Assessment of different pretreatment technologies for efficient bioconversion of lignocellulose to ethanol

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1. ABSTRACT

The future supply of energy to meet growing energy demand of rapidly expanding populations is based on wide energy resources, particularly the renewable ones. Among all resources, lignocellulosic biomasses such as agriculture, forest, and agro-industrial residues are the most abundant and easily available bioresource for biorefineries to provide fuels, chemicals, and materials. However, pretreatment of biomass is required to overcome the physical and chemical barriers that exist in the lignin–carbohydrate composite and pretreatment facilitate the entry of biocatalysts for the conversion of biomass into...
fermentable sugars and other by-products. Therefore, pretreatment of the biomass is necessary prerequisite for efficient hydrolysis of lignocelluloses into different type of fermentable sugars. The physiochemical, biochemical and biological pretreatment methods are considered as most promising technologies for the biomass hydrolysis and are discussed in this review article. We also discussed the recent advancements and modern trends in pretreatment methods of lignocelluloses conversion into ethanol with special focus on fermentation methods.

2. INTRODUCTION

World reserved energy resources are declining at the alarming rate because of the rising requirement of energy by growing population. The requirement of fossil oil in India is rising at an impressive rate of 7.5% per annum. Near about 75% of the total fossil fuel is utilize in India and it is imported from other countries by spending heavy amount (1–10). The problem of declining energy sources, energy crisis and over accumulation of green house gases in our environment, encouraged the search for the another substitute for fossil fuel (11). Energy is an important factor for the economic improvement and urbanization, and it plays a key role in the improvement of daily life. India is a growing economy and presently passing through rapid industrialization and there is a high demand of energy to meet the pace of growth. At present, India is occupying the seventh position in terms of energy production and standing at fifth position as energy consumer in the world (12). Therefore, the upcoming requirement of energy will depend on substitute of energy sources, like water, solar cells, and biomass. Therefore, the production of biofuel chemicals will increasingly depends upon plant biomass (13). Many countries have setup bio energy policies to support and regulate the production and use of fuels from biomass feedstocks. Many countries in the world have setup different green energy policies for increasing the production and use of bio ethanol from lignocellulosic plant substrate (Examples Brazil, China, US, India and Europe). The objectives of these policies are to decrease the dependency on fossil fuels, mainly in oil importing countries. The use of green fuel also facilitates the reduction of greenhouse gases from the environment because released carbon captured by growing plants (14).

In many places of the world, bioethanol is accepted as a positive source for rural and agricultural development, but this is more effective in those countries where agriculture receives high governmental subsidies (15), because of that conditions the terrestrial plants are good option for energy production. The high production rate of terrestrial plants is a favorable substitute for green fuel production. The main goal behind using lignocellulosic biomass for biofuel is to reduce the competition between the food and feed industries moreover, these cellulosic materials also contribute to environmental sustainability. The cellulosic biomass is supplied from different sources in a low cost manner, so it is economically feasible. In different countries the availability of biomass vary and on this basis they are using different biomass for green fuel production for example corn stover, sugarcane bagasse, rice and wheat straw is mostly used in U.S.A., Asia, Europe and South America (16). Several countries have setup policies for the motivation of green fuel production and utilization in large scale in the future. On the other hand biodiesel producing countries includes Ghana, Zimbabwe, Mozambique and South Africa. Among these countries Mozambique and South Africa are considered to be more advanced regulatory and institutional frameworks for biofuel development in Africa (17).

3. COMPOSITION OF BIOMASS AND BIOMASS CONVERSION PROCESS

Lignocellulose is a sustainable, abundant and uneatable plant material, which may be agricultural and forest residues. Plant biomass is comprises of cellulose, hemicelluloses and lignin but their percentage of sugars and phenolic compounds is vary from plant to plant. Lignocelluloses biomass is recalcitrance or resistant against the microbial attack due to the presence of lignin. Cellulose (CH\textsubscript{1.67}O\textsubscript{0.83}) is a homopolymer, linear compound that contain amorphous and crystalline region. Cellulose is made up of repeated D-glucose subunits which are attached by β-1,4 glycosidic bonds. The arrangement of different bonds makes the carbohydrate structure hard and inflexible to attack. Hemicelluloses is a highly branched and heteropolymer structure and composed of pentose (β-D-xylose and α-L-arabinose), sugar acids (α-D-galacturonic, α-D-4-O-methylgalaturonic acids and α-D-glucuronic) and hexose sugars (α-D-galactose, β-D-mannose and β-D-glucose) with little amounts of different types of sugars like α-α-L-fucose and L-rhamnose. The composition of hemicelluloses is depending on different biomass type. Lignin (C\textsubscript{10}H\textsubscript{n}O\textsubscript{5.6}) is highly irregular and amorphous aromatic polymer which is formed by different units of phenylpropane units of syringyl (S), guaiacyl (G) and p-hydroxyphenyl (H). Lignin is attached by covalent bonds with hemicelluloses and provides a protection against the microbial enzyme action. This results in low conversion of the raw lignocellulosic biomass into fuel ethanol (18). Generally, cellulose is a polymer of hexose sugars so it is a starting material for biofuel production. The carbohydrate polymer in plants is synthesized by the process of photosynthesis, in the process of biofuel production the hexose sugars is converted into bioethanol through the fermentation process. The main advantage of biofuel production lies in zero emission of carbon dioxide. As the ethanol
is combusted in any vehicle CO\(_2\) is released as a byproduct into the aerospace, thus, this event has been recognized as cycle for renewable energy which ease reduction of pollution and emission of greenhouse gases (GHG). Additionally, ongoing research stated that the total energy content produced by cellulosic ethanol is three fold greater than that is produced by conventional ethanol by sugar or starch containing crops. However, cellulosic ethanol releases comparatively low amount of greenhouse gases. Therefore, conventional ethanol produced from cereals utilizes fossil fuels for production of heat for various processes and for fermentation, which leads to production of various green house gases. Contradictory lignin is considered as a renewable fuel which does not contribute for production of greenhouse gases therefore, the cellulosic ethanol production will utilize lignin as part of biomass feedstock for production of heat (19). Here in this review article we discussed about the process of biofuel production with particular emphasis on pretreatment methods and their advantages and disadvantages.

4. THE WHOLE PROCESS OF BIOETHANOL PRODUCTION

The bioconversion of lignocellulosic biomass into different types of reducing sugars which are fermented into different fuels like butanol and ethanol is a five step process. These five steps involves pretreatment, hydrolysis, fermentation of sugars, distillation and purification (7, 9). The whole process of biomass conversion is summarized in Figure 1.

