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Review Article

An overview of pre-treatment methods used for bioconversion of lignocellulosic biomasses into valuable products

Raazia Kiran[1], Alya Riaz[1], Muhammad Irfan[1*], Hafiz Abdullah Shakir[2]

[1]*Department of Biotechnology, University of Sargodha, Sargodha Pakistan*
[2]*Department of Zoology, University of the Punjab new campus Lahore Pakistan.*

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Abstract
Lignocellulose for biofuels production is considered as chief renewable biomass source. Ethanol produced from lignocellulosic biomass is extensively recognized as an additive or as a substitute to fossil fuel due to its trivial influence on the environment. Second generation (2G) or cellulosic bio-ethanol is produced from the biomass in three main steps that are pre-treatment, hydrolysis, and fermentation. The pre-treatment method involves the use of different physical processes *i.e.,* size reduction, steaming or boiling, ultrasonication, and bursting, chemical methods *i.e.,* solvents, acids, salts, bases, the physicochemical processes include liquid hot water and AFEX (ammonium fibre explosion), biological methods involving white-rot/brown-rot fungi and bacteria, and quite a few combinations thereof to breakdown the lignocellulose into its components. Pre-treatment process changes cellulose morphological, chemical and physical features, making it prone to enzymatic attack for saccharification. In this review, we have discussed different pre-treatment methods and their effect on recalcitrant structure of lignocelluloses for making it readily available for enzymatic hydrolysis

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INTRODUCTION

Lignocellulose, a common biopolymer, is the most vital resource considered for ethanol production (Zhang and Cai, 2008). Lignocellulosic biomass which can be obtained from hard and softwood, grasses, plant or agricultural wastes, are abundantly found in nature (Ruangmee and Sangwichien, 2013).CO_2 emission has been reduced significantly by producing ethanol using lignocellulosic materials (Binder *et al*., 2009). It has distracted attention as an energy source that lessens greenhouse gas emissions up to 85% (García-Aparicio *et al.,* 2011). Primarily lignocellulosic material is 50%-80% carbohydrates. These are polymers of 5C and 6C sugar units (Wingren *et al*., 2005). Lignocellulosic biomass consists of 40-50% w/w cellulose (Yang and Wyman, 2008).

The structure of lignocellulosic mass is made up of lignin, cellulose and hemicellulose. Cellulose, β1-4 linked chains of glucose molecules are most abundant component of lignocellulose. Polysaccharide layers are connected through hydrogen bonds that provide to cellulose crystallinity and for degradation making it unreachable. Hemicellulose is the second main component of lignocellulosic mass that is comprised of different pentose and hexoses like glucose, arabinose, galactose, xylose and mannose. Lignin is third important lignocellulose component that is comprised of polymerization of three core phenolic elements, sinapyl alcohol (S), p-coumaryl alcohol (H) and coniferyl alcohol (G). Phenolic components ratio of polymerization is different among wood tissues and cell wall layers. Structure of macrofibrils facilitates inorganizational stability of lignocellulose cell wall. Macrofibrils are organized microfibrils that contain lignin, cellulose and hemicellulose (Rubin, 2008).Being a developing country, Pakistan is struggling hard to meet the demand for power generation. Pakistan's economy is largely agricultural-based. Substantial amount of sugarcane, wheat straw, rice husk and maize and other crop

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*Corresponding author: m.irfan@uos.edu.pk

residues in the form of waste are produced in the country. The annual production of crop residues has the potential to generate 76% of the annual electricity requirements of Pakistan. Pakistan has already made a start on a biomass electricity generation strategy using relatively small biomass generator sets that could take all the waste biomass from the surrounding agricultural area (Saeed *et al* 2015).

