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Short Communication

Biological pretreatment of lignocellulosic biomass (Water hyacinth) with different fungus for Enzymatic hydrolysis and Bio-ethanol production Resource: Advantages, Future work and Prospects

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Introduction

Owing to the requirement of sustainable economies and clean environments, interest in bioenergy has sharply risen in recent years [Kumar., *et al*. 2015]. Fossil fuel combustion has created a global anxiety both for the world economy as well as environment. The excessive use of the fossil fuel leads to increase in carbon dioxide level in the atmosphere and also have Significant contribution towards global warming (Silva Lora., *et al*. 2011; Abdel-Fattah and Abdel-Naby 2012). Countries across the world have framed and setup state policies towards the consumption of biomass in order to meet their future energy demands. Besides meeting the energy demands the use of biofuels can also reduce the carbon dioxide to standardized targets as specified in the Kyoto Protocol and will also decrease our reliability on the supply of fossil fuels (Sarkar., *et al*. 2012). Keeping these prerequisites in view there is a pressing demand to adapt towards utilization of biomass as a renewable and clean energy source. Lignocellulosic biomass has attracted a global response in the production and use of biofuels. Lignocellulosic materials is a renewable, abundant and relatively cheap mixture of organic materials, principally containing polysaccharides (~75% dry weight) and lignin (~25% dry weight) [Brown., et al. 2014]. The all type of lignocellulosic plant material is mainly composed of cellulose, hemicelluloses and lignin but the proportion of these components may vary from plant to plant, but collectively they comprise approximately 90% of the dry weight [Dantur., *et al*. 2015]. The Various forms of biomass resources present are grouped in to four different categories such as: i) wood and agricultural products ii) solid waste iii) landfill gas and biogas and iv) ethanol biodiesel. By far wood residues are largest source of biomass for energy production. These residues mainly come from wood product industry including sawmills, furniture manufacturing and paper mills. Municipal solid wastes form the next largest source of biomass for energy production. Fig. 1 shows the different category of biomass.

Biomass obtained from terrestrial plants can generally have 30-50% cellulose, 20-40% hemicellulose and 15-30% lignin's. In plants the lignin (that is composed of phenylpropanoid groups) generally acts as a polymer around the hemicellulose micro fibrils thereby binding the cellulose molecules together and safeguarding them from chemical degradation. Since lignin cannot be converted into sugars and hence is not realistic in case of biofuels production. Their degradation is a high-energy process i.e. requires high amount of energy. On the other hand cellulose and hemicellulose are easily converted into sugars. The use of agro residues towards bioethanol production may address this problem to some extent, however the operation of large-scale plants for cellulosic ethanol production still have a number of limitations such as technical knowledge, high capital investment and high shipping costs of feedstock. Water hyacinth, Eichhornia crassipes, (Liliales: Pontederiaceae), an invasive plant is native to Amazon basin (Barrett and Forno 1982). It is a free floating aquatic plant and a potential raw material for bioethanol production mainly because of its high cellulose content. In the absence of regulatory mechanisms such as natural enemies that are the main agents to keep the weed under check within its native habitat, the plants multiplies at such an alarming rate that manual, mechanical as well as chemical methods fail to bring it under control. The plant serves as a novel feedstock for enzymatic hydrolysis for bioethanol production via different biological pretreatment. Naturally grown perennial vegetations, high cellulose and low lignin content, easily degradable and pest resistance are some of the main attributes that make water hyacinth an ideal biofuels crop. The plant contains high cellulose (20%) and hemicellulose (33%) but is relatively low in lignin content (10%) (Bolenz., et al. 1990; Poddar., et al. 1991; Gressel 2008). The detailed composition polysaccharide of water hyacinth is given in Table 1.

Components	% Composition
Cellulose	25
Hemicellulose	35
Lignin	10
Ash	20
Nitrogen	03

Table 1

Biomass composition of Water hyacinth. Courtesy (Poddar., et al. 1991) (Gunnarsson and Petersen 2007).