4.1. Pretreatment methods

The self-assembly architecture of plant cell wall, with crystalline cellulotic microfibrils intertwining and interacting with lignin and hemicelluloses, forms LCCs or lignin carbohydrate complexes, these are unavailable for cellulases to bind onto the surfaces of cellulotic molecules. Hence, after the reduction in preliminary size from 10 mm to 30 mm by utilizing mechanical methods like chopping, pre-treatment is required to open LCCs, which make structure competent for enzymatic breakdown. The rationale behind to use different pretreatment method is to break the bonds between the lignin and hemicelluloses, increase the porosity and convert crystalline cellulose into amorphous cellulose. Though, pre-treatment method must fulfil these requirements; (i) it should increase the production of pentose and hexose sugars, (ii) prevent loss of sugary compounds (iii) less production of inhibitory compounds and (iv) process should be economically feasible and environmental friendly. The pretreatment processes are to be classified into four different groups (i) chemical pretreatment method, (ii) physical pretreatment method, (iii) biological disintegration method, and (iv) solvent fractionation. The basic features of ideal pretreatment method are that it should maximize yield of sugar derived from...
4.2. Hydrolysis

Hydrolysis process takes place after pretreatment to break down the feedstock's into fermentable sugar for bioethanol production. The two most commonly used hydrolysis methods are acidic and enzymatic. The breakdown of lignocellulosic compound can be done by chemically (e.g. by dilute sulphuric acid) and enzymatically (by lignocellulose degrading enzymes). But here we are focusing on enzymatic process. Enzymatic hydrolysis requires enzymes to hydrolyze the feedstock’s into fermentable sugars. Three types of enzymes that are commonly used for cellulose breakdown such as cellobiohydrolases, β-glucosidases and endo-β—1,4-glucanases. The activity of cellulase enzyme is influenced by the concentration and source of the enzyme. Cellulose will be degraded into reducing sugars under mild conditions (temperature about 45 to 50 °C and pH about 4.8 to 5.0). Moreover, it does not cause corrosion problem in the reactors which can result in high sugar yields. The efficiency of enzymatic hydrolysis is influenced by optimized conditions such as pH, time, temperature, concentration of substrate and loading of enzyme (21). Therefore, the amount of fermentable sugar increases subsequently as the enzyme load increase, and load of cellulose decreases. Enzymatic saccharification of cellulose can be enhanced by using surfactants which function to block lignin. The efficiency of cellulose hydrolysis can be improved by adding Tween 20 and PEG or Polyethylene glycol to increase enzymatic saccharification and therefore reducing the adsorption of cellulase on lignin (22). The limitation of using enzymes in hydrolysis is because they are too costly for economic production of ethanol from biomass. The complete enzymatic hydrolysis is done by the synergistic action of different enzymes which are; exo–glucanases, β– glucosidase and endo- glucanases combinely they are known as cellulolytic enzymes or cellulase. These enzymes works in synergism to combat the crystalline conformation of cellulose, removes celllobiose from terminal or chain free ends and hydrolyse celllobiose for producing glucose. Cellulose degrading enzymes are generally released by fungi e.g. T. reesei, besides Aspergillus, Schizophyllum and Penicillium. Therefore, the hydrolysis of the enzymes is typically carried out under mild conditions e.g. at 40 to 50 °C temperature and 4.5 to 5 pH (23). Enzymatic hydrolysis is the preferred saccharification method because of its higher yields, milder operating condition, higher selectivity and lower energy cost as compared to chemical processes (24).

5. FERMENTATION

Pretreatment is a most important step of bioethanol production, due to this step lignocellulosic biomass is converted to simple sugars which is further fermented into bioethanol and then distillation or purification. The carbohydrate polymer is break down into simple sugary units pentose and hexose sugars, the hexose sugar is easily fermented by microbes in to bioethanol but fermentation of pentose sugars is done by only a few microorganism strains. Therefore, enough research has been conducted in recombinant organism for production of strains that are able to ferment both glucose and xylose into different important chemicals. The method by which hemicelluloses and cellulose breakdown in its sugary units and these sugary units is fermented into bioethanol by the microorganisms at the same time is called simultaneous saccharification and fermentation (SSF). Therefore, the SSF is an ideal way for making the biofuel and chemicals because this is economically feasible and both reactions such as hydrolysis and fermentation preformed in same reactor (25). This method of bioethanol formation includes conversion of fermented sugars into bioethanol followed by distillation process. Meanwhile, fermented CO2 is emitted as a by-product. Therefore, there are different types of feedstock’s that are mainly used for fermentation process and some by-products are released from sugar industries like molasses. While some feedstock’s are fermented directly while other feedstocks needs to be fermented or processed into fermented sugars. Production of ethanol directly from fermentable feedstock’s includes molasses, produced directly from the sugar industry, consist of 45–50% TRS or total reducing sugars and it is considered as a major feedstock for production of bioethanol in India. Therefore, on the basis of the efficiency of recovery of sugar from sugarcane in different sugar mills, different grades of molasses is produced as ‘A’ grade molasses which contains more than 50% total reducing sugars, the ‘B’ grade molasses contains about 45–50% total reducing sugars and ‘C’ grade molasses contains 40–45% total reducing sugars (TRS). Molasses are commonly used in India for production of bioethanol because of its higher efficiency, easy fermentation and cheap cost. Therefore, the ‘B’ grade molasses is commonly utilized for production of bioethanol in India. The ‘B’ grade molasses contains about 45–50% TRS (26). Fermentation of bioethanol can be carried out in batch, fed-batch, repeated batch or continuous mode. In batch process, substrate is provided at the beginning of the process without addition or removal of the medium.

5.1. Solid-state fermentation

Solid state fermentation or (SSF) is a type of fermentation in which the microbes will grow in solid media but moisture is required at optimum level
to support the growth of bacteria and fungi in the absence of free-flowing water. Therefore, the lower moisture content signifies that the fermentation can be carried out by a restricted number of the microbes, majorly, by fungi and yeasts, in spite of that some of the bacteria's can be used. Among these microbes, fungi are considered to be the most adapted towards SSF because they can develop and penetrate their hyphae on and into inner surfaces particle and thereby colonizes the solid substrates. Therefore, the nature of the solid substrate is considered as one of the most essential factors which affects SSF processes and its selection rely upon many factors mainly related with availability and cost. The SSF processes have been appeared particularly suitable route for the production of enzymes by filamentous fungi so they offer natural habitats on which fungus grows better (27). An alternative to traditional submerged fermentation (SmF), SSF have advantages of improved yields, cost competitive, easier products recovery, and lack of foam formation. Furthermore, due to low water contents, contamination risks were significantly eliminated and subsequently the volume of residual wastes also decreases (28). Therefore, the major drawback of this type of cultivation concerns the scale up of the process, largely due to culture homogeneity problems and heat transfer (29). Many studies have been conducted towards the development of bioreactors for SSF systems. Therefore, to overcome this obstacle many studies have been performed by utilizing the SSF systems for the fabrication of various compounds of interest, constituting organic acids, enzymes, and flavours etc.

