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Saccharification of wood and lignocellulosic biomass: A review

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Abstract

Saccharification of wood is gaining commercial importance nowadays as a means of producing cost-effective bioenergy products. The wood waste contains around 65% of carbohydrates in the cellulose, hemicellulose and lignin. If these carbohydrates are changed into simple sugars, they could be used as a source of carbohydrate feed for cattle, chickens and other livestock. The conversion of polymers present in the lignocellulosic biomass of wood into fermentable sugars can be achieved through physical, chemical, physio-chemical and enzymatic pre-treatments methods. To realize the importance of lignocellulosic bioenergy, which is of global interest, enzymatic saccharification step acts as an essential stage to convert polymers to mono-sugars. This mini-review focuses on wood saccharification with improved and efficient pre-treatment, enzymatic saccharification process and detailed of enzymatic saccharification for the production of different bioenergy products are discussed.

Keywords: Wood, lignocellulosic biomass, saccharification, enzymes

Introduction

Saccharification of wood is the process by which carbohydrates can be converted by chemical treatment into simple sugars in wood. The carbohydrates involved are primarily cellulose and hemicelluloses, but are also influenced by the small amount of mono, di, and polysaccharides present in the cell fluid. Wood of most of species contains approximately 70% of carbohydrates, of which cellulose contributes 50% and hemicelluloses contributes approximately 20%. Wood constitutes one of the most significant potential carbohydrate sources for sugar conversion. The conversion into sugars, which can also be called wood hydrolysis, is accomplished by treating wood with strong acids at room temperature or with dilute acids at elevated temperature (usually sawdust, shavings, chips, and other waste materials). During this process, cellulose undergoes conversion into glucose and other sugars and sugar derivatives. The other sugars found are galactose, mannose, arabinose and xylose, and in some instances, fructose also constitutes the relative proportion of this sugars and it varies from species to species. Timber is a relatively expensive commodity in most countries as opposed to sugar. Where possible, the saccharification method would justify using only wood waste and residues that occur in the logging and manufacturing operations. The processes available in India still need to be developed in the wood saccharification industry, they are suited mainly to special situations rarely met in this region (Trotter and Troup, 1940)^[90]. The layers of lignin are obstacles to approaching hydrolysis enzymes hemicellulose and cellulose (Volynets *et al.*, 2017)^[93]. The polymeric sugars in cellulose and hemicellulose need to be hydrolyzed before further fermentation of the reducing sugars from the lignocellulosic biomass (Liao *et al.*, 2016)^[52]. Enzymatic saccharification is an efficient and green method to increase the development of sugar reduction in lignocellulosic biomass from polymer sugars (Manisha and Yadav, 2017; Nizami *et al.*, 2017)^[100, 71]. Developing lignocellulosic biomass technologies processing focuses primarily on waste stream biofuel production and biorafnation processes. Biorafnation products include varied organic chemical compounds, *i.e.*, biomaterials, biochemicals, and biofuels, of which bioethanol, biohydrogen, biodiesel and furan biofuels are the most important (Balat, 2011; Lalak, *et al.* 2014)^[7, 48]. Lignocellulosic biomass processing to hydrogen requires four main unit operations: Pretreatment, hydrolysis, fermentation, and liquid purification. Pre-treatment requires the milling, mincing and

conditioning of biomass; it is a high-cost operation. For most lignocellulosic raw materials, the size reduction of biomass particles is used and is realized by fracturing, crushing, milling or defibration (Wikandari R, *et al.* 2016)^[96].

Industrial processes

1. Dilute-acid processes

The hydrolysis of the dilute acid consists of two chemical reactions. One reaction transforms cellulosic materials into sugar and the other transforms sugars into other chemicals, many of which inhibit the growth of microbes for downstream fermentation. The same conditions that allow the initial reaction to occur concurrently allow sugar and lignin to over degrade, producing inhibitory compounds such as organic acids, furans, and phenols. Lignin is disturbed during the pretreatment cycle and partial lignin is dissolved, thus increasing cellulose susceptibility to enzymes (Yang and Wyman, 2004). Pretreatment with dilute acid is capable of converting hemicellulose found in biomass into soluble sugars and promotes subsequent enzymatic hydrolysis of cellulose (Balat, M. *et al.* 2008; Gomez, LD. *et al.* 2008; Keshwani, DR. *et al.* 2009)^[8, 29, 41]. However, polysaccharide hydrolysis also contributes to the development of products for sugar degradation: namely inhibitors such as furfural and 5-hydroxymethylfurfural (HMF) (Neureiter, M. *et al.* 2002)^[70]. Sugarcane bagasse was pretreated through MW-assisted heating in a dilute sulfuric acid solution (0.2 M); an increase in reaction temperature significantly accelerated the destruction of the bagasse biomass structure and at the same time fragmentation and swelling characterised the pretreated bagasse particles (Chen, WH. *et al.* 2011)^[19].

