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PLANT BIOMASS PRETREATMENT: AN ALTERNATE PROCESS FOR EFFECTIVE BIOFUEL PRODUCTION

Biochemistry

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ABSTRACT
The growing population and developing technologies pose a threat on fossil fuels adequacy. Lignocellulosic biofuel is used as an alternate fuels to the available energies. An efficient pretreatment technique on the recalcitrance of cellulosic polymers and lignin structures can efficiently be broken down to make an abundant energy stored in them into viable biofuel. The emphasis of the present article is on efficient pretreatment methods of them for their pro's and con's, advantages and disadvantages of the combination of pretreatment and biodegradation methods used for hydrolysis of lignocellulosic mass by biological pretreatment in addition to physical and chemical pretreatment methods.

KEYWORDS

INTRODUCTION
Modern world's energy demands are not met by fossil fuels and there is a growing need for alternative fuels from different sources to meet the increased fuel demand and air pollution. Bio ethanol becomes the most effective remedy for fuel depletions which otherwise can be produced from lignocellulosic plant waste accumulated from forestry, agricultural wastes etc.

Lignocellulose is the basic constituent of almost all the plant material. The components of the lignocelluloses are cellulose, hemicellulose and lignin. These three components in turn are composed of polysaccharides, polyphenols and proteins. Lignocellulosic biomass is a fibrous molecule having different physical and chemical properties. The lignopolysaccharides are of energy rich sources with a potential for conversion in to biofuels by making lignocelluloses as the most important group among renewable sources. Although lignocelluloses are the most abundant plant material in nature, the material is highly recalcitrant to natural processes to break the rigid hydrogen and polymeric bonds. Effective pretreatment techniques are needed in order to liberate cellulose from the lignin seal. Other components like acetyl groups, Phenolic compounds are also present in small amounts. Being polymerized structure lignocelluloses have a rigid and recalcitrant nature. The main qualities imparting to the robustness of lignocelluloses are hydrophobicity of lignin polymer, crystallinity of cellulose and lignin- hemicellulose matrix formation. In these cellulose consists of 40 – 60% of dry weight, hemicelluloses is of 20 – 40% and lignin of about 10 – 25%.

Cellulose is a polymeric chain of cellobiose disaccharide units. The main difference between cellulose with other glucan polymers consists of intra and inter molecular bondings are β-1, 4 glycosidic linkages, these extensive O bonds confer to the strong and crystalline matrix to the structure of cellulose. The rigid crystalline structure of cellulose can only be transformed to amorphous cellulose by subjecting to high temperature and pressure. Cotton is one of the most pure forms of cellulose available naturally. The second most abundant polymer hemicellulose also known as “Polyose” is a heteropolymer in nature composed of pentoses. Some examples of hemicelluloses are xylan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan. Hemicelluloses are also found associated with cellulose, by having D-Pentoses and small amounts of L-Pentoses, and their acidified forms glucoronic acid, galacturonic acid etc. All these are found associated to hydrolyze because of their amorphous nature and low molecular weight. Hemicelluloses cover cellulose fibrils and limit availability for enzyme hydrolysis so they are to be removed for efficient cellulose hydrolysis.

Lignin is an organic polymer found in the support tissue of vascular plants, they lend rigidity and are rot resistant. They are cross linked phenolic polymers, and are insoluble in water and alcohols but soluble in weak alkaline solutions. This is the most abundant organic polymer on earth, synthesised from phenylpropanoid precursors i.e., syringyl, guaiacyl, P-hydroxy phenol linked together to form a complicated matrix. High Lignin content is not favourable for bioethanol production. The three components of lignocelluloses are not uniformly distributed within the cell walls and vary according to species, tissues, maturity of the plant.

Pretreatment of Lignocelluloses for production of biofuel - Usage of Lignocellulosic wastes for Bioethanol production has gained importance due to the depletion of fossil fuels and food crisis that may result from usage of edible sugar sources for bioethanol production. First generation biofuels use edible material for biofuel production, from soluble sugars and starch whereas second generation biofuels use Lignocellulosic waste material .Lignocellulosic materials From cellulosic fraction of biomass being an abundant source of energy in its complexity of nature, require efficient pretreatment techniques to extract maximum potential under cost effective conditions.