5.2. Submerged fermentation (SmF)

Submerged fermentation is a type of fermentation in which number of microbes is used for the process of fermentation. In the SmF, the composition of fermentation media has a liquid medium, source of sugar and nutrients. Submerged fermentation is an attractive system for bioethanol production due to different characters which are; i) the fermentation media is uniform in distribution, ii) all conditions are favourable for microbes growth, iii) in this system we can easily modify the growth conditions just like- temperature, oxygen, pH, uniform distribution of media and composition of media, and iv) we can also maintain the temperature by thermal conductivity. Therefore, submerged cultivation includes numerous microbial strains like bacteria, algae fungi and yeast. Media which are used for process it may be synthetic or we can also use lignocellulose residue after hydrolysis. The sugars which are released from lignocellulosic structure can be fermented by use of different microbes into different products of industrial importance by submerged fermentation systems, including ethanol, organic acids, glycerol, butanol, and food additives, etc. Therefore, the conversion of these hydrolysates needs considerable attention because higher yield and productivity can be achieved. Generally the process of hydrolysis is not only employed for extraction of sugars from different structures of lignocellulose, but also for extraction of wide range of compounds proceeding from the lignin or they are originated from degradation of sugars. Therefore, their concentration solely relies on type of raw material and hydrolysis process used. These compounds are generally toxic for the microbes and therefore, lignocelluloses hydrolysate need to undergo detoxification process prior to use the fermentation media which is used for the cultivation and for fermentation of the microbes. Therefore, different kinds of detoxification processes are generally employed like physical, chemical and biological detoxification process to covert inhibitors into inactive compounds in order to reduce their concentration. The effectiveness of detoxification process rely upon the kind of microbial species used and the hemicelluloses hydrolysate employed because each type of hydrolysate displays different level of toxicity and different species of microbes show different degree of tolerance towards inhibitors. There are numerous microbes that have been identified to be employed in fermentation process by submerged cultivation, which constitutes group of algae, fungi, bacteria and yeast. Hence, the fermentation media which has been used in this type of system can be produced by hydrolysis of lignocelluloses or can be formulated artificially.

5.3. Separate hydrolysis and fermentation (SHF)

Separate Hydrolysis and Fermentation (SHF) is a process in which saccharification or enzymatic hydrolysis of polysaccharides and fermentation by use of microbes that are carried out sequentially in separate units. The major advantage of using this process is that the fermentation and hydrolysis both are carried out efficiently at their own optimum pH and temperature. Therefore, about 45-50°C for enzymatic hydrolysis with the help of β-glucosidase and cellulose, and about 30-37°C temperature is required for fermentation of ethanol by use of microorganism (30). However, there is some drawback with SHF process which includes; i) inhibition of glucose and cellobiose on activity of cellulase hence, when the concentration of cellobiose is lowered by 8g/l then the cellulase activity is also lowered by 60%. Therefore, glucose also reduces the activity of cellulases, but the inhibitory effect of cellobiose is more than that of glucose. Contradictorily, glucose is considered as a potent inhibitor of β-glucosidase. Therefore, the activity of glucose is lowered by 75% when the concentration glucose is about 3 g/l; ii) there is always high chances of contamination even when separate vessels are used for fermentation and hydrolysis this is because
the hydrolysis process take place for long duration of time and the released sugars such as cellobiose and glucose provides the chances of contamination with microbes. The preparation of enzymes can be the possible source of contamination hence; it is tedious to sterilize all the enzymes, since all the enzymes must be sterilized with filter because when these enzymes are autoclaved the enzymes are denatured (31). Additionally, we cannot use antibiotics to overcome contamination because adding antibiotics may affect the growth and fermentation of the microbes.

5.4. Simultaneous saccharification and fermentation (SSF)

In this process, hydrolysis and fermentation carried out simultaneously i.e. simultaneous saccharification and fermentation (SSF), which means released sugars is directly used by microbes. This process is more favorable due to the lower capital cost and free from the risk of contamination. The reactor design is simple, convenient because two processes are performed in the same reactor. Which result in increase equipment and operation cost, but the different conditions for two stages including the temperature cycle, pH & other conditions will make the two reactions difficult to operate at the same time. SSF has major drawback that the fermentation and enzymatic hydrolysis need to be performed under specialized conditions, specially with respect to optimum temperature and pH because these conditions always vary. Therefore, hydrolysis is generally rate limiting process in SSF and the optimal temperature of enzyme reaction is much more higher than that of fermentation. Therefore, several thermotolerant species of yeast and bacteria for examples, Kluyveromyces marxianus and Candida acidothermophilum have been employed for simultaneous saccharification and fermentation process in order to elevate the temperature near to the optimum which is used for various enzymatic reactions (32, 33). The major disadvantage of using SSF process is the inhibition of ethanol accumulation over microbes and enzymes. When the concentration of ethanol is about 30g/l then the activity of enzymes is reduced about 25%. Therefore, due to excess accumulation of cellobiose and other sugar monomers in the medium during the enzymatic saccharification, the hydrolysis enzymes are directed for feedback inhibition, in turn reduces the enzyme efficiency. Therefore, the major drawbacks of SSF process are different optimum temperature for enzymes that are used for hydrolysis of the biomass like 45–50°C temperature and about 30°C temperature is required for fermentation of microbes. In order to develop economically feasible SSF process there is need to contrive thermotolerant fermenting yeast and hydrolytic enzymes that are adaptive towards cold temperature. Practically it is very challenging to lower the optimal temperature of cellulases with the use of protein engineering. Hence, to discover any thermotolerant yeast with high ethanol production efficiency can be a great discovery for SSF process. Therefore, the use of thermotolerant yeast in SSF process for bioethanol production offers following advantages; it often improves the efficiency of saccharification by mitigating the feedback inhibition of cellulose, lowering the chances of contamination by reducing concentration of glucose and production of ethanol, reducing total number of steps, that is lowering equipment cost and capital investment, lowering cost of cooling as there is no chiller unit is required, can be used in tropical areas, continuous evaporation of ethanol from broth under reduced pressure. Öhgren et al. (28) reported that by utilizing SSF process 13% more ethanol is produced as compared to SHF process because this process rely upon use of thermotolerant yeast and cold adaptive hydrolytic enzymes. This process is carried out at 40°C at an ambient temperature in a single vessel.

5.5. Simultaneous saccharification and co-fermentation (SSCF)

Simultaneous saccharification and co-fermentation (SSCF) is the process in which the saccharification is carried out simultaneously with the co-fermentation of sugars like pentose and hexose sugars. Hence, the microbes are not able to utilize complete media because are they incapable to ferment pentose sugars. Therefore in SSCF process microbes are able to ferment both pentose and hexose. This process can be operated with fed batch fermentation and at high content of water insoluble solids which simplifies mixing and higher ethanol yield. This process also helps to maintain low concentration of glucose, thereby allows efficient co-fermentation of xylose and glucose (35).