(i). Scholler process

The Scholler process is the most successful of all dilute-acid percolation processes and in Germany and Switzerland it has stood the test of extensive industrial application. A Scholler plant usually contains a 60 cubic meter digester tank, made of steel and lined with acid resistant glass. The digester top has steam and vent lines, and a line for hot diluted acid introduction. A porous tile filter is installed at the bottom. The digester load with 9 to 10 tons of sawdust and chips. The residual chips and dust are heated to approximately 140 °C and fresh acid (less than 1% concentrated) is added at approximately 130 °C and allowed to react approximately half an hour before the sugar solution is extracted as before. For each part of wood used, the total acid used works out to about 12 to 15 parts and the concentration of sugar is about 3 to 4 per cent. The yield of sugar is 40 to 55% of the wood 's

weight, resulting in a recovery of 20% of the wood's alcohol by the Rheinau cycle compared to around 26% of the alcohol (Degoy, E. 2016)^[24].

(ii). Madison wood-sugar process

This method was developed at the laboratory of U.S. forest products in Madison, Wisconsin, and is a variation of the Scholler method with some modifications. The first and major change is the inter-cyclic steaming of Scholler method has been removed so as to effect time-saving of about three hours. Second change is that the 0.5 percent dilute acid is used, and the temperature slowly and steadily rises from 150 °C to 185 °C. Thirdly, the hydrolysate removed constantly from minimum retention time in the digester. There after the hydrolysate is worked either alcohol or for yeast. The hydrolysis process takes around three hours. The sugar yield from Douglas fir (*Pseudotsuga taxifolia*) is found to be 50% by weight, while the average sugar concentration in the solution is 5%. In this method, a number of plants were constructed in Germany during world war I (Trotter and Troup, 1940)^[90].

2. Concentrated-acid processes

A low temperature (100 ° F) and low pressure are used in concentrated acid hydrolysis (about 70 percent acid content). In this process, the rate of cellulose recovery from the initial pretreatment phase and the rate of conversion of cellulose to glucose are much higher (90%) than with dilute acid hydrolysis. This method was developed by USDA. The key advantage of the concentrated process is the high sugar recovery efficiency, which can exceed 90% of hemicellulose and cellulose sugar (Badger, 2002)^[6].

(i) Concentrated phosphoric acid

A pretreatment method has been developed using a highly volatile organic solvent (acetone), together with concentrated phosphoric acid as a non-volatile cellulose solvent, and water for the fractionation of lignocellulose into amorphous cellulose, hemicellulose, lignin and acetic acid under moderate reaction conditions (50 °C and atmospheric pressure). This process achieved its highest sugar yield following enzymatic hydrolysis at an enzyme loading rate of 15 cellulase filter paper units and 60 IU beta-glucosidase per gram of glucan. This was due to the minimal degradation of sugar during fractionation, as well as the high enzymatic digestibility of cellulose (97 percent in 24 hours) during the hydrolysis stage (Zhang, *et al.*, 2007)^[103].

