

# Lignocellulosic Material

## “An Alternative Future Fuel Source”

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**Abstract:** Uncontrolled emission of greenhouse gases due to utilization of fossil fuel has led to increase in the threat of global warming. So as to prevent such damage our natural environment, an alternative fuel source must be identified. Bioethanol production from lignocellulosic waste materials can overcome this problem to some extent if process used for production is economically viable. It is known that abundant plant materials are generated by forestry and agriculture industry. Usability of it is limited because of its rigid structure. So, effective pretreatment is required to liberate the sugar by breaking the complex crystalline structure and by removing lignin content. Pretreatments are given by physical, chemical, physicochemical and biological means. Physical treatments reduce the physical size of biomass whereas chemical treatments remove chemical barriers especially to break recalcitrant components so as to make easy accessibility of cellulose for microbial destruction. Biological pretreatments utilize various enzymes to achieve the accessibility of cellulose for hydrolysis process. To achieve higher efficiency both physical and chemical pretreatments have been applied. Acid hydrolysis, alkali hydrolysis, steam explosion, ammonia fiber expansion, organosolve, sulphite pretreatment, H<sub>2</sub>O<sub>2</sub> pretreatment, ethylene pretreatment, Tween 20 pretreatments etc. are used to break the lignocellulosic material and to liberate fermentable sugars after enzymatic action.

**Keywords:** Bioethanol, Biological Pretreatment, Chemical Pretreatment, Physical Pretreatment, Lignocellulosic materials, Saccharification.

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### 1. INTRODUCTION

The use of sugar and starch as raw material for fuel production competes with their use as foods (Pimental *et al.*, 2008) and supply is not sufficient to meet the recent increasing public demand for fuel. Lignocellulosic biomass provides an alternative attractive fuel source for biofuel production which can replace conventional fossil fuels (Wan and Li, 2011). Lignocellulosic material eliminates the utilization of crop land and because of its larger availability; it provides the domestic source of energy production. This raw material is less expensive and can be produce with lower input of energy, fertilizer and pesticides. Moreover they reduce the effect of climate change as they generate low green house gases.

First generation biofuels are obtained from starch, sugars, and plant oils. These include bioethanol derived from food crops such as sugar beet, cereals, sugarcane and biodiesel obtained from rape, sunflower, soya, and palm, respectively (Ghose *et al.*, 1983). Biofuels derived from lignocellulosic biomass are called as second-generation biofuel which involves hardwood, soft wood, agricultural wastes etc. (Sun and Cheng, 2002).

Lignocellulose is the common name to describe three main constituents of plants, namely cellulose, hemicelluloses and lignin. It is non-starch fibrous based plant material. Cellulose and hemi cellulose can be converted into fermentable sugars but it requires delignification because lignin forms a protective layer and hinders the degradation of lignocellulosic material (Balan *et al.*, 2009; Adsul *et al.*, 2011). Cellulose is composed of D-glucose subunits linked by,  $\beta$ -1, 4 glycosidic bonds forming the dimmer cellobiose. Hemicellulose is formed from D-xylose, D-mannose, D-galactose, D-glucose and L-arabinose etc depending on the source of hemicelluloses and linked together by,  $\beta$ -1, 4 and,  $\beta$ -1, 3 glycosidic bonds.

Physical processes (e.g., size reduction, boiling, ultrasonication etc.), chemical processes (e.g., acid, base, salt, solvents etc.), physicochemical processes (e.g., liquid hot water, ammonia fiber explosion etc.) and biological processes (white rot fungi, brown rot fungi and bacteria) have been developed which alter the structure of lignocelluloses and removes the lignin (Alvira *et al.*, 2010; Geddes *et al.*, 2011). The main goal of pretreatment is to overcome the recalcitrance nature of material and to separate the cellulose from the matrix polymers to make it more accessible for enzymatic hydrolysis.

Pretreatment processes are selected by aiming certain criteria like: (i) To produce highly digestible solids which enhances sugar yields during enzymatic hydrolysis, (ii) It must avoid the degradation of sugars (mainly pentose) including those derived from hemicelluloses, (iii) It minimizes the formation of inhibitors for subsequent fermentation steps, (iv) Recovery of lignin should be possible for conversion into valuable co products, and (v) It must be cost effective by operating in reactors of moderate size and by minimizing heat and power requirements.