In this fermentation the pretreated substrate is hydrolyzed by enzymes/microbes in to the oligomers after in same reactor after the fermentation, sugar (pentose and hexose) is converted to ethanol. For the fermentation of both pentose and hexose sugar required a both type of microbial strain which can efficiently convert the both sugars in to bioethanol. Saccharomyces cerevisiae used for hexose sugar fermentation, but Pichia stipitis utilize both pentose and hexose sugar. This fermentation is a good option because in the limited time period bioconversion is found economically feasible and also has a high production rate. However, the major requirement is of efficient microbes that have ability to ferment broad range of substrates like hexose and pentose sugars as well as have ability to withstand in various types of stress conditions. However, many efforts have been made to develop transgenic microbes that allow co fermentation of both types of sugars namely pentose and hexose sugars. Therefore, three major microbial platforms that has been developed additionally their
performance has been demonstrated in pilot studies these microbial platforms are Zymomonas mobilis, Escherichia coli, and Saccharomyces cerevisiae.

5.6. Consolidated bioprocessing (CBP)

Consolidated bioprocessing (CBP) combines the production of enzyme, hydrolysis of substrate and their fermentation into end product. But for efficient bioconversion we need microbes or engineering of the microbes which can complete the process in a proper way. So use of genetic engineering for improving the yield of product and titles to express a heterologous cellulase system enabling utilization of cellulose (36). There are different microbial strains are identified for CBP system like, Clostridium thermocellum, Thermoanaerobacterium saccharolyticum, Clostridium phytofermentans, Caldicellulosiruptor bescii and yeasts, e.g., S. cerevisiae and thermotolerant K. marxianus. However, most CBP organisms identified and developed, wild or genetically engineered, to date suffer from either low ethanol titer (<3wt %), low growth, or low metabolic yield and/or productive yield (37). Hence, it is noticeable that consolidated bioprocessing process is relatively cheap and need low energy input therefore, few consortia of microbes are needed for fermentation and also for glucose production. Consolidated bioprocessing processes have higher conversion efficiency than other processes. This is economically attractive process because these microbes are efficient cellulase producers. So they become efficient ethanol producer. Therefore, consolidated bioprocessing is considered as a promising cost effective approach for production of LC ethanol, as little expediency are needed to compare SSF and SHF process. Therefore for further development of consolidated bioprocessing process highly engineered microorganisms that are able to produce sufficient hydrolytic enzymes with higher fermentation capacity is needed. To meet this goal we should use directed evolution approach to tailor the biocatalyst and microbes as per the need.

6. DISTILLATION

The cell free fermented broth was preheated upto 90°C and then this fermented broth was sent to degasifying chamber. Bubble cap fractionating column was used to remove any trapped gases from the broth then vapours of ethanol from the analyzer chamber are further taken to the rectifying column and about 94–96% of the rectified ethanol was cooled, trapped, and was collected by reflux action (26).

6.1. Biofuel purification

Generally, the process of distillation has been used for the separation of water and alcohol. Therefore, the distillation process is able to generate a 95% pure ethanol. In order to get pure alcohol many additives and molecular sieves are required to break azetope. Mostly grains or extracted sugar is used in the first generation biorefinery and therefore there are almost no degradation products in the substrate to inhibit enzymes or microbes. Hence, ethanol titer more than 10% is easily achievable allowing an economical distillation process. To avoid distillation process researchers, are looking for different types of biofuels that are not soluble in water and these can be phase separated (38).

7. BREAKDOWNS OF LIGNOCELLULOSIC BIOMASS

The production of biofuel from plant residues is more difficult compared to molasses and starch based substrates, in this process biomass is firstly break into the biomass to its sugary units and then mixture of pentose and hexose sugars must be fermented into bioethanol. But the highest saccharification reaction required a pretreatment method which efficiently breaks the compact structure into its basic units. Therefore, the expensive enzymatic conversion and lower efficiency due to natural recalcitrance of lignocellulose to deconstruction, enzyme cost, compact and crystal structure of cellulose, amount of pentose and hexose sugars with alcoholic groups, and available surface area and porosity form the major bottlenecks in this technology (39).

7.1. Different pretreatment methods: advantages and disadvantages

The main objective of lignocellulosic biomass pretreatment is to make the biomass structure feasible and increase conversion efficiency for hydrolysis. This goal could be achieved by the delignification, depolymerisation, decrystallization and increase surface area of plant biomass for microbial fermentation (40, 41). The pretreatment of raw material could represent up to 20% of the total costs of cellulosic ethanol production (42). Different pretreatment methods which are mostly used are physical, chemical, physico-chemical, and biological pretreatment methods (43) as depicted in Figure 2. In the past hundred million years, lignocellulosic biomass has evolved complex structure and chemical compositions to protect the structural saccharides from outside attack. Plant carbohydrates are the main resource of fermentable sugars, which are compactly associated with lignin, while lignin is the major barrier to enzymatic saccharification of lignocelluloses. Other factors, such as crystallinity and the strong inter-chain hydrogen-bonding network of cellulose, available surface area, the content of acetyl groups, the presence of hemicellulose and its bond with cellulose and lignin, the distribution of lignin and hemicelluloses, as well as the type of lignin, also contribute to the hindrance of
Pretreatment for Lignocelluloses Bioconversion

To enzymatic saccharification. On the other hand, the natural structure of plant biomass also affect the bioconversion process, due to these reasons the pretreatment is required these factor are; i) presence of cuticle and wax, ii) vascular tissues arrangement, iii) thickness of sclerenchymatous tissues, iv) cell wall compactness and their multilayered arrangement, v) requirement of enzyme substrate specificity, vi) plant biomass complexity also effect the mass transport system of plant cell wall. The bioconversion process of lignocellulosic biomass is also affected by composition and the arrangement of cellulose, hemicelluloses and lignin, which vary from plant to plant. Up to now, with the great efforts of researchers, the cost of enzymes has been lowered significantly, and the adaptability and activity of new enzyme products have been improved a lot. However, the cost of pretreatment is still quite high, and hardly meets the requirement of commercial application. Therefore, to a large extent, pretreatment is the main bottleneck for the production of biofuel/ biochemical from lignocelluloses. More efforts should be made to develop more cost-effective pretreatment process. More specific requirements for a good pretreatment method are as follows; i) high yields of fermentable sugars and low sugar degradation; ii) effective delignification or chemical/ structural changes of lignin (e.g. sulfonation of lignin); iii) limited formation of inhibitors and high purity of fermentable sugars; iv) low chemical consumption or efficient chemical recovery; v) low water usage; vi) low energy consumption; v) low cost and environmental benign process; vi) high recovery of hemicelluloses and lignin (44).

7.1.1. Physico-chemical pretreatment

In physio-chemical pretreatment method the composition and structure of biomass is altered by the physical method in the presence of chemicals. The examples of physico-chemical pretreatment is, steam explosion, carbon dioxide explosion, ammonia fibre explosion, and wet oxidation.

7.1.1.1. Steam explosion

In this pretreatment the degradation of lignocellulosic biomass performed by steam, and pressurized steam (20-50 bar, 160-270°C). For this pretreatment the lignocellulosic substrate is kept at 160-270°C temperature and 20-50 bar pressure for some time, all of sudden change the pressure of the system, and depressurized. This process is cost efficient and effective in lignin and hemicelluloses structural modification (45). Therefore, steam
explosion is the most commonly used method for pretreatment of lignocellulosic biomass (46). In this method, the chopped biomass was treated with high pressure saturated steam for about 30 seconds to 20 minutes and then reduces the pressure. Steam explosion is a combination of chemical effects due to the auto hydrolysis of acetyl groups of hemicelluloses and mechanical forces.