Lignocellulosic biomass processing

Production of bioethanol involves pre-treatment, fermentation and hydrolysis of lignocellulosic biomass. Through hydrolysis lignocellulosic biomass is convertedinto fermentable reducing sugars to produce fuels such as butanol and ethanol. The efficiency of hydrolysis process for the conversion of cellulose toits integral sugars is dependent upon pre-treatment step. Enzymatic hydrolysis of lignocellulose is directly affected by accessible surface area (ASA), indirectly by chemical composition (lignin, hemicellulose, acetyl group), factors related to material's structure (particle and pore size, volume, specific surface area SSA), and factors related to cellulose structure (crystallinity of cellulose, degree of polymerization) (Zhao *et al.,* 2012a).Hydrolysis of cellulose is affected by the factors such as porosity of the lignocellulosic materials, crystallinity of cellulose fiber and components of both hemicellulose and lignin. The accessibility to cellulose of acids and cellulase enzymes become tougher due to the presence of hemicellulose and lignin, because of this, the process of hydrolysis is efficiently reduced. The biomass structure and size require pre-treatment to amend it, and also its chemical composition, that's why carbohydrate hydrolysis to monomeric sugars from fraction can be achieved fast with better yields. Acids or cellulase enzymes usually catalyze the hydrolysis, and yeasts or bacteria carry out the fermentation. The hydrolysis process can be enhanced significantly by the reduction of cellulose crystallinity, removal of hemicellulose and lignin, and by increasing porosity through pre-treatment processes (McMillan, 1994). Cellulose accessibility increases by pre-treatments with changing indirect factors such as volume, pore and particle size, surface area (biomass structure-relevant factors), lignin, hemicelluloses, and acetyl group (chemical compositions), and degree of polymerization and cellulose crystallinity (cellulose structure-relevant factors) (Sun and Cheng. 2002). For successful biomass conversion an efficient pre-treatment is critical.

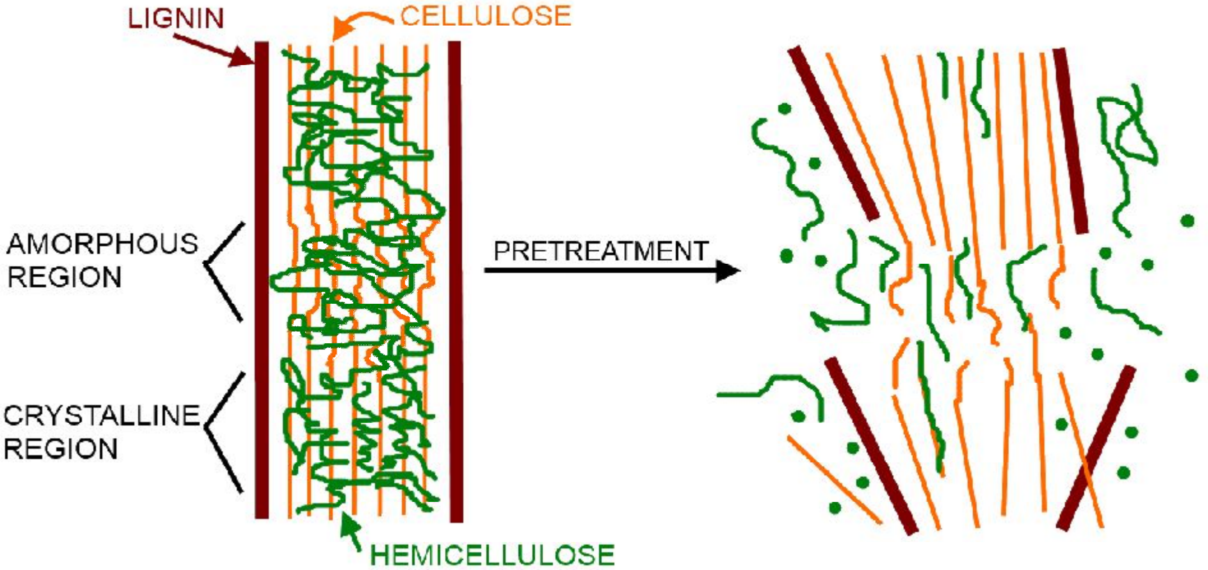

Figure 1. Effect of pre-treatment on structure of lignocellulose (Mosier *et al.,* 2005).

Pre-treatment

Pre-treatment is needed to make cellulose susceptible to enzymatic attack before enzymatic hydrolysis (Mosier *et al.,* 2005). For direct hydrolysis of cellulose, natural lignocellulosic structural and physiochemical properties are persistent. Pre-treatment process changes morphological, chemical and physical features of lignocelluloses and cellulose, making it prone to enzymatic attack for saccharification.