## 2. PHYSICAL PRETREATMENT

Physical pretreatment involves milling and gridding which reduce biomass size and crystallinity. Mass transfer characteristics also improved from reduction in particle size. The energy requirements for physical pretreatments are dependent on the final particle size and reduction in crystallinity of the lignocellulosic material. When physical treatment is the only option, it requires the higher energy than the theoretical energy. This method is expensive and can't be used at a full scale process.

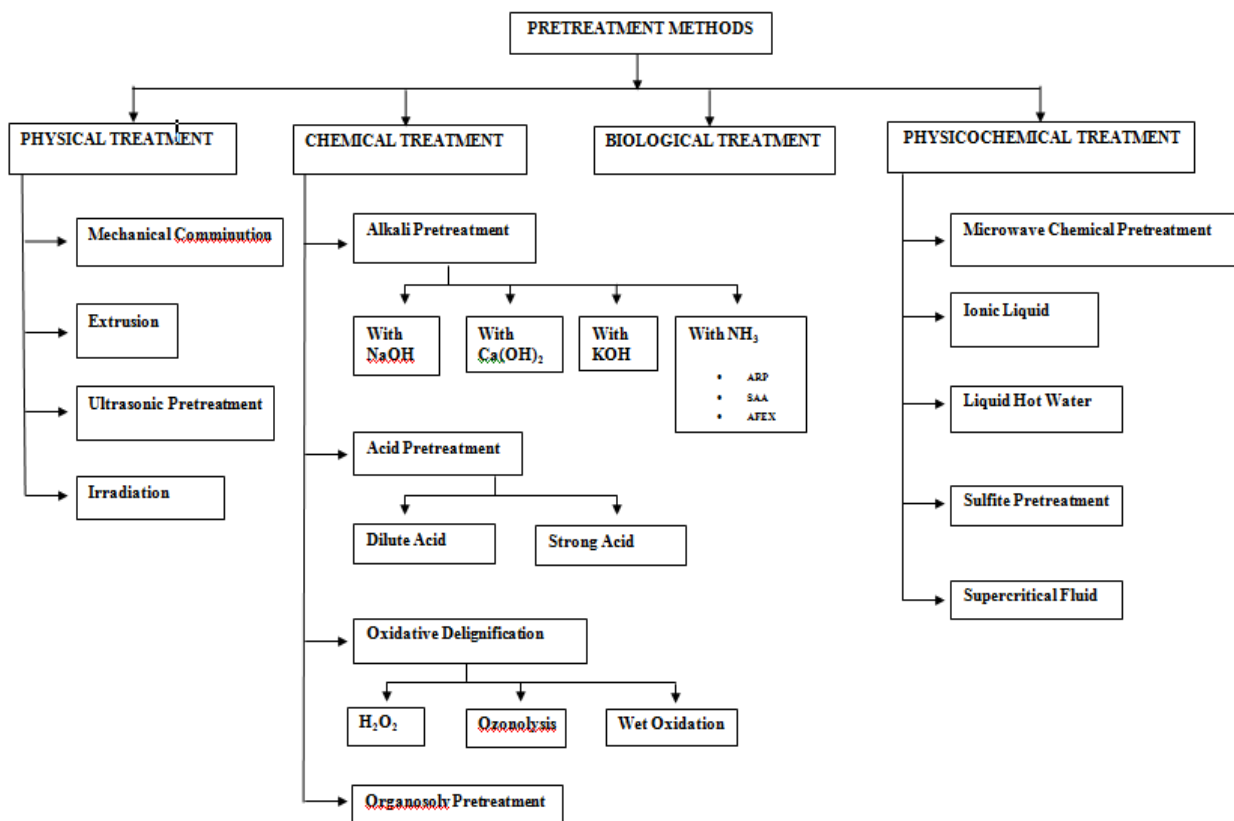


Fig. 1 Various Pretreatment Methods for Lignocellulosic Material

### A. MECHANICAL COMMINATION:

Particle size is reducing to increase the surface area to volume ratio and also to make handling procedure easy. It is eco-friendly pretreatment procedure because it does not involve harmful residue and generation of degradation products. This can be achieved by chipping, milling or grinding. Mechanical pre-treatment is usually carried out to get desired particle size depending on the subsequent processing steps. Mechanical pre-treatment depends on various factors like operating

costs, capital costs, scale-up possibilities and equipment. Power consumption is high which depends on the final particle size and the biomass characteristics especially forest residue requires high energy input. So, sometimes it requires chemical pretreatment prior to size reduction (Zhu *et al.*, 2010).