Pretreatment Techniques - A Pretreatment process can be considered as efficient if the formation of sugars in enzymatic hydrolysis step is greater and carbohydrate degradation is adequate limiting the formation of inhibitory compounds like furfurals and phenols. Cellulose utilization in its crude untreated form is tedious to degrade by enzymatic hydrolysis and consumes large amounts of enzyme. Various limiting factors observed are i.e., surface area, crystalinity of cellulose, Lignin hemicellulose, acetyl content and polymerisation of lignin are taken in to consideration to select suitable pretreatment techniques. The pretreatment methods include physical, chemical and biological for conversion of complex carbohydrates into simple sugar moieties. These simple molecules are further used to produce biofuels in the presence of microorganisms.

A Physical Pre- Treatment -The main objective of physical pretreatments is to increase the surface area by reducing particle size ,depolymerisation, decrystallization of the biomass. Different kinds of physical pre-treatment methods can be implemented based on the hardness and dryness of Biomass. Physical pretreatment can be done by using microwave irradiation, sonication, mechanical beating, Extruding, refining, milling, cavitation etc.

Milling - Milling is generally carried out as the first step of the physical treatment. Several types of milling can be done like ball milling, roll milling, hammer milling, colloid milling and disk milling etc. Depending on the pretreatment method particle size ranges from 10 – 30 mm to 0.2 – 2 mm. Milling requires high energy requirement. To reduce energy costs wet disk milling can be adopted as it is a reasonable method of pretreatment. Wet disk milling has a draw back when compared with ball milling. The concentrations of glucose and xylose are higher when treated by ball milling comparatively.

Extrusion-The process is carried in an extruder, which consists of one or two screws that spin in to a tight barrel with temperature control. When a biomass is allowed through the barrel it is subjected to friction, shear force, an increased temperature and pressure. At finishing the biomass is released from pressure suddenly which causes structural changes. In this process the biomass experiences heat compression and shear force, which causes physicochemical altercations of biomass. Hence it is a thermo-Physical treatment there is no furfural and HMF formation due to moderate temperatures. There is a possibility for easy scale up and continuous operation. According to Yoo et al.(2011), 94.8% of glucose conversion is possible after enzymatic hydrolysis by applying extrusion method.

Cavitation - Cavitation is produced by passing ultrasonic waves through the liquid medium and hydrodynamic cavitation produced using hydraulic systems. This method is called accoustic cavitation. In this method microbubbles called cavitation were developed when ultra sound waves are propagated in liquid medium due to repeating pattern of compressions and rarefactions, expansion of cavitation to an unstable size and collapse, lead to a temperature up to 5000K and pressure up to 180 MPa. Shear forces damage the cell walls of Lignocellulosic cellular material. However higher sonication power adversely affects pretreatment processes by hindering travel of bubbles from transducer to the liquid medium. But cavitation through ultrasound one can achieve good and high results.Cavitation is generated by forcing fluid through cavitating devices in hydrodynamic system. The flow of fluid leads to a sudden pressure drop, leading to collapse large magnitudes of energy altogether leading to dissolution of biomass.

Microwave Irradiation - Microwave Irradiation **(MI)** can alter the structure of cellulose and degrade lignin to an extent, degrade waxy surfaces and enhance chances of enzymatic degradation of Lignocelluloses. M.I causes disruption of chemical bonds by polarized macromolecules aligning with the poles of electromagnetic field, thus resulting in denaturation. Advantages of M.I method are process time is shorter, uniformity and selectivity are high, less energy input than conventional heating.

Freeze Treatment - Few studies have been carried out on freeze treatment of biomass. According to study on rice straw biomass with above treatment, there was a significant increase in enzyme digestion. There has been a great attention for this method due to less negative environmental impact and non-usage of dangerous chemicals. This is a green method.

Thermal -Temperature 60°C to 270°C have been studied extensively for methane and biofuel production based pretreatments at temperature above 200°C, recalcitrant soluble organics, toxic, Inhibitory products were produced. Thermal treatment temperature was optimized at 160°C -180°C while at higher temperature biodegradability is reduced and inhibitory products were formed.