7.1.1.2. Ammonia fiber explosion (AFEX)

Ammonia fiber explosion or AFEX is another type of physico-chemical pretreatment in which lignocellulosic biomass is treated with liquid ammonia at relatively moderate temperature (90–100°C) for a period of 30–60 min. followed by a rapid pressure release (47). This pretreatment method is similar to steam explosion pretreatment, but liquid ammonia is also used at high temperature and pressure and all of sudden pressure is released. AFEX is a pretreatment method with numerous benefits which are eco friendly, high energy efficient, gentle reaction temperature and no formation of inhibitory compound. The used ammonia is again usable which make the process cost efficient (48). The presence of liquid ammonia make the process more effective, which result the highly compact structure is converted into smooth structure and increase unfolding of structure. The structure of cellulose changes into native cellulose I to cellulose III. The sudden release of pressure disrupts the crystal structure and crystallinity of cellulose material. AFEX treatment is also effective in the delignification and degradation of hemicellulose from the lignocellulose. The effect of AFEX pretreatment is reported in bamuda grass, and the chemical structure of substrate is not changed but the sugar yield is increased upto 94.8% after the enzymatic hydrolysis (49). In switch grass 93% of glucan conversion observed. But in corn stover near about 100% cellulose and 80% hemicellulose converted into fermentable sugars (50). Ammonia fiber explosion has been reported to be ineffective for biomass with higher lignin content (~25%) (51). AFEX increases the digestibility of lignocellulosic biomass by removing the least acetyl groups by deacetylation process (52). The main advantage of the ammonia pretreatment is that it does not produce inhibitors for the downstream biological processes, so water wash is not necessary. AFEX pretreated corn stover resulted in 70% glucan conversion after 72 h of hydrolysis. Ethanol fermentation of AFEX treated (at 6% w/w glucan loading) corn stover resulted in 93% of ethanol yield (53). Teymouri et al. (50) optimized the conditions such as ammonia loading, temperature, blowdown pressure, moisture content of biomass and residence time in the AFEX process. They observed that AFEX can achieve more than 90% conversion of cellulose and hemicellulose to fermentable sugars for a broad variety of lignocellulosic materials.

7.1.1.3. Carbon dioxide explosion

The CO₂ explosion or carbon dioxide explosion is much similar to ammonia fibre explosion and steam explosion. It is believed that carbon dioxide reacts with carionic acid that is carbon dioxide in water, thereby improves the rate of hydrolysis. Therefore, the pretreatments as CO₂ molecules have a similar size property to those of water and ammonia making them capable of penetrating into small pores of lignocellulosic material. In contrast with steam explosion, supercritical CO₂ explosion needs lower temperature and is also less costly in comparison with AFEX, making it an ideal choice among the explosion type methods. Besides, CO₂ explosion possesses many other advantages such as non-toxicity and non-flammability (54). CO₂ explosion is a perfect choice among the explosion type of pretreatment. Therefore, yield is comparatively low as compared to ammonia explosion and steam pretreatment method but comparatively high as compared to enzymatic hydrolysis.

7.1.1.4. Chemical pretreatment

Chemical pretreatment method utilizes different chemicals that are acidic, alkaline, and oxidants in nature and therefore, theses chemicals cause destruction of organic compounds (55). But alkali and acid pretreatment is most commonly used pretreatment method because of their economic feasibility and their effectiveness. The alkali pretreatment is more effective in the removal of lignin and disperses compact structure into plants fiber. Alkaline pretreatment is more effective in degradation of the ester linkages between the hemicelluloses and lignin, thus significantly promote the solubilization of hemicelluloses and lignin, resulting in the exposure of cellulose to enzymes. Thus, for delignification process complex reagent sodium hydroxide or NaOH is used in chemical pretreatment method. However, accompanied with lignin removal, substantial hemicellulose was also dissolved, which led to plenty waste of substrate material. Therefore as compared to NaOH sodium hydroxide the calcium hydroxide Ca(OH)₂ is economically cheap and safe. Cao et al. (56) in his study reported that during lime pretreatment method, the rigorous structure of the cornstalk can be disrupted and more cellulose can be exposed to the surface, this intern increases H₂ yield and improves the biodegradability of substrate. Therefore, Ca(OH)₂ can be easily recovered by using lime kiln technology, which is suggested to be more propitious pretreatment of lignocellulosic biomass for production of H₂. The acid pretreatment method is most desirable pretreatment method for pretreatment of lignocellulosic substrates not only, because it leads to the degradation of the lignin, but also the microbes used in hydrolysis are able to acclimatize at low pH.
Therefore, further research is required to improve the economics of these pretreatments and construct effective solvent recovery procedures (43).

7.1.1.5. Ionic liquid pretreatment (ILs)

The use of ionic liquids (ILs), is another alternative for pretreatment of lignocellulosics materials (60). ILs pretreatment has been emerged as a promising technology toward environmentally benign conversion of lignocellulosic residues into high value cellulosic fiber as sustainable raw material for biocomposite fabrication. Therefore, ionic liquids are globally recognized materials of future. Ionic liquids are solvents that can be employed in pretreatment steps to achieve following objectives that lead to the degradation and to reduce the biomass recalcitrance are cellulose amorphization, deacetylation of hemicellulose, delignification, and their unusual properties appeals towards most diverse technological areas like environmental chemistry, chemical industry, nanotechnology and medicine. ILs are most effective pretreatment agent and used for extraction of many useful chemicals like ethanol, biodiesel, and other biofuels can be obtained from algae, which is considered as the most widespread organisms on the surface of the Earth. Ideal products

Figure 3. Degradation products from lignocellulose as a result of pretreatment under acidic conditions.
are sorbitol, alkylglycosides and glucose esters. Ionic liquids has emerged as an alternative pretreatment method used for degradation of lignocellulosic biomass. These ionic liquids are evolved with unique ability of dissolving whole biomass rather than dissolving individual subcomponent of lignocelluloses. Pu et al. (63) in their study observed different properties of the anion that are extremely essential for the solubility of ionic liquids in lignin. These ILs can reduce the crystallinity of the cellulose by partial removal of lignin and hemicelluloses therefore enhances digestibility of biomass. These ILs ionic liquids form hydrogen bonds with cellulose at very high temperature due to presence of different anions such as formate, acetate, alkyl phosphonate and chloride. Lee et al. (49) in their study founded ([Emim][OAc]) or 1-ethyl-3-methylimidazolium acetate that extract lignin selectively from wood with less crystalline cellulose remaining. The ionic liquid ([Emim][OAc]) or IL 1-ethyl-3-methylimidazolium acetate is recognized as an effective pretreatment agent for treatment of different biomass materials (64). Ionic liquids (ILs) have attracted much attention in both academicians and industries as promising solvents for a diverse range of applications. The economic efficiency can be improved by recycling and reuse of ILs. In the last few decades, several attempts have been made, by the researchers, for recovery and recycling of ILs. Structures of some ionic liquids are given in Figure 4.