Numerous pre-treatment techniques involves: (1) physical (grinding, milling), (2) chemical (acidic, alkaline), (3) thermal (steam), (4) biological (fungi) and combination of these. Pre-treatment of biomass increase its porosity, particle size and decrease cellulose crystallinity, hence surface area and enzymatic susceptibility increase (Fig. 1). In bioethanol production, pre-treatment is a key step that characterizes process chief commercial cost. An optimal pre-treatment method should be capable of, increasing fermentable sugar release, degradation of cellulose, enhancing enzymatic hydrolysis, increasing glucose production and above all should be economically and environmentally feasible (Alvira *et al.,* 2010). Generally pre-treatment processes can be divided into distinctive types: physical (grinding and milling), physicochemical (steam pre-treatment/auto-hydrolysis, hydrothermolysis and wet oxidation), chemical (dilute acid, alkali, organic solvents and oxidizing agents), electrical, biological or a combination of these. These pre-treatment techniques have promise for cost effective pre-treatment of lignocellulosic materials for their biological conversion to chemicals and fuels.

Physical

Physical pre-treatment involves pyrolysis, mechanical commination (Sun and Cheng. 2002). Singh and Chouhan (2014) used 212 $\mu\mathrm{m}$ size biomass in reactor, as large size particles do not transfer heat efficiently. Larger particles size cause resists heat transfer which affecting bio-oil production. Gas and charcoal production also increases by large particle size whereas small particle size has higher potential for heat transfer and bio-oil production. Smaller size particles and increases in temperature increase heating rate and decreases charcoal yield (Jun *et al*., 2009).

Mechanical

In this method, simply the biomass break mechanically into smaller pieces. Mechanical commination comprises chipping, milling, grinding and need high energy involvement (Sun and Cheng, 2002). Particle size reduces through a combination of chipping, milling and grinding. Generally, after grinding or milling particle size reduction is 0.2mm to 2mm and after chipping 10mm to 30mm (McMillan, 1994). Cellulose crystallinity mainly disrupts by mechanical commination, reduced degree of polymerization, through biomass degradation cellulose specific surface area enhances into smaller particles and make cellulose more prone to enzymatic hydrolysis. Limitations for mechanical commination are it is energy intensive, cost expensive and time consuming. In cellulose accessibility, a major hurdle is that it has no role in delignification. This pre-treatment as compared to chemical pre-treatment is less effective and hence is not often used as a pre-treatment technique merely (Zheng *et al.,* 2009).

Irradiation

Gamma rays, electron beam irradiation and microwave irradiation are used for physical pre-treatment of lignocellulosic biomass (Taherzadeh and Karimi, 2007). High energy radiations cause significant solubilisation and partial delignification of hemicellulose, by partially removing acetyl group. It decreases degree of polymerization, cellulose crystallinity and increases available surface area (Zheng *et al.,* 2009).

Chemical pre-treatment

Among all types of pre-treatment techniques, it is the most studied. Generally chemical pre-treatment methods are acidic, alkaline, fibre/freeze explosion, ammonia, ionic liquids, catalysed steam-explosion, liquid hot water (pH-controlled) and organosolv pre-treatments (Zheng *et al.,* 2009). Use of reagents like acids, alkali, salts, solvents and oxidants have shown some promising results. The development and integration of chemical-based pre-treatment have potentially scaled up the production of ethanol to commercial levels (Bensah and Mensah, 2013).Chemical pre-treatment processes are more efficient and fast as compared to biological and physical pre-treatment methods (Mosier *et al.,* 2005).