The other biomass products that act as the sources for ethanol production are beet, wheat, sweet sorghum, corn etc. Unlike fossil fuel, biofuels has the advantages of being renewable, cleaner and producing no green house gases. Lignocellulosic biomass is an attra Pretreatment of lignocellulosic biomass is the first step toward the conversion of lignocellulose to ethanol. Different pretreatments of the lignocellulosic biomass remove the hemicellulose and lignin, reduces cellulose crystallinity and increase the porosity of the material. The different types of pretreatment methods used are: physical, chemical and biological. The physical and chemical pretreatment methods require high temperature followed by acid and alkaline treatment. Therefore, such pretreatment methods increase the cost of treatment process with neutralization or washing step that results in the loss of sugars. Biological pretreatment applies microorganism especially fungi and their enzymes system to delignify cellulosic feedstock. Fungi with high selectivity of lignin degradation over cellulose are important for successful microbial pretreatment of different fungus. Ethanol, one of the most excellent tools to overcome the vehicular pollution, contains 35% oxygen that facilitates in the complete combustion of this fuel and therefore minimizes the harmful and toxic tailpipe emissions. It also decreases the aerosol emissions that otherwise would pose a number of health hazards. In this regard production of lignocellulosic bioethanol from the widely available waste biomass like water hyacinth can serve a dual purpose. In the present era petroleum industries are keen and committed to use of ethanol as fuel, as it is likely to benefit the oil industry as well as sugarcane farmers in the long run. Much like most developing countries, majority of India's labour force make their livelihood by working in the agricultural sector; therefore, in India there is predominantly high potential for bioethanol to raise generate employment, raise incomes, and to contribute towards the rural development. Pretreatment of lignocellulosic biomass is the first step toward the conversion of lignocellulose to ethanol. Different pretreatments of the lignocellulosic biomass remove the hemicellulose and lignin, reduces cellulose crystallinity and increase the porosity of the material. The different types of pretreatment methods used are: physical, chemical and biological. The physical and chemical pretreatment methods require high temperature followed by acid and alkaline treatment. Therefore, such pretreatment methods increase the cost of treatment process with neutralization or washing step that results in the loss of sugars. Biological pretreatment applies microorganism especially fungi and their enzymes system to delignify cellulosic feedstock. Fungi with high selectivity of lignin degradation over cellulose are important for successful microbial pretreatment of different fungus. Ethanol, one of the most excellent tools to overcome the vehicular pollution, contains 35% oxygen that facilitates in the complete combustion of this fuel and therefore minimizes the harmful and toxic tailpipe emissions. It also decreases the aerosol emissions that otherwise would pose a number of health hazards. In this regard production of lignocellulosic bioethanol from the widely available waste biomass like water hyacinth can serve a dual purpose. In the present era petroleum industries are keen and committed to use of ethanol as fuel, as it is likely to benefit the oil industry as well as sugarcane farmers in the long run. Much like most developing countries, majority of India's labour force make their livelihood by working in the agricultural sector; therefore, in India there is predominantly high potential for bioethanol to raise generate employment, raise incomes, and to contribute toward the rural development.

The present study emphasis optimization of different pretreatment parameter (soaking time, concentration, temperature) during the pretreatment of lignocellulosic substrate with alkaline NaoH by using the method for optimum release of cellulose. The optimum parameters obtained in Taguchi experiments were further analyzed by High performance liquid chromatography (HPLC). For hexosans and pentose's. Besides the structural changes in untreated(native) and pretreated lignocellulosic biomass were also analyzed by FTIR(Fourier transform infrared spectroscopy) and SEM(scanning electron microscopy) analyses. The goal of pretreatment process is to break down the lignin structure and disrupt the crystalline structure of cellulose, so that the acid or enzyme can easily access and hydrolyze the cellulose. Pretreatment can be the most expensive process in biomass-to-fuels conversion but it has great potential for improvement in efficiency and lowering of costs through further research development 9-13. Pretreatment is an important tool for biomass-to-biofuels conversion processes and is the subject this review article.