B. CHEMICAL PRETREATMENT METHODS

To modify the complex nature of carbohydrates it is the practice of laboratories and industries to use several types of chemical methods such as hydrolysis by acids or alkali, oxidation, and Ionic or organic solvent treatments. These methods can convert the hard complex bio organic molecules into simple molecules. All these methods or any one of them may be adopted depending on the degradation efficiency on lignocelluloses into cellobioses or glucose units.

Acid Hydrolysis - Acid pretreatments are the most commonly employed pretreatments employed for Lignocellulosic disintegration. Acid pretreatment breaks down polysaccharides in to monosaccharides. Using high concentration of acids at low temperature may be economical but results in equipment damage due to corrosiveness, production of fermentation inhibitors like HMF and furfural. In the case of low concentration acids are worked at higher temperatures generation of Inhibitors is much lower . Sulphuric acid is the most commonly used in acid pretreatment of lignocelluloses. During dilute acid pretreatment, the higher the temperature, the lower the retention time and vice-versa is maintained for example at 180°C the biomass can be retained for 5-10 min, and at 140°C for 30 min depending on the nature of the biomass. This pretreatment results In disruption of hydrogen bonds, covalent bonds and vanderwaals forces breaking the hemicellulose and reduction of cellulose (Taherdanank et al.(2016) in an anaerobic method used diluted sulfuric acid to treat wheat waste to improve biomethane production, at temperature 121°C obtained a 15.5% higher methane yield after pretreatment for 120 minutes compared with untreated wheat plant waste. Redding et al.(2011) used 1.2% sulphuric acid for treating Coastal Bermuda grass at 140°C for 30 min., 94% of total sugar yield was achieved theoretically. Usage of organic acids like maleic and fumaric acids also gives desirable results.

Alkaline Hydrolysis - Solubility of cellulose and hemicellulose is lesser compared to acid pretreatment method. This method of pretreatment induces chemical swelling of particulate fibers at an elevated P^H and disruption of cross linkages between hemicelluloses, lignin and cellulose. Delignification, de-esterification of ester bonds. Alkaline pretreatment can be carried at room temperatures, retainment time, being the main drawback which possibly varies from hours to a few days which makes this process a much time lagging procedure. Energy requirement to process is very limited with few or no changes to the experimental environment. Sodium Hydroxide, Potassium Hydroxide (KOH), Calcium Hydroxide ($CaOH_2$), Ammonia are generally used for alkaline hydrolysis. Combination methods are also studied for effective pretreatment. In a study NaOH was first used and the biomass was recovered for pretreatment with $Ca(OH)_2$. This method showed an increase glucose and xylose content by 2.1% of the total concentration. Sambusiti et al.(2013) have studied the effect of alkaline. NaOH on ensiled sorghum forage and noticed an increase in methane yield by 2.5% comparitively with the untreated biomass, there were no inhibitory products in the process.

Ioinization - The natural products have anions and cations with a melting point less than 100° C. Ionic Liquid (IL) Pretreatments are environmentally friendly and have a wide range of applications including pretreatment of lignocellulosic biomass. IL's have high thermal stability and low or negligible vapour pressures. Fu and Mazza(2011) reported 29% lignin removal from triticale straw using (EMIM)cl at 150°C for 1.5 hours; 30% lignin removal was reported by Wei et al. with (BMIM)cl as on IL solvent. A few examples of IL fluids are (EMIM)cl, (BMIM)cl, (EMIM)Ac. (EMIM)cl is accepted for its better performance than the other two. Main drawbacks of IL pretreatment are time lag and functioning at high temperatures. A slight change in lignocellulosic composition was also observed. The most important of the drawback being incompatibility of the cellulose enzyme with IL fluids which result in unfolding and inactivation of the enzyme. Though IL's can be recycled, its an energy intensive process .Viscosity of the fluid when pretreatment is taking place is another disadvantage and IL's are quite expensive. To overcome most of the disadvantages water mixtures of IL's can be used and recycled easily.