Acidic pre-treatment

Acidic reagents are widely used for lignocellulosic biomass pre-treatment (Alvira *et al.,* 2010). Dilute acid pre-treatment efficiently recovers maximum of it as dissolved sugars and removes hemicellulose. Cellulosic sugar production enhances by hemicellulose removal and for complete hemicellulose degradation about 100% glucose produce (Mosier *et al*., 2005). Dilute acid pre-treatment is done at modest temperature *i.e.,* $< 160℃$ and at increased temperature$> 160℃$ (Silverstein *et al.,* 2007). Dilute acid pre-treatment is more effective for cellulose hydrolysis when process is carried out at high temperature (McMillan, 1994). Dilute acids expose cellulose by removing hemicelluloses and making it available for enzymatic degradation (Silverstein *et al*., 2007). The unstructured hemicelluloses in LB hydrolyse quicker in acidic media into soluble sugars andseveral oligomers than cellulose (Samuel *et al*., 2010; Foston and Ragauskas, 2010; Tao *et al*., 2011) by disrupting the xylosidic bonds and cleavage of acetyl ester groups (Chen *et al*., 2012; Tang *et al*., 2011). The lignin seal is broken down in substitution reaction accompanied by condensation reaction that prevents the dissolution (Candido *et al*., 2012; Pingali *et al*., 2010). Degradation of amorphous regions in cellulose leads to an enlarged cellulose fibrils causing (Foston and Ragauskas, 2010) a boost in the crystallinity index of the biomass (Li *et al*., 2010; Samuel *et al*., 2010). In dilute acid pre-treatment, for continuous operations, relatively high temperatures and pressures acquires high reaction time (Tao *et al*., 2011; Menon and Rao, 2012). In acid pre-treatment, the dried biomass is crushed and then soaked in water and sunken in dilute acid solution under certain temperatures for specific periods. This pre-treated substance is then filtered to split the filtrate liquid from the substrate which is washed and neutralized before saccharification. The hydrolysis process would become more effective with an increase in the hydrogen ion concentration of acid hence the more negative pKa value (Bobleter, 1994). Low acid consumption is a major pro in terms of cost saving (Galbe and Zacchi, 2002). Concentrated acid treatment, like HCl and H_2SO_4, has been extensively used for lignocellulosic cellulose hydrolysis, as they are strong hydrolytic agents (Sun and Cheng, 2002). It operates at mild temperature conditions with enhanced monomeric sugar production, and no more enzymes are further required for hydrolysis.

Sulfuric and hydrochloric acids are widely used in dilute acid hydrolysis. Sulfuric acid pre-treatment causes partial release of oligosaccharides, monosaccharides and lignin monomers (Xu *et al.,* 2012). Dilute sulfuric acid is beneficial in effectively producing soluble carbohydrates and removing most of the hemicelluloses (Manzoor *et al*., 2012).Dilute sulfuric acid at lower than 0.1% acidic levels is effective. With low acidic loadings, its yield increased cellulose digestibility, higher hemicellulose sugars (Kumar *et al.,* 2009a). Furthermore, phosphoric and sulphuric acid in low concentrations tend to release essential nutrients (S and P) that boost downstream fermentation (Zhang *et al*., 2012). The process involves 2 main stages: in the first stage, a more dilute acid is used to solubilise the hemicelluloses in biomass substrate, while in the second stage higher concentrations to hydrolyse the cellulose and the remaining hemicellulose of acid is used (Kazi *et al*., 2010; Talebnia *et al*., 2010). Phosphoric (H_3PO_4) and sulphuric (H_2SO_4) acids are economical and are competent in hydrolysing lignocelluloses, although the former gives a mild effect and is friendly to the environment (Bensah and Mensah, 2013). Hydrochloric acid (HCl) attacks biomass better than sulphuric acid (H_2SO_4) (Demirbas, 2008) whereas nitric acid (HNO_3) gives high cellulose to glucose conversion yield (Tutt *et al*., 2012) although both of the acids (HCL and HNO_3) are expensive as compared to sulphuric acid. The pre-treatment of biomass with dilute acid at high temperature attains high reaction time hence improving the cellulose hydrolysis (Kumar *et al*., 2009). After pre-treatment process, the enzyme can hydrolyze the cellulose into fermentable sugars as it will be less crystalline (Satimanont *et al*., 2012). Dilute acid has been used to pre-treat various lignocellulosic biomasses like hardwood, softwood, hardwood, agricultural residues, herbaceous crops, agricultural residues, municipal solid waste and wastepaper. Being effective and inexpensive technique, sulfuric acid pre-treatment has been most extensively studied. Dilute sulfuric acid pre-treatment drawbacks are: (1) neutralization of pre-treated biomass prior to fermentation, (2) corrosion of expensive construction materials, (3) reverse solubility of gypsum with calcium hydroxide upon neutralization, (4) degradation products formation, (5) fermentation inhibitors release, (6) neutralization salts disposal, (7) particle size reduction of material is required and, (8) economic feasibility due to recent increase in sulfuric acid prices (Zheng *et al.,* 2009). Dilute acid pre-treatment is costly as compared to physicochemical pre-treatment methods like AFEX of steam explosion, negative impact on biomass enzymatic hydrolysis (Kumar *et al.,* 2009a).