Structure and Types of Lignocellulosic Biomass

Lignocellulose biomass is produced in nature through photosynthesis achieved by solar energy. Lignocellulose Biomass is a sustainable abundant and uneatable plant material. Conversion of Biomass means organic matter is convert into simplest form the reaction is the process of photosynthesis in the presence of solar radiation, can be represented as follows:

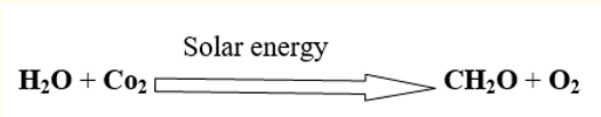

Figure 1

Biomass can be converted into fuels through a number of different processes, Including solid fuels combustion, digestion, hydrolysis, fermentation and catalyzed reaction.

Availability of Biomass resources fall into three categories

- Biomass in its traditional solid mass (Wood and Agricultural residue: it can burn directly and get the energy)
- Biomass in non- traditional form (converted into liquid fuels: the biomass can be converted into ethanol and methanol to be used as liquid fuels in engines)
- To ferment the biomass anaerobic ally to obtain a gaseous fuels called biogas (in biogas=55to65%, methane 30 to 40%, Co_2 and rest impurities i.e.H_2, H_2S and some N_2)

Characterization of biomass

The Characterization of the conversion process of the lignocellulosic Biomass biofuels requires large array of method and analytical system to extract the meaningful parameters necessary to describe the solid materials and the conversion liquor. The Biofuels Research Laboratory has been equipped with analytical system.

These system include HPLC for fluorescence, UV-Vis and Refractive index (RI), detection, liquid chromatography.

Typical Types of Biomass Components

- **Cellulose:** Cellulose is the main structural constituents in plant cell wall and is found in an organized fibrous structures. The linear polymer consists of D-glucose subunit linked to each by(B-1,4) glycosidic bond. A polysaccharide in which D-glucose is linked uniformly by B-glycosidic bonds. Its molecular formula is ($C_6H_{12}O_6)_n$. The total hydrolysis of cellulose yields D-glucose (a monosaccharide), but partial hydrolysis yields a disaccharide (cellobiose) and polysaccharides in which n is in the order of 3 to 10 cellulose has a crystalline structures and great resistance to acids and alkaline. Cellulose must be broken down into their corresponding monomers (sugars) so that microorganism can utilize them.
- **Hemicellulose:** A polysaccharide whose unit are 5-corbon monosaccharide's including D- xylose and D-arabinose, and 6-corbon monosaccharide including D-mannose, D-gulactose and D-glucose. The average molecular formula is $(C_5H_8O_4)_n$. Because the degree of polymerization n is 50 to 200, which is smaller than that of cellulose, it breaks down more easily then cellulose and many hemicellulose are soluble in alkaline solution. The main feature that differentiates hemicellulose from cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. These monosaccharide includes pentose's(xylose, rhamnose, and arabinose), hexoses(glucose, mannose, and galactose) and uronic acid.
- **Lignin:** lignin is a complex large molecular structure containing cross-linked polymers of phenollic monomers. Its complex 3-dimensional structure. Its present in the primary cell wall, imparting structural support, impermeability, and resistance against microbial attack. Cellulose, hemicellulose, and lignin are universally found in many kind of biomass, and are the most plentiful natural carbon resources on Earth. The presence of lignin in lignocellulose leads to a protective barrier that prevent plant cell destruction by fungi and bacteria for biomass conversion to fuels.
- **Starch:** Starch like cellulose, Starch is a polysaccharide whose constituents unit are D-glucose, but they are linked by α- glycosidic bonds. Owing to the difference in the bonds structures, cellulose is not water soluble, while part of starch is a soluble in hot water (amylase, with a molecular weight of about 10,000 to 50,000 accounting for 10%-20% of (starch) and part is not soluble (amylopectin, with a molecular weight of about 50,000 to 100.000.Accounting for 80%-90%starch).starch is found in seed, tubers, root, stems, and has a very high value as food.
- **Protein:** These are macromolecular compounds in which amino acids are polymerized to high degree. Properties differ depending on the kinds and ratios of constituents amino acids, and the degree of polymerization proteins are not a primary components of biomass, and account for a lower proportion than do not the previous three components.
- **Other components (organic and inorganic):** The amount of the other organic components vary widely depending on species, but there are also organic components with high value, such as glycosides (representative

examples include rapeseed oils, palm oils, and other vegetable oils) and sucrose in sugarcane and sugar beet. Other example are alkaloids, pigments, trepans, and waxes. They have very high added value as pharmaceutical in residents. Biomass comprises organic macromolecular compounds but it also contains inorganic substance (ash) in trace amount, The primary metal elements include (Ca, K, P, Mg, Si, Al, Fe, and Na substances and their amounts differ according to the feed stock type.