#### **B. EXTRUSION:**

It is novel and promising pretreatment process. The material is subjected to heating, mixing and shearing which results in physical and chemical modifications during the passage through extruder. Extruder provide high shear, effective and rapid mixing and rapid heat transfer (Karunanithy and Muthukumarappan, 2010). Barrel temperature as well as screw speed disrupt the lignocellulosic material which leads to defabrication and increase accessibility to enzymatic hydrolysis. It is widely acceptable treatment because various modifications are carried out like addition of chemicals, removal of materials, application of high pressure and temperature. This technique is majorly used to increase enzymatic accessibility of wheat bran, soya bean hull (Lamsal *et al.*, 2010), switch grass and corn stover (Karunanithy and Muthukumarappan, 2010).

#### **C. ULTRASONIC PRETREATMENT:**

This method is widely utilized for the treatment of sludge particularly derived from sewage treatment plants. Experiment carried out by using pure cellulose (Carboxyl Methyl Cellulose) indicates that when suspension of cellulose is provided along with irradiation energy, the enzymatic reaction rate is increased by 200% (Imai *et al.*, 2004). The reason is still not known but may be breakages of hydrogen bonds are responsible. The energy provided in this case (130 kJ/g) which is higher than the energy of the Hydrogen bond 0.12 kJ/g cellulose (Bochek, 2003).

#### **D. IRRADIATION:**

Enzymatic hydrolysis can be improved by irradiation like gamma rays, microwave and electron beam. Effect of irradiation can be improved with combination of acid treatment to lignocellulosic materials (Kumakura *et al.*, 1984; Mamaret *et al.*, 1990). Oligosaccharide is decomposed at high irradiation above 100 MR (Kumakura and Kaetsu, 1983). The effect of irradiation depends on the presence of lignin as well as crystallinity and density of material. The major drawback of this method is, it can't be applied at industrial level because of its high cost.

### **3. CHEMICAL PRETREATMENT**

Chemical pretreatment involves the utilization of various chemicals to disrupt biomass structure, structural alteration of lignin, cellulose swelling and partial decrystallization of cellulose and partial solvation of hemicelluloses.

#### **A. ALKALI PRETREATMENT:**

Alkali pretreatment increase cellulose digestibility and they are more effective for lignin solubilization, exhibiting less effect on cellulose and hemicellulose than acid or hydrothermal process. It causes less sugar degradation compared to acid pretreatment and more effective on agricultural residues than on wood material (Kumar *et al.*, 2009). Previous studies indicate that alkali process removes uronic acid and acetyl group present in hemicellulose and thus enhances the accessibility of the enzyme that degrades hemicellulose (Chang and Holtzapple, 2000). It also hydrolyzes ester linkages that join xylan and hemicelluloses residues (Sun and Cheng, 2002).

##### **➤ Pretreatment with Sodium Hydroxide:**

NaOH can be widely employed to lignocellulosic material. It is applied in dilute as well as in concentrated form. Dilute NaOH separate the bonds between lignin and carbohydrate, thus it increase the surface area and disrupt the lignin structure. It also decreases the degree of polymerization and crystallinity. Concentrated form of alkali increased the delignification. Increased pH from 10 to 13 removes lignin from 40 to 80% w/w at 140°C dry wheat straw (Pedersen *et al.*, 1998). NaOH has been reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24-55% to 20% (Kumar *et al.*, 2009). Zhao *et al.*, recorded an increase in delignification, from 52.3% to 75.5%. Positive results have come out from combined treatment of NaOH and other agents such as peracetic acid, urea, hydrogen peroxide and polyelectrolyte. Normally, NaOH treatment is carried out at room temperature or at elevated temperature but recently cold NaOH (-5°C) or NaOH/urea (-20°C) are used to treat plant fiber and cotton cellulose respectively. Bamboo

was subjected to ultrasound irradiation and NaOH/urea pretreatment at low temperature (-12°C) which break the recalcitrant material and produce high reactive cellulose (Li *et al.*, 2010).

