volume prior to completion of fermentation, the fermentation may stop if temperatures are held above 33oC. This effect has been attributed to intracellular ethanol being produced more rapidly than it can be transported through the cell membrane, causing inhibition of intracellular fermentation enzymes. Thus, in the course of fermentation, the temperature should not exceed 32oC, especially once the ethanol begins to accumulate. The temperature optimum for the growth of yeast is about 25oC. This temperature can be increased slightly by use of a rich nutrient medium. Since yeast growth and fermentation both produce heat, cooling may be necessary to maintain the desired temperature. Ethanol: A limitation of ethanolic fermentation is the capacity of the microbe to tolerate this solvent, because ethanol inhibits alcoholic fermentation, which limits the concentration of ethanol which can be produced by a given strain of yeast. The maximum concentration of ethanol which can be produced by yeast varies with species and a maximum upto 20% ethanol by volume can be produced. The results of mating experiments with yeasts indicate that ethanol inhibition is a polygenic phenomenon, that it is a result of multiple enzyme and cell physiological functions. The degree of inhibition is also related to other environmental factors, in particular high sugar concetration and high temperature which cause the inhibition to be more severe. Brewers' yeast (S. cerevisiae var. carlsbergensis) ceases to ferment at about 6% ethanol by volume, whereas bakers' yeast (S. cerevisiae var. diastaticus) stops fermentation at about 12% ethanol by volume, and wine yeast (S. cerevisiae var. ellipsoideus) at about 15% ethanol by volume. Ethanol which is produced during fermentation (autogenous ethanol) is more inhibitory to cell growth than that from an exogenous source. Addition of ethanol to actively fermenting yeast cultures results in rapid reduction of fermentation and growth rates due to its influence on protein synthesis and irreversible denaturation of enzymes. Ethanol has also been shown to exhibit non-competitive inhibition of yeast growth, that is, independent of other inhibitors which may be present. The toxic effect of ethanol has also been attributed to damaging the cell membrane or changing its properties. The extent of ethanol tolerance of certain yeasts is highly strain dependent and appears to be related to the unsaturated fatty acids and the fatty acyl

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composition of the plasma membrane. The intolerance exhibited by some yeasts appears to be related to low cellular lipid content and the inability of the plasma membrane to maintain, during high rates of ethanol production, a high ethanol flux through the plasma membrane and out of the cell to avoid intracellular accumulation. Nutrients: Most yeasts grow well on a variety of amino acids, purines, and pyrimidines as the sole source of nitrogen. Mixtures of amino acids are better that any single amino acid. About 15 mg of assimilable nitrogen per 100 ml of fermentation solution is sufficient to assure good fermentation rates. The nitrogen can also be supplied in the form of ammonium salts, urea, corn steep liquor or distillers' malt. The distillers' malt have the advantage of containing vitamins and minerals necessary for maximum yeast growth. Yeasts require trace amounts of biotin, thiamine, pyridoxine, calcium pantothenate, and inositol for maximum growth and fermentation rates. These vitamins also regulate yeast metabolism, vitamins being generally either coenzymes or precursors for fully active enzymes. Vitamin requirements are strain dependent. Candida utilis and Hansenula anomala are vitamin independent and synthesize all their own needs, whereas biotin and pantothenate are essential for all strains of Saccharomyces. Phosphorus in the form of phosphate is an important ionic factor determining the rate of fermentation. It is required at a concentration of about 0.16 mM/g cells for optimum fermentation performance. Yeast growth also depends on compounds containing potassuim (K), sulphur (S), and traces of zinc (Zn), iron (Fe) and copper (Cu), required in concentrations of 0.1-1 mM. For anaerobic growth of yeasts, ergosterol and unsaturated fatty acids are beneficial additive while some thermophilic yeast strains have requirements for choline or leucine. Yeast inoculum: Provided that temperature, pH, sugar concentration, assimilable nitrogen, vitamins and minerals are at their optimal values, a controlling factor is the amount of yeast added in the inoculum, or the "pitch" as it is often called in industrial operations. Using a larger inoculum of yeast will decrease the time required for growth, and alcoholic fermentation will set in sooner. For commerical production of alcohol, yeast is usually added to provide a starting population of 7-10 million cells/ml, about 0.2g of dry yeast/l of broth. At this inoculation level, fermentation is usually completed within a day or two, depending upon sugar concentration. Much higher inocula, up to 10 or more g cells/l, result in much more rapid fermentation, and is also beneficial in resisting inhibition and suppressing contamination. Dissolved oxygen: Ethanol fermentation is not a purely anaerobic process. Dissolved oxygen concentration is an important variable for industrial alcohol production. In many instances, active starter cultures are grown under aerobic or semiaerobic conditions to improve yeast yields and growth rates. Yeasts are unable to multiply for more than four to five generations in solutions containing less than 1 ppm oxygen, even if sufficient nutrients, such as sugar, nitrogen, and vitamins, are present. It is the dissolved oxygen in the solution which limits the population of yeast in the fermentation medium. Once the dissolved oxygen is depleted during fermentation, the yeast growth slows and eventually ceases. Also during the time the metabolism of yeast switches from aerobic respiration to anaerobic alcoholic fermentation, and the synthesis of alcohol occurs until the sugar is depleted or until a limiting ethanol concentration is obtained. Oxygen is utilized by yeasts for at least two separate functions: • as the ultimate electron acceptor in oxidative phosphorylation, that is for growth and respiration; and • as an essential nutrilite in lipid, ergosterol and nicotinic acid biosyntheis. During respiration yeasts have a high demand for dissolved oxygen which promotes complete oxidation of glucose to carbon dioxide and water via the tricarboxylic acid cycle at the expense of ethanol accumulation via fermentation (Pasteur effect). However, the extent of this respiration is tightly controlled by the concentration of fermentable carbohydrates in the medium. In the presence of high

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sugar concentrations, ethanol is formed during aerobic growth and the phenomenon is known as 'aerobic fermentation'. This happens due to the operation of the so-called reverse Pasteur, or Crabtree effect which results in the repression of both the synthesis and the activity of the respiratory enzymes, notably some Krebs cycle enzymes under fully erobic conditions. The Pasteur and Crabtree effect are illustrated in Figure 7 Conditions 3 and 4 are carried out exclusively for alcohol production, whereas, condition 2 is the mode for fodder yeast production, and condition 1 is practised during production of bakers' yeast. The Crabtree effect is also applied in brewery operation, where due to high initial sugar concentration (condition 1) a substantial degree of aeration of the wort prior to pitching, up to about 20% oxygen saturation, is practised to get maximum yeast yield, high viability, growth rate, and overall fermentation rate. The effect of dissolved oxygen is critical in continuous yeast growth or single cell protein production, where it is usually the limiting factor. It is also important in continuous fermentation, in order to maintain yeast health as well as a satisfactory fermentation rate. The exact level of the minimal oxygen required is strain-dependent and varies with environment. At oxygen concentrations below 1 ppm in the broth,

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