Fuels

Lignocellulose materials	Cellulose %	Hemicellulose %	Lignin %
Hard wood stem	40-50	24-40	18-25
Soft wood stem	45-55	25-35	25-35
Nut shell	25-30	25-30	30-40
Corn cobs	45-35	30-35	10-15
Grass	25-40	35-50	10-30
Paper	85-99	0 – 0	0-15
Leaves	15-20	80-85	0 – 0
News paper	40-55	25-40	18-30

Table 2

Overview of the conversion of Biomass to fuels

Action of microorganism an enzymes on biological sources can leads to the production of mostly ethanol and, less commonly, propanol and butanol. These agents carry out the fermentation of sugar, starch, hemicellulose, or cellulose with cellulose fermentation being the most difficult, Biobutanol which can also called biogasoline, is often claimed to provide a direct replacement for gasoline, because it can be used directly in a gasoline engines, similarly to the way in which biodiesel can be used in diesel engines. There has been extensive research on the conversion of lignocellulose materials to fuels, as especially ethanol, in past few decades.

The conversion of biomass includes the hydrolysis of various components in the lignocellulosic materials to fermentable reducing sugar and the fermentation of the sugars to flues such as ethanol and butanol. The pretreatments step is mainly required for efficient hydrolysis of cellulose to its constituent sugars. The hydrolysis is usually catalyzed by acids or cellulose enzymes, and the fermentation is carried out by yeast or bacteria. The factor affecting the hydrolysis of cellulose include porosity (Accessible surface area) of the biomass materials, cellulose fiber crystallinity, and content of both lignin and hemicellulose19. The presence of lignin and hemicellulose makes the accessibility of cellulose enzymes and acid to cellulose more difficult, thus reducing the efficiency of the hydrolysis process pretreatment is required to alter the size and structure of the biomass. As well as its chemical composition, so that the hydrolysis of the carbohydrate fraction to monomerics sugar can be achieved rapidly and with greater yields. The hydrolysis process can be significantly improved by removal of lignin and hemicellulose, reduction of cellulose crystallinity and increase of porosity through pretreatment process. In the hydrolysis process, the sugars are released by breaking down the carbohydrate chains, before they are fermented for alcohol production. The cellular hydrolysis process include 1) Acid hydrolysis and 2) An enzymatic hydrolysis. In traditional method developed in the 19th and early 20th centuries, hydrolysis is performed by reacting the cellulose with an acid. Dilute acid can be used under conditions of both high temperature and pressure, or concentrated acid can be used at lower temperatures and atmospheric pressure.

The decrystallized cellulosic mixture of acid and sugar molecules. The dilute-acid process is a hares process that leads to the formation of toxic degradation products that can interfere with fermentation. Cellulose chain can also be broken down into individual glucose sugar molecules by enzymes known as cellulose-cellulose refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoan that catalyze the hydrolysis of cellulose. However there are also celluloses produced by plants and animals. Lignocellulosic materials can similarly be enzymatically hydrolyzed under relatively mild condition (50oc and pH 5) enabling effective cellulose breakdown without the formation of by products that would otherwise inhibit enzyme activity.

Bakers yeast, or saccharomyces cerevisiae, has been traditionally used in the brewing industry to produce ethanol from hexoses. Because of the complex nature of the carbohydrates present in lignocellulosic biomasses, five carbon sugars such as xylose and arabinose, derived from the hemicellulose portion of lignocellulose, are also present in the hydrolysis.