➤ **Pretreatment with Calcium Hydroxide:**

Ca(OH)<sub>2</sub> which also known as lime is investigated as pretreatment agent because of its various positive characters like safe handling, cheaply availability and easy recovery. It removes acetyl group from hemicellulose reducing steric hindrance of enzymes and enhancing cellulose digestibility (Mosier *et al.*, 2005). Lime was proved to be successful agent to treat corn stover (Kim and Holtzapfle., 2006) or poplar wood (Chang *et al.*, 2001) at temperature from 85-150°C and for 3-13 h. It is also applied to switch grass and sugarcane bagasse. For pretreatment purpose, lime and water are added to the Lignocellulosic materials at temperatures ranging from ambient to 130°C. Sometimes in the presence of oxygen lime enhances the delignification process (Falls and Holtzapfle, 2011). Combined treatment of lime and dilute sulfuric acid was given to Miscanthus sp. (Guo *et al.*, 2013). Lime can be recovered via precipitation to CaCO<sub>3</sub> using CO<sub>2</sub>. Pretreatment with lime has lowered the cost and less safety requirements compared to NaOH and KOH.

➤ **Pretreatment with Potassium Hydroxide:**

KOH is applied to low lignin containing feedstock such as rice hulls and grasses. On treatment with KOH, lignin is slightly removed and structure of feedstock is altered to make easy accessibility towards the enzymatic hydrolysis. Switch grass was treated by 0.5% KOH which retain 99.26% sugar at 21°C after 12 h (Sharma *et al.*, 2012).

➤ **Pretreatment with Ammonia:**

Feedstock is treated with aqueous ammonia at elevated temperature. This process reduces lignin content, decrystallizes cellulose and also removes hemicelluloses. Treatment can be applied in three different forms i.e. Ammonia Recycle Percolation (Kim *et al.*, 2003), Soaking in Aqueous Ammonia (Kim *et al.*, 2008) and Ammonia Fiber Explosion method.

**Ammonia Recycle Percolation (ARP):** Aqueous ammonia is used to treat biomass packed in column reactor (Kim *et al.*, 2003; Kim and Lee, 2005). Ammonolysis carry out the destruction of lignin and hydrolysis of hemicelluloses. It breaks hydrogen bonds in cellulose to loss crystallinity. Thus it becomes accessible to enzymatic hydrolysis (Schuerch 1963; Mittal *et al.*, 2011). Liquid fraction is used to recover ammonia and to get lignin and other sugars. Solid fraction sent to a crystallizer and then washing step is carried out to extract sugar. Pretreatment of corn stover indicate 73.4% delignification and 85.5% enzymatic digestibility (Kim and Lee, 2005).

**Soaking in Aqueous Ammonia (SAA):** It is operated at low temperature which efficiently removed lignin and minimizing the interaction with hemicelluloses. It increases surface area and pore size for easy enzymatic hydrolysis. Destarched barley hull was treated by 15-30% of aqueous ammonia at 30-75°C for 12 h to 77 days indicates that 66% lignin was solubilized and 83% of glucan and 63% of xylan were saccharified (Kim *et al.*, 2008). Similarly hot water treated corn stover was treated by aqueous ammonia which hydrolysed 92% xylan and 75% lignin (Kim and Lee, 2006).

**Ammonia Fiber Explosion (AFEX):** In this process biomass is treated at 60-100°C temperature and 250-300 psi pressure for 5 minutes. It leads to swelling of lignocelluloses, disruption of hemicelluloses structure and decrystallization of cellulose. Cellulose and hemicelluloses obtained are converted into fermentable sugars (Mosier *et al.*, 2005). Lignin is not affected but the main advantage is that nearly all the ammonia can be recovered. This treatment can applied to rice straw, municipal solid waste, newspaper, sugar beet pulp, sugar cane bagasse, corn stover, switch grass etc. Thus, it is a potential technique for pretreatment of lignocellulosic material (Holtzapfle *et al.*, 1992). AFEX pretreated corn grain gave 1.5-3.0 fold higher enzymatic hydrolysis compared to untreated substrate (Shao *et al.*, 2010). It gave 70% glucan conversion after 72 h of hydrolysis for 6% glucan loading which gave 93% ethanol yield.