Alkaline pre-treatment

Study on alkaline pre-treatment of crop residues was found effective for enzymatic digestion with low lignin content. Alkaline reagents disassociate polymers of lignocellulosic wall entirely (Li et *al.,* 2014). Lower pressure and temperature are used in alkaline pre-treatment, as associated to other pre-treatment methods. Instead of seconds or minutes time required for pre-treatment is in hours and days (Mosier *et al.,* 2005). Ester intermolecular bonds (crosslinking hemicellulose, lignin and xylan) saponification depicts alkaline treatment mode of action (Sun and Cheng, 2002). The alkaline action expands lignocellulosic biomass permeability, by disordering lignin and carbohydrate polymers ester bonds. Due to lignin solubilisation, cellulase enzymes have increased access to inner structure of lignocellulosic materials (Singh *et al.,* 2015). Alkaline hydrolysis employs the alkaline solutions such as, calcium or sodium hydroxide and ammonia for the pre-treatment of biomass. For lignocellulosic biomass pre-treatment, alkaline reagents such as sodium hydroxide are extensively used (Alvira *et al.,* 2010). It significantly removes the lignin seal and also part of hemicelluloses to make cellulose available for enzymatic digestion. This is chiefly a process of delignification in which considerable amounts of hemicelluloses are also dissolved. Using base results in breaking of the ester and the glycosidic side chains which causes alteration in structure of lignin, cellulose inflammation, incomplete decrystallization of cellulose and biased solubilizing of hemicelluloses (Ibrahim *et al*., 2011). The employment of mainly lower temperatures and pressures in contrast to dilute acid treatment, alkali pre-treatments cause low sugar degradation and caustic salts are also recovered. Disadvantages include requirement of long reaction times and high concentration of base at the room temperature. The alkaline reagent reduces the chances of attack of enzymes on cellulose and hemicelluose by removing the acetyl and acid substitutions on hemicellulose (Sills and Gossett, 2011). Pre-treatment of sodium hydroxide increases the glucose and ethanol production by improving the degradation from enzymatic hydrolysis and fermentation steps (Teater *et al*., 2011). In ammonia pre-treatment the main effect is delignification of biomass without any noteworthy outcome on contents of carbohydrate. Ammonia has been very effective reagent for the pre-treatment of substrates like agricultural residues and herbaceous feedstock that have low lignin contents. It is appropriate for simultaneous saccharification and co-fermentation in view of the fact that cellulose and hemicelluloses are not lost by pre-treated biomass. A delignification reagent can decrease the lignin content to very low level in treated biomass and ammonia has been proven to be a great one (Kim *et al*., 2009).

Torrefecation

A treatment of biomass thermally which consists of heating it to a modest temperature (200 and 300°C) under inert or nitrogen atmosphere is called torrefaction (Prins *et al*., 2006). This process depolymerizes the long polysaccharides by removing the moisture and low weight organic components. Due to torrefaction there is no inhibition in enzymatic hydrolysis and it can be easily grinded saving energy. On the other hand a number of dehydration and de-carboxylation reactions increases the friability and loss of mass of wood

Ozonolysis

In this, through ozone treatment, lignin content of lignocellulosic biomass is degraded, improving treated biomass in vitro digestibility (Kumar *et al.,* 2009a).It degrades the lignin structure by cutting the aromatic rings while the cellulose and hemicellulose are hardly sullied. It can degrade various lignocellulosic biomasses like cotton straw, bagasse, poplar dust, wheat straw, pine and peanut. Ozonolysis can operate in meek environment. Hence it is environmentally friendly as because of its short half-life it does not leave any residues. Unlikely the need for large amount of ozone makes it very expensive (Sun and Cheng, 2002).