The process of bioethanol production

The conversion of lignocellulosic biomass into different type of reducing sugar and also sugar are fermented into different fuels like butanol and ethanol, A five method to treatment of lignocellulose biomass- Pre-treatment, hydrolysis, fermentation of sugar, distillation and purification.

Pre-treatment method

The main objective of pretreatment method is to break the bonds between the lignin and hemicellulose, increase the porosity and convert crystalline cellulose in to amorphous cellulose.

Through treatment method must fulfill these requirement (1) Increase the production of pentose and hexose sugar, (2) Prevent loss of sugary compounds (3) Inhibitory compounds will not be formed which effect the conversion process and (4) Economically feasible. The self-assembly architecture of plant cell wall, with crystalline cellulosic micro fibrils intertwining interacting with lignin and hemicellulose, forms LCCs or lignin carbohydrate complexes, these are unavailable for celluloses to bind onto the surfaces of cellulosic molecules. Hence, after the reduction in preliminary size from 10mm to 30mm by utilizing mechanical methods like chopping, pretreatment is required to open LCCs, which make structure competent for enzymatic breakdown. The pretreatment process are to be classified into four different groups: like chemical, physical and biological pretreatment method and solvent fractionation. The basic features of ideal pretreatment method is that it should maximize yield of sugar derived from hemicellulose and celluloses, and in the same time it should also minimize the expenditure of energy and impact of environment. Therefore, the major problem is that none of them are able to fulfill all the criteria.

Detoxification

After pretreatment, lignin and partial hemicellulose are dissolved in liquid prehydrolysates. The free hemicellulose polymer is hydrolyzed to a mixture of monomeric and oligomeric sugars, including xylose, mannose, arabinose, galactose and glucose released from the cellulose hydrolysis. The residual solid part, cellulose and lignin can be filtered and washed (Ren, Wang et.al 2009 During thermo-chemical pretreatment, inhibitory compounds such as weak organic acids (acetic, formic and levulinic acids), furan derivatives, i.e. furfural, 5-hydroxymethylfurfural (HMF) and phenolic compounds (phenol, vanillin, p-hydroxybenzoic acid) will be generated. These compounds are toxic to microbial fermentation and will decrease the fermentation yield. These toxic compounds can be categorized into four groups: 1) sugar and lignin degradation15 These toxic compounds can be categorized into four groups: 1) sugar and lignin degradation products, 2) compounds released during pretreatment, 3) fermentation products and 4) heavy metal ions (Olsson and Hahn-Hägerdal 1996). The category and concentration of toxic compounds are mainly associated with feedstock species and pretreatment conditions. Other fermentation variables, such as pH conditions, temperatures and dissolved oxygen concentrations will also affect the inhibition level (Clark and Mackie 1984; Lopez, Nichols., et al. 2004; Mussatto and Roberto 2004; Liu and Blaschek 2010; Zhao, Peng., et al. 2012). Detoxification step is needed to remove the toxic compounds before fermentation. Several methods, including biological, physical, and chemical ones, have been proposed to transform inhibitors into inactive compounds or to reduce their concentration (Larsson, Reimann., et al. 1999; Palmqvist and Hahn-Hägerdal 2000; Klinke, Thomsen., et al. 2004; Lopez, Nichols., et al. 2004; Mussatto and Roberto 2004; Park, Yun., et al. 2005; Feng, XueBing., et al. 2009; Pienkos and Zhang 2009; Parawira and Tekere 2011).

Hydrolysis

Hydrolysis process takes place after pretreatment to break down the feedstock 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 sulfuric acid) and enzymatically (by lignocellulose degrading enzymes). But here we are doing discussion about enzymatic process. Enzymatic hydrolysis requires enzymes to hydrolysis the feedstock into fermentable sugar. Three types of enzymes.