## B. ACID TREATMENT:

Acid pretreatment utilize different acids as catalyst which have stronger effect on hemicelluloses and lignin. Its main goal is to solubilize the hemicelluloses fraction to make cellulose more accessible to enzymatic hydrolysis. Treatment with acids can be classified into two groups, treatment with concentrate acid or with dilute acid. Dilute acid pretreatment is preferred for ethanol production because concentrated acid treatments are responsible for generation of many inhibitory compounds. Moreover equipment corrosion and acid recovery are another major drawback associated with concentrated

acid utilization. Dilute acid treatment can be given at high temperature (180 °C) during a short period of time or at lower temperature (120 °C) for longer retention time (30-90 minutes). It solubilise the hemicelluloses especially xylan and converting hemicelluloses to fermentable sugars.

Various acids like H<sub>2</sub>SO<sub>4</sub>, HCl, H<sub>3</sub>PO<sub>3</sub> and HNO<sub>3</sub> are used to treat lignocellulosic material. Types of acids used are depends on the nature of feedstock. Depending on the treatment temperature sugar degradation products like furfural, Hydroxyl Methyl Furfural (HMF) and aromatic lignin degradation components are formed. These inhibitory components may interfere in the subsequent fermentation step.

➤ **Dilute Acid Pretreatment:**

Dilute acid pretreatment is cheap and effective because of easy availability and cheapness (Kim *et al.*, 2005). Dilute acid treatment require high temperature (120-210 °C) and pressure to achieve considerable saccharification. Usually 0.2-2.5% v/v acid concentration is selected (R. Kumar *et al.*, 2009). Dilute HNO<sub>3</sub> treatment gave highest glucose concentration compared to dilute H<sub>2</sub>SO<sub>4</sub> in case of rye straw (Lenihan., 2010). Nitric acid pretreated biomass releases the byproducts which can't be washed by simple washing (Tutt *et al.*, 2012). Dilute H<sub>3</sub>PO<sub>4</sub> were applied on bamboo and corn cob at 170 °C for 45 minutes and 140 °C for 10 minutes respectively yield high sugar (Hong *et al.*, 2012). Thin branches and leaves of olive tree were treated with dilute H<sub>2</sub>SO<sub>4</sub> (Conc. 0.2-1.4% and temperature 170-210 °C). Highest sugar yield i.e. 48.6% was obtained at 170°C and 1.0% acid concentration (Cara *et al.*, 2008). Combined treatment of HCl and H<sub>2</sub>SO<sub>4</sub> do not have any significant role in saccharification (Heredia-Olea *et al.*, 2012). However, when combined treatment of H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub> were given to oil palm, it releases highest xylose (Zhang *et al.*, 2012). Weak acid treatment releases a good amount of hemicelluloses sugars but sometimes its further degradation produce furfural and hydroxyl methyl furfural which acts as a strong inhibitors to fermentation process.

➤ **Strong Acid Pretreatment:**

This treatment involves the utilization of concentrated acid because they are strong hydrolytic agents for cellulose (Sun and Cheng, 2002). Moreover, they provide flexibility for feedstock selection and give high monomeric sugar. Various acids like H<sub>2</sub>SO<sub>4</sub> (65-86%), H<sub>3</sub>PO<sub>4</sub> (85%), HCl (41%) were used for the pretreatment purposes. Pretreated content is diluted with the help of deionised water for saccharification to take place at moderate temperature (70-121°C). Strong H<sub>3</sub>PO<sub>3</sub> is effective at low temperature and dissolves cellulose in presence of H<sub>2</sub>O, forms no inhibitory compounds and yields high sugar upon hydrolysis compared to weak acid treatment. Process efficiency is affected by process time, temperature, acid concentration and acid/biomass ratio.

The major drawback of this method is the formation of different types of inhibitors like furans, carboxylic acids and phenolic compounds at low pH which affect the microbial fermentation (Taherzadeh *et al.*, 1999) as well as increases the downstream processing cost. Another drawback of this treatment is corrosion of reactor wall and also the recycling of acid to reduce its cost. Many companies commercialize the strong acid hydrolysis of lignocellulosic biomass for microbial fermentation (Bluefire ethanol, 2010; Biosulfurol, 2010).

### C. OXIDATIVE DELIGNIFICATION:

Various oxidizing agents such as H<sub>2</sub>O<sub>2</sub>, ozone and air or oxygen are used to treat lignocellulosic materials. They react with aromatic ring and convert lignin polymer into acid like carboxylic acid. So neutralization is carried out prior to fermentation. These agents also affect the hemicelluloses fraction and make easy accessibility towards the enzymatic hydrolysis.