Solvent Pretreatment - Organic solvent extraction process focuses on removal of lignin from Lignocellulosic biomass by using organic liquids and water mixtures [with or without addition of catalysts like acid/base] to hydrolyze lignin and lignin- carbohydrate bonds. Though hemicellulose is also solubilized in this process, pure lignin can be obtained as a by-product. In turn lignin removal tends to increase availability of cellulose and hemi cellulose for enzymatic hydrolysis. Solvents used in this method are acetone, ethanol, methanol, ethyleneglycol etc. which have a very low boiling point which becomes the main draw back, along with inflammability and volatility of these mixtures.

Wet Oxidation -Oxygen, air and water are employed in this kind of pretreatment under high temperature and pressure to maintain and increase dissolved oxygen. Wet oxidation enhances contact between organic matter and molecular oxygen. WO technique's efficiency is to fractionate Lignocellulosic material by bringing about solubilisation and hydrolysis of hemicelluloses and delignification of comparative furfural formation is much lower than other pretreatment techniques. Usage of alkali was recommended to further reduce inhibitory compounds 89% lignin removal was achieved by Banerjee et al. (2009) while managing above 66% cellulose content. Chandra et al. worked on increasing biodegradability of effluent mixtures from a distillery and succeeded by using wet oxidation. Enhanced biogas yield was achieved over untreated effluent. Despite many advantages of WO, It is cost effective treatment technique.

Ozonolysis - Ozone has a potential to degrade Lignin in Lignocellulosic materials. Ozone gas is a very strong oxidant, interacts with recalcitrant compound and increases their biodegradability. Lack of by products from degradation makes it easier in subsequent steps, Garcio-Cubero et al.(2009) achieved enzyme hydrolysis yield up to 88.6%, a higher produce compared to not ozonolysed substrate, Yu et al. (2011) hydrolysed the total lignin content on loblolly pine and mixed southern hardwood pulps with maximum hexose, pentose concentration around 80%. Interactions between lignin and ozone depends on substrate and process parameters ozone reacts with Olefinic, aromatic and Phenolic compounds through initial electrophilic attack and hydroxylation of the aromatic ring, changing partially acid insoluble lignin in to acid soluble lignin. Carbohydrate interactions of ozone are a bit slower than those with lignin. The main disadvantages of this technique are cost of ozone is higher and expensive upscale processes which make it a less sought method. Subsequent studies are being done to bring down the costs and reap the benefits of this technique.

C. BIOLOGICAL PRETREATMENT

Several pretreatment techniques are applied for the conversion of biomass for microbial or enzymatic break down, so as to make the substrate more available for the enzymatic activity. There are a wide variety of bacteria and fungi like Bacillus, Streptomyces, Candida, and Aspergillus produce a broad spectrum of cellulolytic and lignolytic enzymes which break down complex sugars to simple sugars for further fermentation. Biological pretreatment is an environmental friendly method as there are no chemicals involved in the process. There is not much environment change required and it is a very economical way of treatment. Saranya et al 2014. studied phase separated disintegration pretreatment using $CaCl_2$ along with bacteria. At their pre designed optimum temperature, they achieved deflocculation and biosurfactant producing potency of bacteria. Kavitha et al 2017. investigated biological pretreatment based on bacteria on the ability to liquify *Chlorella vulgaris* (Algae), *Phanerochaete chrysosporium* is a common white rot, was studied for lignin degrading abilities as of only cellulose is degraded by brown and soft fungi . Many parameters of the substrate affect the production of enzymes by the microorganism, like particle size, moisture content, P^H, time and temperature which in turn can affect the cellulose and lignin degradability.

There are many enzymes involved in biological degradation of lignocellulosic biomass. Different enzymes play different role in hydrolysis. The main group of enzymes is celluloses which come under glycosyl hydrolases, three main celluloses being endoglucanases, exoglucanases and glucosidases. Hemicellulases act on hemicellulose, Glycosidic hydrolase, Carbohydrate esterase being the modules. The other group of enzymes is Lignases, Lignolytic enzymes act on lignin and are mainly produced by fungi .Pectinases act on pectin and breaks down poly galacturonic acid (Gal A). Various other multiple enzymes like accessory enzymes take part in hydrolysis of wood.