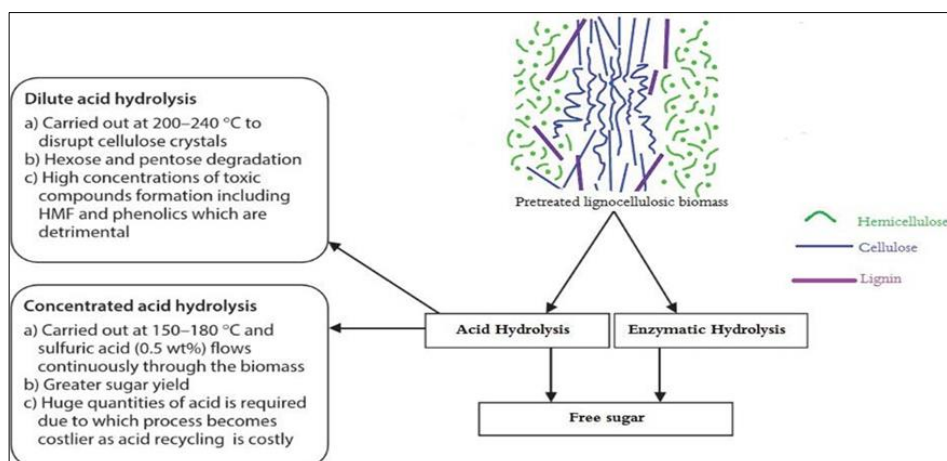


Fig 1: Hydrolysis process for lignocellulosic biomass. (Kuila, A. *et al.* 2016).

Enzymatic saccharification of lignocellulosic material

Reflecting the scarcity of energy with increasing prices and protein supplies in relation to world population growth, the possible uses of lignocellulosic materials for chemicals, food, and fuel in the future have been given greater attention. The most important component of renewable biomass is lignocellulosic materials, which constitute a large proportion of agricultural, industrial and municipal waste. Enormous quantities are available worldwide. With the recent development in cellulase technology, enzymatic saccharification of woody waste has received considerable interest. The recovered sugars can be converted by fermentation using yeasts into ethanol or single-cell protein. Nonetheless, there are still a number of problems to be solved for large-scale commercial implementation of this method. For example, certain pretreatments are required to increase substrates susceptibility to enzymatic attack. One of the major economic factors is the cost of this process. A considerable amount of work on improving the susceptibility of lignocellulose materials has been carried out in the past (Kazumasa Shimizu, 1981) [32].

Pretreatment for Enzymatic Saccharification

Pretreatment removes or weakens the barriers that cause

recalcitrance, so that the raw one's feedstock transforms into a substratum suitable for enzymatic saccharification. A pretreatment that is technically successful will result in a high enzymatic digestibility of the cellulose in the pretreated solids and a high recovery of hemi cellulose sugars / oligomers in the liquid fraction with minimal formation of microbial and enzymatic process inhibitors (Jonsson, L.J, *et al.* 2016). Removal of hemicelluloses or their acetyl groups, degradation of the lignin sheath, decreased crystallinity and degree of polymerisation of cellulose or increased porosity of substrates are some of the mechanisms behind the enzymatic digestibility of various pretreatments. Hydrothermal Pretreatment with Dilute Acid (HPT-DA), also called pretreatment with dilute acid or dilute-acid prehydrolysis, is a highly applicable process for industrial implementation. This is achieved by combining biomass with diluted acid, typically sulfuric acid below 2% (w/w) and heating the suspension for a short period to about 200 °C (Zhao, X.B., *et al.* 2012).

Figure 2 describes the mechanism by which lignocellulosic biomass produces ethanol. A suitable pretreatment is required for the alteration of the biomass structure and its overall chemical component to facilitate the rapid and efficient access and hydrolysis of carbohydrates to fermentable sugars (Maitan-Alfenas, G.P *et al.* 2015; Njoku, SI, *et al.* 2012) [57, 72].