➤ **Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>):**

2% H<sub>2</sub>O<sub>2</sub> at 30 °C leads to dissolution of about 50% lignin and also most of the hemicelluloses have been degraded. The enzymatic hydrolysis after the pretreatment yields as high as 95% fermentable sugars.

➤ **Ozonolysis**

Ozonolysis can be performed at room temperature and at normal pressure. It is widely utilize to treat wheat straw and rye straw (Garcia-Cubero *et al.*, 2009), cotton straw (Silverstaien *et al.*, 2007), bagasse and poplar among others (Kumar *et al.*, 2009). Ozone is strong oxidizing agent which is associated with delignification process (Shatalov, 2008). It reacts with



lignin based aromatic compounds and produce degradation products. Ozone is sparged over reaction mixture to solubilize lignin and hemicellulose for specific period of time and temperature. 90% sugar yield was obtained during combined pretreatments of ozone and wet disk milling process (Barros *et al.*, 2013). Ozonolysis yields 89.7% glucose and 48.8% xylose under optimized condition in case of sugar cane bagasse. It requires large amount of ozone which make process economically unviable.

#### ➤ **Wet Oxidation:**

Wet oxidation (WO) is an alternative to steam explosion which is widely used pre-treatment process (Ahring *et al.*, 1999). This treatment utilizes air or oxygen along with water at high temperature and pressure for delignification (McGinnis *et al.*, 1983). At industrial level, it is used to treat waste having high organic matter by oxidation of suspended or soluble materials at high temperature (150-350 °C) and high pressure (5-20 MPa) (Jorgensen *et al.*, 2007).

At 185 °C temperature and 5 bar pressure for 15 minutes 67% of cellulose is obtained from rice husk while 7% and 89% hemicelluloses and lignin were removed (Beccari *et al.*, 2001). Similarly WO treatment with Na<sub>2</sub>CO<sub>3</sub> and subsequent enzymatic hydrolysis of oilseed rape, winter rye and faba produced ethanol yield of 70%, 66% and 52% respectively (Tanaka *et al.*, 1997).

Wet oxidation is comparatively costly because it requires high pressure oxygen and chemicals like Na<sub>2</sub>CO<sub>3</sub>. This problem can be overcome by using air instead of oxygen in a modified process known as wet air oxidation (WAO). WAO was used to treat shea-tree sawdust which results in maximum sugar yield of 263.5 mg glucose/g dry biomass at optimum conditions of 150 °C/45 min/1% H<sub>2</sub>O<sub>2</sub>/10 bar air (Heo *et al.*, 2003).

#### **D. ORGANOSOLV PRETREATMENT:**

Organosolv treatment utilizes aqueous or organic solvents to extract lignin. Various solvents like methanol, ethanol, acetone, glycerol etc are used to treat lignocellulosic material. This process solubilizes hemicelluloses and lignin degradation into water, CO<sub>2</sub> and various carboxylic acids (Bierre *et al.*, 1996). Solvents are mixed with water, added to feedstock and then it is heated to 100-250 °C temperature. If treatment is carried out at 185-210 °C, it requires addition of acids to accelerate the hydrolysis treatment (Nayhun *et al.*, 2010). Delignification increased and yields higher xylose when acid was added (Zhao *et al.*, 2009). Thermal wet oxidation enhances anaerobic biodegradability and methane from different biowastes (Lissens *et al.*, 2004). Organosolv treatment produces highly digestible cellulose substrate from all types of materials. Moreover it minimizes the absorption problem of cellulolytic enzymes to lignin which is reflected in lower enzyme dosage.

The main drawback involves (1) Costly to operate because of the requirements of high temperature and pressure (2) Requirement of mineral acids create an environmental concern (3) Corrosion due to acid use (4) Produce furfural, hydroxyl methyl furfural and phenols in pre-hydrolysates obtained after solvent treatment (Girio *et al.*, 2010; Zhu and Pan, 2010) (5) Need washing step to prevent lignin from precipitation (6) Recovery of expensive volatile organic solvents require efficient control system and additional energy requirements.