7.1.2. Biological pretreatment

Biological pretreatment is a eco-friendly and economically feasible process for biomass pretreatment, due to low chemical & energy input, high substrate reaction specificity and higher yield of sugary product. Biological pretreatment is based on the use of microbial strains, which are able to degrade lignocellulosic biomass. Brown rot and soft rot fungi are generally used by researchers for the degradation and increasing the rate of enzymatic hydrolysis (65). Brown rot fungi, have highest capacity to degrade cellululosic biomass. The white rot fungi generally belongs to class basidiomycetes and used majorly for biological pre-treatment method. Hence, white rot fungi secretes different lignolytic enzymes like lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase and in the presence of veratryl alcohol (VA) and Mn (III), manganese peroxidase and lignin peroxidase are respectively oxidized by hydrogen peroxide. Therefore, the oxidation of lignin is caused by the oxidized form of manganese peroxidase and lignin peroxidase enzymes. Laccase enzymes catalyses the oxidation of phenolic compound of lignin with the help of lignin oxidizers like 3-hydroxyanthranilic acid (HA) and 2,2’-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS). Many researchers have reported that white rot fungi such as Ceriporiopsis subvermispora, Phlebia subserialis, Phanerochaete chrysosporium, and Pleurotus ostreatus can completely remove lignin. Singh et al. (26) reported that Dichomitus squalens is effective in rice straw and increase the lignocelluloses degradation rate. As compared to different chemical and physical pretreatment method biological pretreatment method is relatively slower but it consumes less energy with better environmental footprint. Therefore, the use of fungus for pretreatment method prior to the pyrolysis is essential to improve its performance (66). But biological pretreatment and its enzymatic hydrolysis is also affected by some factors which are biomass particle size, moisture content, temperature, pretreatment time and pH depicted in Table 1 (54). Schizophyllum commune is a ubiquitous white-rot fungus with a cosmopolitan distribution that can degrade complex plant biomass, including the recalcitrant lignin (67). Therefore, the genome of Schizophyllum commune encodes an extensive catalog of genes implicated in lignocellulose degradation. Its lignocellulolytic enzyme pool is expected to provide a prospective enzyme source for biotechnological applications (68). One of the most effective methods used for the enzymatic saccharification is fungal pretreatment method which utilizes wood rot fungi. Therefore, Gloeophyllum trabeum, the brown rot fungi, produces different enzymes leads to the depolymerisation of hemicelluloses and celluloses in wood. Hemicelluloses is the type of branch polymer which consist of sugar monomers and glucose theses hemicelluloses form cross linking to maintain the structural integrity of the cell wall. Therefore, the action of different xylanases with different specificities and action are needed for complete hydrolysis of xylan (69). Different microbial species are employed for production of xylanase at commercial scale include, Trichoderma reesei, Aspergillus niger, Humicola insolens and Bacillus. Exoxygenases and endoxylanases are required to break up the cross linking between hemicelluloses. Therefore the microbes that are able to produce lignin degrading enzymes are produced by Ceriporia cerata, Cyathus sterkolerus, P. chrysosporium, Pycnoporus

Figure 4. Types of ionic liquids.
cinnarbarinus, C. subvermispora, P. chrysosporium and Pleurotus ostreaus (52). Trichoderma reesei, was unable to degrade lignin although it is a good producer of cellulolytic and hemicellulosic enzymes. Potumarthi et al. (70) in their study utilizes biological pretreatment method for pretreatment of rice husks with the help of the fungus Phanerochaete chrysosporium which resulted in 44.7 % reducing sugars. Similarly, Pinto et al. (71) also utilizes biological pretreatment method for treatment of wheat straw by submerged and solid state fermentation methods with the help of white-rot basidiomycetes like Ganoderma resinaeum, Iripex lacteus, Bjerkandera adusta, Phanerochaete chrysosporium, Fomes fomentarius, Euc-1, Lepista nuda, Trametes versicolor and were tested. Therefore, Trametes versicolor was proved to be better strain as compared to other strains for enzymatic hydrolysis of holocellulose. Now the biofuel production is based on lignocellulosic waste so in this era also focused on genetically modified plants that easily degraded by microbes (72). In the same line metabolic engineering is considered as an emerging field which utilizes the recombinant DNA technologies for direct production of bioethanol. Table 2 showed the production of bioethanol from different feedstock's sources containing sucrose.