Combination of Pretreatment Methods: - All above Pretreatment methods mentioned above are seen to have some advantages and at the same time some disadvantages. A combination of various Pretreatment methods can be carried out to reap maximum benefits from the process of Pretreatment. Physico chemical pretreatments like steam explosion which involves thermo-mechano chemical alteration of lignin and break down of hemicelluloses by combined forces like high temperature, shear forces and pressure. AFEX or Ammonia fiber explosion pretreatment, uses liquid ammonia and is based on steam explosion concept AFEX can be optimised. Under high pressure, moderate temperature for a time span of more than 30 min., pressure release Ammonia gas expands and further cleaves lignocellulosic complex Co_2 explosion is also carried on in the same pattern as AFEX ,as Ammonia and Co_2 have same expansion capabilities and can result in similar degradation of lignocellulosic complex. Co_2 explosion should be operated at lower temperature, non-toxicity and non-flammability of Co_2 are added advantages. Kim and Hong (2000) used Co_2 method on aspen wood with 73% moisture at 165°C for 30 min., Co_2 at 21.37 Mpa which resulted in higher reducing sugar content. Liquid hot water method uses high temperatures and pressures to maintain liquid state. No chemicals are added in this process, the slurry resulted is rich in cellulose, water and insoluble materials. This method is 80% effective in solubilising hemicelluloses and an efficient method to treat cornstover, wheat straw, rye straw, Bagasse etc., known by works done already. Wet Oxidation is already discussed above. Various other combination pretreatments are combination of Alkali and acid at dilute concentrations was done for example NaOH + per acetic acid, at 75°C and time 2.5 hours, 92.04% of reducing sugars were yielded by this method. Combination of alkali and ionic

liquids, dilute acid and steam, supercritical Co_2 and steam explosion, organosolv and biological, biological and dilute acids, biological and steam explosion, microwave and alkali, dilute. Acid and microwave, Ionic liquid and Ultrasonic and other compatible combination pretreatments were carried out and betterment of results were reaped.

CONCLUSION

The abundant resources of lignocellulosic biomass can be turned in to value added products, fuels and by products which can replace fossil fuels and industrially important products. Though resources are abundant, the crystallinity of cellulose and lignin- hemicelluloses matrix make it almost impossible for biological conversion of lignocelluloses to sugars for further fermentation many pretreatment methods which have been already worked on, which have their own pro's and con's have been studied and implemented by many researchers to make the lignocellulosic complex manageable resource. Pretreatment is a cost effective process. The bottle necks being energy requirement, environmental effects are taken in to consideration to propose new perspectives. Production of inhibitors is one more hurdle in the process of pretreatments. On the other hand genetically modified microorganisms and plants are yet to decide the future prospects.

A suitable pretreatment method should disrupt the H – bonds, break down cross linked matrix of hemicelluloses and lignin, raising the porosity and surface area of cellulose for enzymatic hydrolysis. These above pretreatment methods can be suitably designed based on the lignocellulosic biomass and celluloses whether produced by microorganism or pre synthesised and quantified celluloses. Many different groups of microorganisms produce a broad array of cellulose enzymes like endo – 1, 4 , B – D – glucanase, B – 1,4 – endogulcanhydrolase, carboxymethyl cellulose (CM case), aricellose, celludextrinase cellulose A. These enzymes work by modifying the structure of lignin, cellulose and hemicellulose. Celluloses are produced by a variety of bacteria like Pseudomonas fluorescens, Bacillus Subtilis, E-coli and SerratiaMarcescens and species like Clostridium, Cellulomonas, Bacillus, Pseudomonas, Fibribacter, Ruminococcus, Butyrivibrio, etc., and Fungal species like Aspergillus, Rhizopus, Trichoderma, Fusarium, Neurospora, Pencillium etc., Actinomycetes likeThermomonospora, Thermo actinomycetes are also efficient in degrading Lignocelluloses enzymatically. From above all organisms' fungi are the group of organisms which efficiently degrade celluloses, with their two types of extracellular enzymes like hydrolytic and oxidative catalytic systems. (Hydrolytic system works on lignin by degrading the phenyl rings by producing hydrolases. During this process value added by products are formed from lignin.

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