### **4. PHYSICOCHEMICAL PRE-TREATMENT**

#### **A. Microwave-Chemical Pretreatment:**

Microwave based treatment involves the incorporation of thermal as well as non thermal processes. Microwave uses the ability of direct interaction between a heated object and an applied electromagnetic field to increase heat (Hu and Wen, 2008). When lignocellulosic material was treated by this technique, it selectively heats the more polar part and disrupts recalcitrant structure. Biomass first immersed in the water along with various chemicals to yield high amount of sugars. NaOH were found to be more effective compared to Na<sub>2</sub>CO<sub>3</sub> and Ca(OH)<sub>2</sub> for the treatment of switch grass (Hu and Wen., 2008; Keshwani and Cheng, 2010). Rice straws were also treated for by the same method which partially disrupt the lignin and make cellulose more accessible to enzymatic hydrolysis (Singh *et al.*, 2010).

Microwave treatment along with acid leads to the degradation of hemicellulose content. Crystalline form of xylose is obtained when treated by microwave/acid /alkali as well as by microwave/acid/alkali/H<sub>2</sub>O<sub>2</sub> pretreatment. Enzymatic

hydrolysis yield less glucose compared to microwave, acid, alkali and H<sub>2</sub>O<sub>2</sub> treatment. The advantages of this treatment are: produce less quantity of inhibitors and cost effective.

#### **B. Ionic Liquids:**

Ionic liquids are salts which are composed of large organic cations and small inorganic anions. They are liquid at room temperature and have notable characteristics like nonflammability, thermal stability, chemical stability, wide liquid temperature range, good solvating properties for various types of feedstock (Hayes, 2009). They dissolve large amount of cellulose (Swatloski *et al.*, 2002). They convert lignocellulosic material into fermentable sugars by two pathways: (1) Biomass treatment to improve enzymatic hydrolysis and (2) Transformation from heterogeneous to homogeneous system by dissolution in the solvent. They help to separate lignin and increase cellulose accessibility under optimum condition without using alkaline or acidic solutions. They also not formed the inhibitory compounds. Ionic liquids are used to pretreat lignocellulosic material like bagasse (Dadi *et al.*, 2006), wood (Lee *et al.*, 2009) wheat straw (Li *et al.*, 2009). Reducing sugars were released from wheat straw i.e. 54.8% when treated with 1-ethyl-3-methyl imidazolium diethyl phosphate after being enzymatically hydrolyzed for 12 h (Li *et al.*, 2009). When biomass was treated using ionic liquids, toxicity to enzymes and fermentative microorganism must be evaluated (Yang and Wyman, 2008; Zhao *et al.*, 2010)

#### **C. Liquid Hot Water (LHW):**

It is a hydrothermal treatment which uses water at high pressure and lead to changes in the structure of lignocellulosic material. It doesn't require any catalyst or chemical. It uses high temperature (150-230 °C) for variable time ranges from seconds to hour (Girio *et al.*, 2010; Hu *et al.*, 2008). This treatment solubilized hemicelluloses but do not affect the cellulose and lignin. Lignin is partially depolymerised and solubilized but only water can't leads to complete delignification (Cara *et al.*, 2002). LHW removes 80% hemicelluloses to enhance the enzyme digestibility for e.g. corn stover (Mosier *et al.*, 2001), sugarcane bagasse (Laser *et al.*, 2002) and wheat straw (Peres *et al.*, 2007; Peres *et al.*, 2008).

Advantages of LHW involves: (1) It has cost saving potential (2) Catalysts are not required (3) Low corrosion allows construction of low cost reactor (4) Produce lower lignin product which act as a inhibitors. The main limitations of this pretreatment are: (a) requires high amount of water (b) requires high energy input (c) It is not developed at commercial level.

#### **D. Sulfite Pretreatment:**

This treatment is widely used to treat woody biomass which has recalcitrance nature. Wood chips are treated with the help of sodium sulfite or sodium bisulfate (Na<sub>2</sub>SO<sub>3</sub>, NaHSO<sub>3</sub>) followed by mechanical size reduction (Zhu *et al.*, 2009). Effectivity of this treatment depends on the dissolution of hemicelluloses, depolymerization of cellulose, partial delignification, partial sulfonation of lignin and increasing surface area through disc milling (Tian *et al.*, 2010). Sulfonation is enhanced in presence of volatile organic solvents e.g. ethanol which reduce surface tension. Corn cob was treated with sulfite and ethanol yields 81.2% glucose. Lignin is hydrolysed and dissolved in organic phase and is easily recovered in the pure form. Combined action of sulphite and formaldehyde yields high sugar. Corn stover treated with sulphite at 140 °C yields 92% lignin removal and 78.2% total sugar after enzymatic hydrolysis. The major advantages of this treatments are (1) To produce lower amount of degradation product i.e. furfural and hydroxyl methyl furfural, (2) High sugar yield, (3) Effective lignin removal, (4) Recovery of biomass components in less chemically transformed forms. Drawback involves: (a) Sugar degradation at severe condition, (b) Large volume of water is required in post treatment washing; (c) High cost is associated in recovery of chemicals.