Enzymatic hydrolysis

After pretreatment, the pretreated lignocellulosic biomass need to be converted to soluble and fermentable sugar through chemical hydrolysis (such as diluted acid hydrolysis and acid hydrolysis) or enzymatic hydrolysis (Ladisch, Lin., *et al*. 1983; Taherzadeh and Karimi 2007). Products after hydrolysis are illustrated in Cellulose from lignocellulosic biomass will be hydrolyzed to monosaccharide glucose; the hemicellulose can be mainly hydrolyzed to xylose and small amount of mannose, acetic acid, galactose, and glucose. A significant disadvantage of acid hydrolysis is that under high temperature and high pressure, sugar will be degraded to undesirable compounds: xylose is further degraded to furfural and similarly, hexose degradation will generate 5-hydroxymethyl furfural (HMF) (Dunlop 1948; 18 Ulbricht, Northup., *et al.* 1984; Gullu 2003; Binder and Raines 2009; Capuano and Fogliano 2011; Rosatella, Simeonov., *et al*. 2011). Using enzymatic hydrolysis can get rid of these degradations with 100% selective conversion of cellulose to glucose. Over the past half century, significant researches have been conducted to assess the ability of highly specific cellulolytic enzymes to depolymerize the cellulosic component of lignocellulosic substrates. However, efficient, rapid and complete enzymatic hydrolysis with low protein loadings still remains one of the major technical and economical bottlenecks in the overall bioconversion process (Walker and Wilson 1991; Xia and Cen 1999; Chang and Holtzapple 2000; Berlin, Maximenko., et al. 2007; Arantes and Saddler 2010; Zhao, Song., *et al*. 2011). Limitation factors relate to: 1) substrates aspects including lignin/hemicellulose association, degree of cellulose crystallinity and polymerization, extent of sur-

face area and etc.; and 2) enzymes aspects like end-product inhibition, need for synergism, irreversible enzyme adsorption and etc. (Coughlan 1985; Zhang and Lynd 2004; Mansfield, Mooney., *et al.* 2008; Sannigrahi, Miller., *et al*. 2010). Enzymatic saccharification of cellulose is generally viewed as a heterogeneous reaction system. Cellulases in aqueous environment react with the insoluble, macroscopic and structured cellulose. This process is typically catalyzed by cellulases from bacteria and fungi (Ryu, Kim., *et al*. 1984; Himmel, Ruth., *et al*. 1999; Lee, Evans., *et al*. 2000). The widely accepted mechanism for enzymatic cellulose hydrolysis (Figure 2) involves synergistic actions by endoglucanses (EG, endo-1,4-b-D-glucanases, or EC 3.2.1.3.), exoglucanases or cellobiohydrolases (CBH, 1,4-b-D-glucan cellobiohydrolases, or EC 3.2.1.91.), and β-glucosidases (BGL, cellobiases or EC 3.2.1.21).

Figure 2

SHF and SSF

Two principal configurations that use enzymes for saccharification are separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) (Wingren, Galbe., *et al.* 2008; Ask, Olofsson., *et al*. 2012). In SHF process, enzymatic hydrolysis and fermentation are carried out separately, which makes it possible to run each process under their optimal conditions. In addition, SHF offers the possibility of cell recycling, whereas in SSF it is not possible to separate cells and solid raw material particles. Its primary limitation is the end-product inhibition of the cellulolytic enzymes (Alfani, Gallifuoco., *et al.* 2000; Tomás-Pejó, Oliva., *et al.* 2008). SSF integrates the enzymatic hydrolysis of cellulose with microbial fermentation for target biofuels or chemicals production. This allows released sugars from the hydrolysis to be rapidly consumed by microorganisms, thereby minimizing end-product inhibition on the cellulolytic20 enzymes. SSF is attractive for lower capital costs, lower process time and minimum loss of sugars by using same reaction vessel. One disadvantage is that since conditions for both enzymatic hydrolysis and fermentation have to be the same, therefore, typically suboptimal for both (Wyman, Spindler., *et al*. 1992; Hari Krishna, Janardhan Reddy., *et al.* 2001; Öhgren, Bengtsson., *et al*. 2006; Ask, Olofsson., *et al*. 2012). Choice of configuration should be determined by a tradeoff of advantages and drawbacks associated with motivations.