### Table 1. Advantages and disadvantages of different pretreatment methods

<table>
<thead>
<tr>
<th>S. No</th>
<th>Pretreatment methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Physicochemical pretreatment</td>
<td>Steam explosion</td>
<td>Lignin transformation, hemicelluloses solubilization, Cost-effective</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AFEX</td>
<td>Increase surface area of cellulose, and absence of inhibitory substances formed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Co2 explosion</td>
<td>Increase surface area of cellulose, and absence of inhibitory substances formed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wet oxidation</td>
<td>Delignification, breakdown of hemicellulose and decrystalization of cellulose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LHW</td>
<td>The maximum part of hemicelluloses and lignin is dissolved but the insoluble part of carbohydrates is homogenously distributed in the cell wall</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Electrical catalysis</td>
<td>Not produce inhibition compounds, cost-effective, increases surface area, and remove lignin effective cleanliness</td>
</tr>
<tr>
<td>2</td>
<td>Chemical pretreatment</td>
<td>Ionic liquid pretreatment</td>
<td>Environmental, large temperature range</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Concentrated acid</td>
<td>High sugar conversion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dilute acid</td>
<td>Fast and don not need recycle acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alkali pretreatment</td>
<td>Room temperature, destroy lignin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organosolv pretreatment</td>
<td>Obtain pure lignin, cellulose and hemicelluloses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxidation pretreatment</td>
<td>Environmental, remove lignin effectively</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ozonolysis</td>
<td>Reduces lignin content, no toxic residues</td>
</tr>
<tr>
<td>3</td>
<td>Physical pretreatment</td>
<td>Mechanical splintered</td>
<td>Reduce particle size and cellulose crystallinity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microwave</td>
<td>Simple operation, energy-efficient, short time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ultrasonic</td>
<td>Improve accessibility and reactivity of cellulose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-energy electron radiation</td>
<td>Reduce cellulose polymerization degree</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-temperature pyrolysis</td>
<td>Decompose cellulose rapidly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biological pretreatment</td>
<td>Degrades lignin and hemicellulose Low energy consumption</td>
</tr>
<tr>
<td>S. No.</td>
<td>Feedstock</td>
<td>Yield, tonnes ha⁻¹</td>
<td>Sugar content, % w/w</td>
</tr>
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<td>--------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| 1     | Sugar beet pulp                   | 4717360 tonnes annum¹ | Total carbohydrate 80%: Rhamnose 2.4%  
Arabinose 23.0%  
Galactose 6.2%  
Glucose 25.9%  
Mannose 1.0%  
Xylose 1.7%  
Galacturonic acid 14.4% | S. cerevisiae                        | Fermentation at 30°C for 24 h and other conditions not available                  | 0.5 g ethanol per g of glucose from sugar beet pulp | (73)      |
| 2     | Sweet sorghum stover              |                    | S. cerevisiae  
Fermentation at 30˚C, 48 h and inoculum 0.27 g∙L⁻¹ | New Aule Alcohol yeast and New Aule Baker’s yeast | Alcohol yeast-74.8 g∙L⁻¹, Yp/s 0.4 g∙g⁻¹ and Baker’s yeast-102.9 g∙L⁻¹, Yp/s 0.7 g∙g⁻¹ from 300 gL⁻¹ sugar concentration | 91.9 g ethanol·kg⁻¹ native sorghum | (74)      |
| 3     | Sugarcane molasses                 | 62 - 74 tonnes ha⁻¹ | 31% sucrose and 15% invert sugars  
New Aule Alcohol yeast and New Aule Baker’s yeast | Fermentation at room temperature, pH 4.3 for 72 h, inoculum 1% w/v | 128.7 g·L⁻¹, Yp/s 0.6 g·g⁻¹ from 250 g·L⁻¹ sugar concentration |                                      | (75)      |
| 4     | Cassava stems                      | 403 tonnes ha⁻¹    | Cellulose 28.9%, 9.7%  
Hemicellulose 21.1%, 32.3% Klasson      | S.cerevisiae or Rhyzopus spp                        | Fermentation conditions not available  
Stems: 5.2 g ethanol 100 g⁻¹ stems  
Peelings: 2.6 g ethanol 100 g⁻¹ peelings |                                      | (76)      |
| 5     | Sugarcane molasses                 |                    | Saccharomyces species isolated from molasses  
New Aule Alcohol yeast and New Aule Baker’s yeast | Fermentation at 30°C for 144 h, inoculum 0.5 g·L⁻¹ | 128.7 g·L⁻¹, Yp/s 0.6 g·g⁻¹ from 250 g·L⁻¹ sugar concentration |                                      | (77)      |
| 6     | Rice straw                         | 34.3 million tonnes annum¹ | Cellulose 32% - 47%  
Hemicellulose 13% - 27 %  
Saccharomyces species isolated from molasses  
New Aule Alcohol yeast and New Aule Baker’s yeast | S. cerevisiae and Candida tropicalis | Fermentation at 37°C for 72 h and inoculum 1.2 g yeast in 10 mL YP medium | 25.1 g·L⁻¹, Yp/s 0.4 g·g⁻¹ from 250 g·L⁻¹ sugar concentration | (78)      |
| 7     | Rice straw                         |                    | Glucan 44.8%  
Xylan 20.8%  
Lignin 18.3% | Mucor hiemalis                        | Fermentation organism at 37°C, pH 5.5, 72 h and inoculum 1 g dry biomass L | 12.8 g·L⁻¹, 154 g ethanol·kg⁻¹ rice straw, 83% ethanol yield | (79)      |
| 8     | Corn HSG and PFC varieties         | 7.2 tonnes ha⁻¹    | Starch 67.3%,  
total sugar 7.4%  
and PFC: starch 73.6%, total sugar 1.2% | S. cerevisiae ATCC 96581                        | Fermentation at 30°C, pH 4.2, 96 h and inoculum 2 mL per 100 mL media | From HSG: 0.4 g ethanol·g⁻¹ dry corn, 141.5 g·L⁻¹  
From PFC: 0.4 g ethanol·g⁻¹ | (80)      |
<table>
<thead>
<tr>
<th>S. No</th>
<th>Feedstock</th>
<th>Yield, tonnes ha⁻¹</th>
<th>Sugar content, % w/w</th>
<th>Microorganism</th>
<th>Fermentation</th>
<th>Ethanol</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Sweet sorghum juice</td>
<td></td>
<td></td>
<td></td>
<td>S. cerevisiae strain BY4741 Fermentation at 30˚C, pH 5.2, 48 h, inoculum 5*10⁸ cells mL⁻¹</td>
<td>Ethanol 115.2 g∙L⁻¹, 87.1% of the theoretical ethanol yield, Yp/s 0.4 g g⁻¹</td>
<td>(81)</td>
</tr>
<tr>
<td>10</td>
<td>Cassava</td>
<td>Liquid waste (1% total solids): 8.9 - 10.6 tonnes and Wet cassava bagasse: 0.9 - 1.1 tonnes from 1 tonne of dry cassava processed</td>
<td>Carbohydrate 76.6% Starch 60.8% Fibre 15.8% Protein 0.8%</td>
<td>S. cerevisiae</td>
<td>Fermentation using S. cerevisiae at 40˚C - 50˚C, pH 4.6 - 5.5, 8 h and inoculum 0.2 g dry biomass</td>
<td>2.7 g ethanol 15 g⁻¹ cassava cellulosic waste, 32.4% w/w ethanol concentration</td>
<td>(82)</td>
</tr>
<tr>
<td>11</td>
<td>Wild cassava</td>
<td></td>
<td></td>
<td>Caloramator boliviensis (Thermoanaerobe)</td>
<td>Fermentation at 60˚C, pH 7, 48 h and inoculum 50 mL overnight culture per 240 mL media</td>
<td>33.0 g∙L⁻¹, 1.7 mol∙mol⁻¹, 85% of the theoretical ethanol yield</td>
<td>(83)</td>
</tr>
<tr>
<td>12</td>
<td>Sugar cane bagasse</td>
<td>276 kg bagasse tonne⁻¹ of sugarcane</td>
<td>Cellulose 52% Hemicellulose 20% Lignin 24%</td>
<td>S. cerevisiae</td>
<td>Fermentation 5 days and other conditions not available</td>
<td>11.8 g ethanol L⁻¹</td>
<td>(84)</td>
</tr>
<tr>
<td>13</td>
<td>Sweet sorghum juice from three varieties: GK-coba; Mn-4508; SS-301</td>
<td></td>
<td></td>
<td>Zymomonas mobilis and S. cerevisiae mixed culture (1:1)</td>
<td>Fermentation at 30˚C, 4 days, inoculum 5 mL of 48 h old liquid seed cultures</td>
<td>45.2 mL L⁻¹; 1075.4 L ha⁻¹ 46.9 mL L⁻¹; 1318.2 L ha⁻¹ 50.2 mL L⁻¹; 1232.6 L ha⁻¹</td>
<td>(80)</td>
</tr>
<tr>
<td>14</td>
<td>Cassava flour</td>
<td></td>
<td></td>
<td>S. cerevisiae;</td>
<td>Fermentation at 30˚C, pH 5.5, 72 h and inoculum 1.5*10⁷ cells mL⁻¹ At lab scale: 17.2% v/v, 86.1% of the theoretical Ethanol yield; At pilot scale: 16.5% v/v, 83.6% of the theoretical ethanol yield</td>
<td>Ethanol yield</td>
<td>(85)</td>
</tr>
<tr>
<td>15</td>
<td>Sweet sorghum juice</td>
<td></td>
<td></td>
<td>S. cerevisiae; yield</td>
<td>Fermentation at 30˚C, 48 h, inoculums 5*10⁸ cells mL⁻¹</td>
<td>Ethanol 133.5 g∙L⁻¹, 87.6% of the theoretical</td>
<td>(86)</td>
</tr>
<tr>
<td>16</td>
<td>Sugar cane bagasse</td>
<td></td>
<td>Cellulose 42% Hemicellulose 25% Lignin 20%</td>
<td>Pencillium funiculosum, S. cerevisiae</td>
<td>Fermentation at 37˚C, pH 5, 144 h and inoculum 15 g L⁻¹</td>
<td>Ethanol 100 g∙L⁻¹, 121.2 L of ethanol tonne of sugarcane bagasse</td>
<td>(87)</td>
</tr>
<tr>
<td>S. No.</td>
<td>Feedstock</td>
<td>Yield, tonnes ha(^{-1})</td>
<td>Sugar content, % w/w</td>
<td>Microorganism</td>
<td>Fermentation</td>
<td>Ethanol</td>
<td>Reference</td>
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<td>-------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>17</td>
<td>Sweet sorghum bagasse (SSB)</td>
<td></td>
<td>Cellulose 34% - 45% Hemicellulose 25% - 27% Lignin 18% - 21%</td>
<td>Active dry yeast</td>
<td>Fermentation at 30°C and inoculum at 1:10 volume ratio of yeast medium to fermentation broth</td>
<td>Total sugar yield of 90.9 g sugar 100 g(^{-}\text{dry SSB}), Ethanol 6.1 g L(^{-1})</td>
<td>(88)</td>
</tr>
<tr>
<td>18</td>
<td>Sugar beet molasses and thick juice</td>
<td>54 - 111</td>
<td>Total sugars in Sugar beet molasses: 53.0% and in thick juice: 60.0%</td>
<td>Immobilized yeast;</td>
<td>Fermentation at 30°C, pH 5.5, 144 h, inoculum 1 g L(^{-1})</td>
<td>From molasses: Y(<em>{p/s}) 0.5 g(^{-}\text{g}), 96.8%, 83.2 g L(^{-1}) and from thick juice: Y(</em>{p/s}) 0.4 g(^{-}\text{g}), 90.6%, 132.4 g L(^{-1}) from 300 g L(^{-1}) sugar concentration</td>
<td>(89)</td>
</tr>
<tr>
<td>19</td>
<td>Sugar beet raw, thin and thick juice and molasses</td>
<td></td>
<td>In raw juice:13.4% Thin juice:13.0% Thick juice: 58.3% Molasses: 50.1%</td>
<td>Commercial yeast strain;</td>
<td>Fermentation at 30°C, 60 h, inoculum 3 g L(^{-1})</td>
<td>From raw juice: 0.08 v/v Thin juice: 0.08 v/v Thick juice: 0.08 v/v Molasses: 0.07 v/v from an initial sugar concentration of 130 g kg(^{-1}) media</td>
<td>(90)</td>
</tr>
<tr>
<td>20</td>
<td>Cassava</td>
<td>36.3 tonnes ha(^{-1}) annum(^{-1})</td>
<td>Starch 76% - 81%</td>
<td>Dry bake's Yeast</td>
<td>Fermentation at 30°C, 48 h, pH 5.5, and inoculum 10 mL yeast suspension having O.D 3.8 - 4.0 at 450 nm</td>
<td>558 g ethanol kg(^{-1}) cassava starch, fermentation efficiency 98.4%</td>
<td>(91)</td>
</tr>
</tbody>
</table>
8. HURDLES IN PRETREATING THE BIOMASS