#### **E. Supercritical Fluids:**

These are compounds that are in a gaseous form but are compressed at temperature above their critical point to a liquid like density. This treatment removes lignin effectively and increase substrate digestibility. Ethanol addition enhances the lignin extraction. Many fluids like water, CO<sub>2</sub> and ammonia are widely used. CO<sub>2</sub> is nontoxic, noninflammable, leaves no harmful residues, inexpensive and readily available (Gao *et al.*, 2010). Formation of carbonic acid in aqueous solutions leads to the polymer hydrolysis. CO<sub>2</sub> explosion is cost effective and formations of inhibitors are less compared to other pretreatments (Zheng *et al.*, 1998). Enzymatic hydrolysis after CO<sub>2</sub> explosion was checked for woody material (Kim and

Hong, 2001) and also for agricultural residues such as rice straw (Gao *et al.*, 2010). CO<sub>2</sub> utilization could be an attractive alternative to reduce costs but requirements of high pressure is the major concern.

## 5. BIOLOGICAL PRETREATMENT

Biological pretreatment utilizes the microorganisms such as brown, white and soft rot fungi to degrade lignin, hemicelluloses and little part of cellulose. White rot fungi are the most effective for the treatment of lignocellulosic materials (Sun *et al.*, 2002). Rice straw were treated using four white rot fungi (*Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, *Trametes versicolor* and *Pleurotus ostreatus*) and increases the enzymatic hydrolysis (Taniguchi *et al.*, 2005). Office paper wastes were treated with two bacterial strains (*Sphingomonas paucimobilis* and *Bacillus circulans*) for better enzymatic hydrolysis (Kurakake *et al.*, 2007) and obtained 94% sugar. Biological treatment not only removes lignin but also removes specific antimicrobial components (Srilatha *et al.*, 1995).

Biological pretreatment requires lower energy input, no chemical and mild environmental conditions. The only drawback of this treatment method is, the rate of biological hydrolysis is usually very low, so requires long residence time (Cardona and Sanchez, 2007; Sun and Cheng, 2002; Tengerdy and Szakacs, 2003).

## 6. CONCLUSION

To reduce the serious environmental threat i.e. global warming more efficient, sustainable and eco-friendly technology must be develop to prevent green house gas emissions which would otherwise are responsible for environment pollution. Lignocellulosic materials have immense potential to be converted into biofuel. So, various pretreatments (Physical, Chemical and Biological) were applied to make hydrolysis process eco-friendly. One important outcome is that not a single treatment offers 100% conversion of lignocellulosic materials into fermentable sugars. Although combination of more than two treatments provide a promising results but still extensive research is required in this area to develop more efficient treatment process. When lignocellulosic materials are treated with acid or alkali first and then with cellulase enzyme leads to better saccharification. So, by standardizing the time period for acid or alkali treatment and also for enzymatic treatment better saccharification is achieved. After successful development of effective pretreatment method for lignocellulosic material, it is responsible for the generation of greater amount of fermentable sugar. This leads to greater production of bioethanol by using appropriate microorganism. Bioethanol can be used as a fuel in vehicles as a substitute of petrol and diesel. Now a day ethanol is used as a fuel as a blending with petrol. The remaining lignocellulosic materials after saccharification process can be used for production of fertilizers, as an energy source by burning or it can be used as a feed constitute for animals. Treated lignocellulosic material can also be utilized for the preparation of cake which is used as an absorbent. This cake can efficiently treat the waste water to reduce the load of total organic dissolved matters or it can also be used to treat industrial effluents which have many hazardous chemical particles. So, problems regarding environmental pollution can be reduced by effectively using lignocellulosic materials as an alternative fuel source.

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