Different pretreatment Advantage and disadvantage

The main aim of lignocellulosic biomass pretreatment is make the 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 (chundawat., *et al*; 2010, ye and Berson, 2014). The raw material pretreatment step could represent up to 20% of the total costs of celluloses ethanol production (Yang wgman; 2008). Different pretreatment method which are methods (Ren., *et al*.; 2016) in the post hundred million years, lignocellulosic biomass has evolved complex structures and chemical composition to protect the sturural 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, DP, and the strong inter chain hydrogen-bonding network of cellulose, available surface area, the content of acetyl groups the presences 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 lignocelluloses of enzymatic saccharification. On the other hand the natural structure of plant biomass also affect the bioconversion process, due to these reason the pretreatment is required these factor are. (1.) presence of cuticle and wax, (2). Vascular tissues arrangement, (3). Thickness of sclerenchymatous tissue, (4). Cell wall compactness and their multilayered arrangement, (5). Requirement of enzymes substrates specificity, (6). Plant biomass complexity also effect the mass transport system of plant cell wall the conversion process of lignocellulosic biomass is also affected by the concentration composition and the arrangement of cellulose, hemicelluloses and lignin which plant to plants. Up to now, with the great efforts of human, the cost of enzymes has been improved a lot. However, the cost of pretreatment is still quite high, and hardly meet the requirement of commercial application. Therefore, to a large extant, pretreatment is the main bottleneck for the production of biofuels/biochemical from lignocelluloses, more efforts should be made to develop more cost-effective pretreatments process methods are as follows; (1). High yields of fermentable sugars and low sugars degradation; (2). Effective delignification or chemical /structural changes of lignin(e.g. sulfonation of lignin); (3). Limited formation of inhibitors and high purity of fermentable sugar; (4). Low chemical consumption or efficient chemical recovery;(5). Low energy consumption;(6). Low water usage;(7). Low cost and environmental benign process;(8). High recovery of hemicelluloses and lignin (Huanfei Xu, Bin Li; 2016).

Chemical pretreatment

Chemical pretreatment method utilizes different chemical like alkali, oxidants and strong acid, Therefore, these chemicals cause destruction of organic compounds (Li *et.al*; 2012). 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 disperse compact structure into plants fibers. Alkaline pretreatment is more effective in degradation of the ester linkages between the hemicelluloses and lignin, resulting in the exposure of cellulose to enzymes Thus, for delignification process complex reagents sodium hydroxide or NaoH is used in chemical pretreatment method However, accompanied with lignin removal. Substantial hemicellulose was also dissolved, led to plenty waste of substrate materials (Ran., *et al*; 2010). Therefore as compared to NaoH the calcium hydroxide $Ca(OH)_2$ is economically cheap and safe.(Cao., *et al*. 2012) in their study reported that during lime pretreatment method, the rigorous structure of the corn stalk can be disrupted and more cellulose can be exposed to the surface, this in term increases H_2 yield and improves the biodegradability of substrates. Therefore, $Ca(OH)_2$ can be easily recovered by using lime kiln technology, which is suggested to be more propitious pretreatment lignocellulosic biomass for production of H_2. The acid pretreatment method or most desirable pretreatment method for pretreatment of lignocellulosic substrates not only, because it leads the degradation of the lignin, but also the microbes used in hydrolysis are able to acclimatize. Therefore, the primary reaction that take place during acid pretreatment method is the hydrolysis of hemicelluloses into different monosaccharide's, mean while the condensation and precipitation of lignin take place. Strong acidic pretreatment may result in the production of inhibitory by-products such as hydroxymethylfurfural (HMF) and furfural (Ariunbaatar., *et al*; 2014).

(Cao., *et al*; 2009) in this study reported that corn Stover pretreated with diluted sulfuric acid or H2So4 at 1210C for about 30-180 minutes can improves solution rate of lignin and hemicellulose and the maximum hemicellulose solution rate was achieved with H2So4 concentration of 1.69% and reaction time of 117min. Acid pretreatment also substrate, Such as Jatropha curcas shells, Over 70% of hemicellulose could be derived after being treated with acid (Martin., *et al*. 2015). But a removal of inhibitory product also necessary, which increase the yield of product and make process cost efficient. The main target of pretreatments delignification of biomass, and maximum use of cellulose and hemicelluloses with their conversion into fermentable sugars. (Ren., *et al*. 2016), (Sun., *et al*. 2016) Although most solvents used in these processes can be recycled from the reactor to reduce the operational cost in the high price and potential hazards of handlings large volumes of organic solvent limit the utilization of these pretreatments Therefore, further research is required to improve the economic of these pretreatments and construct effective solvent recovery procedures (Ren., *et al.* 2016).