Organosolv pre-treatment

For organosolv pre-treatment of lignocellulosic materials, organic solvents and their aqueous solutions are used. Lignin synchronised with hemicellulose solubilization are removed by organosolv pre-treatment method. Catalysed (base or acid) or uncatalyzed treatment, at 100 to 250℃ with organic solvent or aqueous organic solvent mixture, causes distraction of internal hemicellulose and lignin bonds (Zhao *et al.,* 2009). Hemicellulose solubilisation and substrate digestibility enhances by catalysed treatment. Organosolv pre-treatment uses ethylene glycol, ethanol, methanol, triethylene glycol, acetone, tetrahydrofurfuryl alcohol glycerol, aqueous n-butanol and aqueous phenol (Taherzadeh and Karimi, 2008). Oxidation of Lignin increases carboxyl group, that's why substrate hydrophilicity increased (Zhao *et al.,* 2012b). Lignin removes in results of pre-treatment due to of lignin-hemicellulose and lignin internal bondshydrolysis. Lignocellulosic biomass structure loosens by lignin and hemicellulose dissolution results in depolymerisation and increase in ASA. In the pre-treatment, organic solvents used must be removed and recycled (evaporated and condensed), to decrease the functioning expenditures. In an organic medium lignin is solubilised hence providing the residual cellulose for enzymatic hydrolysis. Organosolv is an effective approach for pre-treatment. Lignin recovered after precipitation is highly pure and is sulfur free making its molecular weight low. Hence due to fiscal basis as in considering the cost and its properties, the aqueous ethanol is preferred but high pressure is required for the process hence increasing the cost of the equipment (Zhao *et al*., 2009).

Aqueous ammonia pre-treatment

In this process through reactor (column reactor) aqueous ammonia passed in a flow covering lignocellulosic biomass, named as ammonia recycled percolation pre-treatment ARP. Lignocellulosic biomass is reacted with 5-15wt% ammonia conc. with about 5ml/min percolation rate, at 140-210℃ fixed reactor temperature, for 5-90min time. ARP pre-treatment efficacy is mostly affected by ammonia concentrations, amount of liquid through put, reaction time and temperature (Mosier *et al.,* 2005).

Physiochemical

Lignocellulosic biomass is pre-treated to make it available to enzymatic attack. Numerous pre-treatment methods with diverse mode of action and process characteristics are used. Physiochemical pre-treatments are very imperative; like using organic solvents (organosolv pre-treatment) *e.g.,* ethanol, steam pre-treatment with count of acid SO_2, H_2SO_4 or organic acids, using ammonia or lime alkali pre-treatment (Mosier *et al.,* 2005).

Liquid hot water pre-treatment

In liquid hot water (LHW) pre-treatment, biomass is immersed in liquid water at high temperatures around 180–230°C and high pressures. It does not require any chemical reagents furthermore 50% of substrate is dissolved which more or less completely removes hemicelluloses along with 5–20% removal of cellulose also. Lower concentrations of inhibitory derivatives are generated by LHW which is a environmental friendly approach (Hendriks and Zeeman, 2009). For lignocellulosic biomass pre-treatment, liquid hot wateris a hydrothermal process (Taherzadeh and Karimi, 2008). Liquid hot water pre-treatment involve biomass water saturation sequential heat treatment, at 120-200℃ temperature, under pressure for 5-15min reaction time (Kim *et al.,* 2009), and at controlled 4-7 pH to decrease sugar degrading products and production of monomeric sugar (Mosier *et al.,* 2005). LHW pre-treatment solubilizes hemicellulose, making cellulose more responsive to successive enzymatic attack, and prevents inhibitors formation like hydroxymethyl furfural HMF and furfural (Alvira *et al.,* 2010). This pre-treatment technique, accomplished easily with little energy usage and no trouble in handling or chemical recovery ignoring the apparatus erosion (Cybulska *et al.,* 2010).

Steam explosion pre-treatment

This pre-treatment is one of the extreme favourable physiochemical pre-treatment. Steam explosion is recognized as a significant pre-treatment technique for the production of bioethanol and biogas, from more than ten different lignocellulosic biomass materials (Taherzadeh and Karimi, 2008). Lignocellulosic biomass composition and cell wall structure modification occur in this. Highly saturated pressure steam is applied to biomass and rapidly releasing cause explosive decompression. It is also named as thermo mechano chemical method due to its lignocellulosic biomass degradation through heat of steam (thermo), glycosidic bonds hydrolysis (chemical) and moisture expansion (mechano) (Sun and Cheng. 2002). Chemical impact encompasses by steam explosion, as hemicellulose connected acetyl groups produce acetic acid that catalyses the hydrolysis and decomposition of glucose or xylose. Enzymatic availability increasing due to hemicellulose removal and reduction of particle size (Mosier *et al.,* 2005), it is regarded as cost effective and environmentally feasible pre-treatment method (Alvira *et al.,* 2010). Catalysed (base, acid) water heat pre-treatment is generally known to increase cellulose accessibility for enzymatic hydrolysis of cellulases. Usually 50-300mM dilute sulfuric acid, at 100-200℃ temperature is used to degrade the lignin structure and make cellulose prone to enzymatic hydrolysis (Yang and Wyman, 2008). Kang *et al.* (2013) reported for steam explosion SO_2 catalysed as an efficient, cost effective pre-treatment method, for generation of bio-ethanol from softwood. In physiochemical pre-treatment, steam explosion is the most favourable. High pressure steam and rapid release results in explosive decompression which modifies the structure of cell wall and biomass composition.