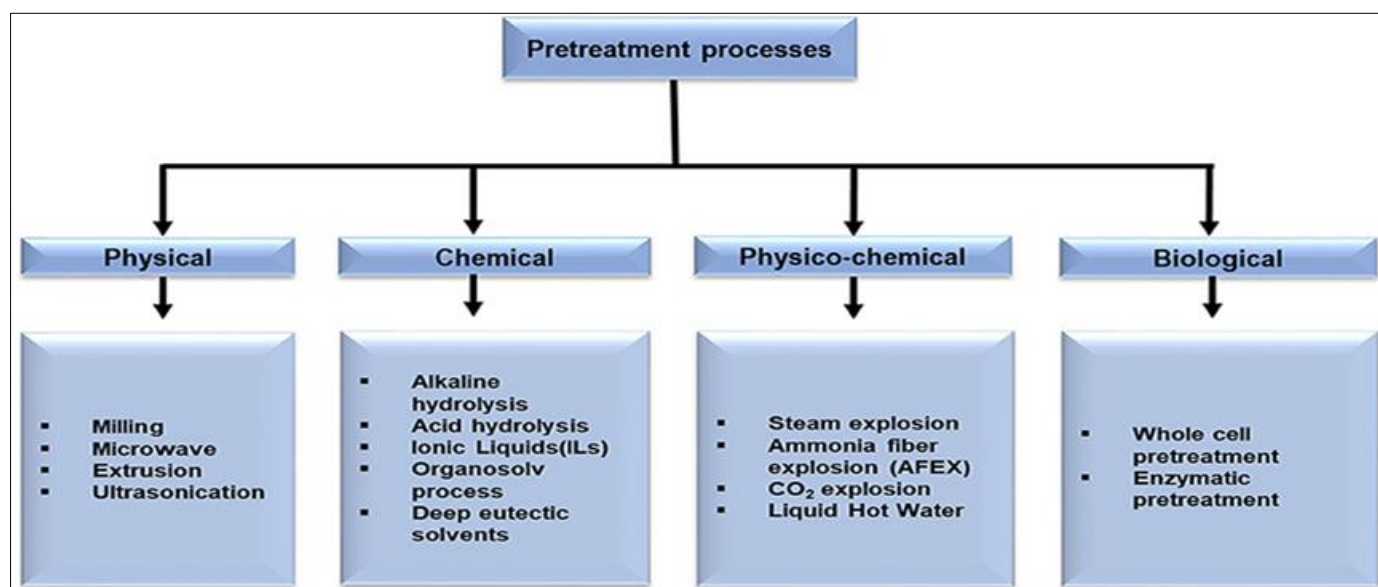


Fig 2: Flowchart representing methods in pretreatment processes. (Baruah, *et al.* 2018) [12].

Pretreatment methods

Steam-Explosion

Steam explosion, also known as autohydrolysis, is the physico-chemical pretreatment process most commonly used for any lignocellulosic biomass (Chandra, R.P. *et al.* 2007) [18]. Delignification, because of its ability to disturb cellulose crystallinity (Balat, M., *et al.* 2007) [9], and simple hemicellulose hydrolysis (Lee, J. M., *et al.* 2010) [49]. Steam explosion is close to that of acid-based chemical pretreatment except that more concentrated sugars are produced during steam explosion since the biomass is easily heated by steam and much less moisture occurs in the reactor (Jorgensen, *et al.* 2007) [39]. This pretreatment causes the breakdown of biomass components due to the expansion of moisture and hydrolysis of glycosidic bonds by the organic acid produced during the process (Avellar, B.K., *et al.* 1998; Jacquet, N., *et al.* 2010) [4, 37]. The method of steam pretreatment is interesting, making it easier to extract lignin, hemicellulose hydrolysis and increase

the surface area for cellulose hydrolysis, but it still suffers from a variety of problems such as low hemicellulose sugar yield, incomplete interruption of the lignin-carbohydrate matrix (Chiaromonti, *et al.*, 2012) and generation of degraded products like hydroxymethylfurfural (Martin-Sampedro, R., *et al.* 2012) [60] and soluble phenolic compounds are inhibitory to microbial growth (Jorgensen, H., *et al.* 2007) [39]. It is a problematic for long term ethanol production.

Organosolv pre-treatments

Pre-treatment with Organosolv following the advantages: (1) Sustainable solvents can often be quickly recovered through distillation and recycled for pretreatment; (2) chemical recovery in organosolv pulping processes may isolate lignin as a solid material and carbohydrates as a syrup, both promising as chemical feedstocks (Aziz, S., *et al.* 1989) [5]. Many organic solvents used for pretreatment with lignocellulose are either polar protic or polar aprotic solvents.

A fraction abundant in cellulose, a fraction of organosolv lignin and a fraction soluble in water sugars (mainly hemicellulose-based sugars), acid-soluble lignin, products for the breakdown of carbohydrates, organic acids and other constituents. Many organic solvents that are used are bulk commodity chemicals, so their cost is low compared to solvents that dissolve cellulose, such as ionic liquids. Despite this, the expense of organosolv pretreatment and corrosion issues continues to be factors that affect their large-scale adoption and they need successful recovery and recycling strategies (Zhanying Zhang, *et al.* 2016)^[104].