Biological pretreatment

Biological pretreatment is ecofriendly and economically feasible process for biomass pretreatment, due to low chemical and 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. Researching major challenging constituent of plants for biological degradation and increasing the rate of enzymatic hydrolysis (Chiaramonti., *et al*;2012). Brown rot fungi have a highest capacity to degrade cellulosic biomass. The white rot fungi generation belongs to class basidomycetes and used majorly for biological pretreatment method. Hence white rot fungi secreates different lignolytic enzyme like lignin peroxidase, Manganese peroxidase (Mnp) and in the presence of varatryl alcohol. Therefore, the oxidation of lignin is caused by the oxidized from of manganese peroxidase and lignin peroxidase enzyme. As compared to different chemical and physical pretreatment method biological method is relatively slower but it consumes less energy with better environment footprint. Therefore, the use of fungus for pretreatment method preior to the Pyrolysis is essential to improve its performance (Ken., *et al*;2016). But biological pretreatment and its enzymatic hydrolysis is also affected by some factor which are. Biomass particle size, moisture content, temperature, pretreatment time and PH (Haghighi., *et al*;2013). One of the most effective method used for the enzymatic saccharification is fungal pretreatment method which utilizes wood rot fungi. There are the several products different enzyme leads to depolymerisation of hemicellulose and celluloses in wood. Hemicellulose is the type of branch polymer which consist of sugar monomer and glucose these, hemicellulose from 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(Binod., *et al*;2012). Developed a new pretreatment technology that convert the lignocellulosic material into desired product in a cost effective manner, Now the biofuels production is based on lignocellulosic waste and ecofriendly so in this era also focused on genetically modified plants that easily degraded by microbes (Zeng, Zhao, Yang and Ding;2014). Metabolic engineering is considered as an emerging. Field which utilizes the recombinant DNA technologies for direct production of Bioethanol.

Future work

Extensive research has been done on the development of advanced pre-treatment technologies to prepare more digestible biomass to ease bioconversion of biomass into cellulosic ethanol. Due to its reactivity at mild condition, chemical pre-treatment of lignocellulosic biomass forms the basic of several proprietary. Cellulosic ethanol production configuration and technologies that have been developed by various research group and companies for development at various levels, usually with financial support bodies. Therefore, the future research on pre-treatment would be focused on the following areas.

- First, The reduction of water and chemical uses.
- Second, The recovery of carbohydrate and value added by products to improve the economic feasibility.
- Third, Development of clean delignification yielding benefits of co-fermentation of hexose and pentose sugars with improved economics of pre-treatment.
- Fourth, Fundamental understanding of pre-treatment mechanism and the relationship between the biomass structure feature and enzymatic hydrolysis.
- Fifth, Reduction of the generation of inhibitators such has furfural 5-HMF and acetic acid which could significantly inhibit enzymatic hydrolysis and fermentation of biomass.

Prospective

By virtue of its low water requirements high productivities in semiarid lands, adaptability to high temperatures, resistance to drought and proven high sugar/product conversion energy crop that could be employed in a bio refinery scheme as a feedstock and comparable to traditional bioenergy feedstock's (eg. high carbohydrate content). Likewise, even it Agave has a smaller average then corn or sugar cane, the impact of an agave based bio refinery would cause minimal increases in environmental impact even it initially the total quantities of ethanol might be rather small but significant in the would market. However, it is necessary to better understand the effects of thermo chemical pre-treatment and subsequent downstream processing in agave recalcitrance to assess the optimum route for a larger production scale of biofuels and value added products. Finally, further studies, such as life cycle and sustainability assessment of a biorefinary from different agave species and regions, are necessary to examine the environmental impact and cost effectiveness of a functional plant where the production of diverse value-added products could potentially improve overall economical.

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