Pyrolysis

This pre-treatment involves breakdown of substrate through heating at high temperature. It is used with other techniques in combination, as for results optimization with acid hydrolysis (Sun and Cheng, 2002).

Ammonia fibre/ freeze explosion

Liquid ammonia exposure used in physicochemical pre-treatment technique at increased temperature and pressure to lignocellulosic biomass, for a time duration and sharply reducing pressure, as like in steam explosion (Kumar *et al.,* 2009a). AFEX pre-treatment method resembles with steam explosion pre-treatment. In AFEX pre-treatment, per kg of dried lignocellulosic material is treated with one to two kg of liquid ammonia, at 90℃, and reaction time 30 min. AFEX pretreatment significantly increases sugar production of different herbaceous crops and grasses (Sun and Cheng, 2002). AFEX results in lignin solubilisation, hemicellulose hydrolysis and depolymerisation, increases porosity and decreases cellulose crystallinity subsequently improving enzymatic hydrolysisrate (Mosier *et al.*, 2005). Although AFEX pre-treatment enhance rate of cellulose hydrolysis of grasses, influence on high lignin content biomass (*i.e.,* soft wood, hard wood) is insufficient. After pre-treatment to keep the environment safe and to decrease production cost ammonia must be recycled (Sun and Cheng, 2002).In liquid ammonia pre-treatment called AFEX, the substrate along with liquid ammonia is kept under high temperature and pressure and abruptly releasing the pressure similar to steam explosion approach. Due to high pressure the ammonia is vaporized which is recovered and recycled (Bals *et al*., 2011).

Ionic liquid (IL) pre-treatment

Ionic liquid pre-treatment used to treat Switch grass that results in amorphous cellulose regeneration and degradation of hydrogen bonds, which makes cellulose prone to cellulase (Sun and Cheng, 2002).In contrast to organic solvents the ionic liquid salts are formed by large organic cations and small inorganic anions. ILs have low vapor pressure, posses possible electronic interaction due to presence of organic cations and have high chemical and thermal stability (Zavrel *et al*., 2009).

Wet oxidative pre-treatment

In this technique lignocellulosic biomass is treated with water or oxygen for 30min and at a temperature higher than 120℃ that is between 148 and 200℃ (Palonen *et al.,* 2004). This is a suitable pre-treatment method for lignocellulosic biomass having slight lignin content (Galbe and Zacchi, 2002).

Lime pre-treatment

Lime is present as slaked lime $Ca(OH)_2$and quick lime CaO that is available in least prices, at alkaline pH it can be a alternative for removal of lignin. Substrate is treated with 0.1g slaked lime $Ca(OH)_2$/g of biomass lime and water H_2O 5-15g/g of biomass for 1-2h at 100℃. Woody biomass have increased lignin content that's why lime pre-treatment is less efficient for it as compared to herbaceous plants and agricultural residues. As compared to ammonia and other bases like NaOH, lime works slowly. Lime pre-treatment cause delignification of biomass and also improves digestibility of cellulose during hydrolysis by opening cellulose structure and decreasing adsorption of unproductive cellulases. Lime is easily accessible in deposits of limestone with relatively safe handling. Biomass is eroded with water, and saturated with carbon dioxide CO_2, which produce precipitates of calcium carbonate. Lime can be renewed and separated for recycling and served to a limekiln (Chang *et al.,* 1998). Optimum conditions for lime pre-treatment reported as $Ca(OH)_2$ 0.075 g/g of biomass, H_2O 5g/g of biomass, at 120℃ temperature for 4 h. Lime pre-treatment provide more surface area with less elimination of hemicellulose and slight removal/change in lignin structure (Bridgwater, 2003).