Lignocellulose pretreatment process is a multi-scale and non-uniform structure interaction system. The complex and dynamic heterogeneous structure is the key factor influencing the transport and reaction processes, which result in the large differences in pretreatments results. However, many problems still exist in each pretreatment process, and it remains in experimental stage. The major problems in pretreatment processes are summarized as follows; i) different pretreatment methods have different key points, so evaluating various pretreatment methods directly through the test data is not accurate. Therefore, a scientific, economically feasible, and highly productive pretreatment method should be developed on the basis of the evaluation standard, ii) investigations on physical and chemical reaction mechanisms of pretreatment technology research are deficient, thus determining an excellent pretreatment method is difficult. Overlapping discipline should be developed to broaden the ideas to further understand the influence of lignocellulosic structure on cellulase and hemicellulase digestion during processing. The mechanism should be further studied to determine a suitable reaction model and optimize and improve the existing pretreatment technologies iii) Many pretreatment technologies only consider cellulose enzymolysis rate, hydrolysis rate, sugar yield, and removal rate of lignin apparent indexes, which cannot explain theoretically the involved physical chemistry in the transfer and reaction processes on the preprocessing result. Therefore, an innovative process is necessary, iii) Many pretreatment processes require optimal reaction conditions and high cost, and cause environmental pollution.

9. CONCLUSIONS

The utilization of lignocellulosic biomass for production of bioethanol requires efficient production technology which is environmentally sustainable and cost effective. The purpose of these pretreatment methods is to eliminate the limiting factors. Presently, different methods are used for this purpose which has several advantages and disadvantages. Application of using these pretreatment methods can be selected on the basis of cost and type of lignocellulosic materials. Though, the effects of various pretreatment processes on lignocellulose composition and sugar yield have been extensively investigated. However, different pretreatment methods have been rarely compared. Many pretreatment processes can improve the efficiency of lignocellulosic biomass pyrolysis and the production of chemicals, although these processes have yet to be developed for industrial applications. Different pretreatment methods of lignocellulosic materials for improving the bioethanol production have been discussed in this article. On the basis of this review, we proposed the following research prospects; a) lignocellulosic biomass components should be extensively investigated to obtain complex high-value chemicals through highly efficient separation and create an economically feasible follow-up process, b) existing pretreatment methods should be optimized by combining saccharification and fermentation c) the effect of lignocellulosic structure to enzymolysis should be further elucidated, d) an efficient, eco-friendly, low cost, and simple operation pretreatment process should be developed to improve the existing methods, e) the physical and chemical reaction mechanisms of pretreatment should be explored in detail to establish reasonable pretreatment models and to optimize process conditions, f) a new pretreatment process should be explored to alter the structure of lignocellulosic biomass. This can be achieved to improve chemical production and promote industrial lignocellulose applications. Interdisciplinary studies may also provide opportunities to solve energy crisis and to promote the safe use of chemicals.

10. ACKNOWLEDGEMENTS

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**Key Words:** Biomass; Lignocellulose; Pretreatment; Fermentation; Bioethanol, Review

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