Carbon dioxide explosion pre-treatment

A technique called carbon dioxide explosion uses CO_2 as supercritical fluid (SC–CO_2) for pre-treatment of biomass. Zheng *et al*. (1995) first reported that by using Avicel substrate, pre-treatment can be done by SC-CO2 explosion pre-treatment. Avicel is a commercially available pure form of cellulose. Results of glucose yield were good after testing the method on recycled paper and sugarcane bagasse (Zheng *et al.,* 1998).This pre-treatment method resembles to heat explosion and ammonia fibre (Sun and Cheng, 2002). Lignocellulosic biomass pre-treatment through supercritical CO_2 explosion (SC-CO_2) works at lower temperature as compared to steam explosion and have little cost then ammonia explosion. Possible mechanism suggested that carbonic acid (CO_2 dissolved in H_2O) would have amplified hydrolysis. Carbon dioxide (CO_2) molecules have same size as that of water and ammonia would enter through tiny openings reachable to the molecules of ammonia and water (H_2O). Carbon dioxide (CO_2) hydrolyses hemicellulose and cellulose, and acidic decomposition of monosaccharides avoids through low temperature. Pressure explosive release break cellulose structure and available surface area for hydrolysis also increases (Kumar *et al.,* 2009a). CO_2 used in this method is a green solvent. For one mole of ethanol produced, one mole of $CO_2$is released as a byproduct. This CO_2released is reused for pre-treatment of biomass without emitting it into the atmosphere. SC-CO_2 increases the acidity and it diffuses into the crystalline structure of cellulose which helps in the pre-treatment process. Later through the explosion, area for the attack of cellulase enzyme is increased as it deteriorates the structure of cell wall (Gu, 2013). Supercritical CO_2 (SC-CO_2) in a high pressure vessel explosion increases lignocellulose biomass digestibility or at high pressure 1000-4000 psi, SC-CO_2 delivered. In biomassCO_2 penetrates at high pressure, hydrolyse hemicellulose by reacting with water and forming carbonic acid. Biomass structure disrupts with pressurized gas and its susceptibility increases (Zheng *et al.,* 1995). Carbon dioxide (CO_2) explosion pre-treatment is cost effective, enhances reachable surface area, hemicellulose and lignin structures are not modified, and inhibitory compounds does not produce (Kumar *et al.,* 2009a).

Biological

In biological pre-treatment microorganism's ability uses to destroy lignin structure that make easy accessibility to hemicellulose and cellulose (López-Abelairas *et al*., 2013). Species of fungi are used in biological pre-treatment for degradation of lignin. These processes are environment friendly (Sanchez, 2009).Brown, soft rot and white fungi degrades lignin and hemicellulose (Sun and Cheng, 2002). Fungal enzymes decompose the plant cell wall. White rot and brown fungi produces H_2O_2 (hydrogen peroxide) that release free hydroxyl radicals (•OH) by Fenton reaction. Lignin and polysaccharides in plant cell wall are cleaved through the attack of •OH causing the penetration of lignocellulolytic enzymes produced by fungi (Dashtban *et al.,* 2009). Diverse lignocellulosic materials have been effectively pre-treated using white rot basidiomycete, *Irpex lacteus* (Lopez-Abelairas,*et al.,* 2013). Use of microbes like *Phanerochaete chrysosporium* commonly known as white-rot fungi, is viable for biomass delignification (Sánchez, 2009).From different lignocellulosic biomass *P. chrysoporium* can degrade excessive amounts of lignin. Biological pre-treatment is beneficial over conventional chemical pre-treatment methods due to its low cost and less negative influences on environment (Sun and Cheng, 2002). Also it does not produce toxic compounds, but at drawback for minor decrease in sugar production longer residence time (Lee, 1997) almost 14 days, than other methods (Sun and Cheng, 2002).

Conclusion

Demand of fuel is expected to increase in near future due to augmented industrial use for the improvement of a country's economy. Fermentation of sugars in fuel ethanol industry is based on several technologies which are derived from starch and sugar crops. The lignocellulosic biomass includes agricultural residues, wood products, yard waste and forestry residues etc which are renewable resource and affordable fuel ethanol can be produced through them. These biomasses approximately contains around 50-80% carbohydrates that are pre-requisite in the ethanol production. However the physiochemical and structural composition of lignocelluloses is a major hurdle to be overcome for direct enzymatic hydrolysis. Hence an effective pre-treatment is mandatory to provide the cellulose acquiescent to enzymatic hydrolysis

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