Enzymatic saccharification of lignocellulosic biomass for bioethanol production

When the developed countries and living conditions rise, the demand for energy is increasing rapidly. At the other side, fossil fuel depletion produces an energy deficit that indicates a serious need for renewable energy resources (Hook, M. *et*

al. 2013)^[35]. The best theory to fill this energy gap is to use sustainable and renewable resources such as biomass from lignocellulosic sources (Anwar, Z., *et al.* 2014)^[2]. Bioethanol due to high energy density, lower CO₂ emissions, higher air-fuel ratio and reduced vaporization heat is one of the promising renewable energy sources with a high potential for fossil fuels substitution (De Oliveira, MED., *et al.* 2005; Bansal., *et al.* 2016)^[23, 11]. Bioethanol, based on the raw material, is distinguished as first and second-generation ethanol. First-generation bioethanol is primarily produced from C₆ sugars such as sugar beets, cereals and sugar cane, while second-generation bioethanol is generated from renewable lignocellulosic biomass and industrial by-products or residues. (Naik, S.N., *et al.* 2010; Ho, D.P., *et al.* 2014)^[69]. Lignocellulosic biomass ethanol production comprises three main steps: (1) Pretreatment, (2) Enzymatic saccharification / hydrolysis, and (3) Fermentation (Madadi, M., *et al.* 2017)^[56].

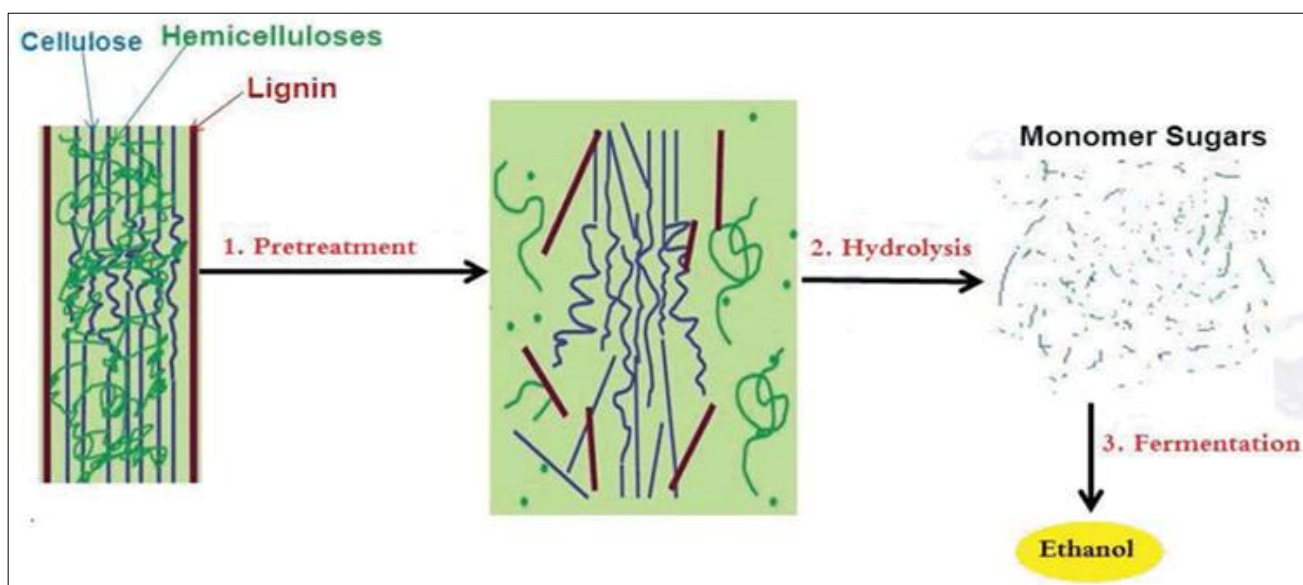


Fig 3: Process of ethanol production from lignocellulosic biomass (Madadi, M., *et al.* 2017)^[56].

Enzymatic saccharification of lignocellulosic biomass for conversion to biofuels and bio-based chemicals

The phase-out of fossil fuels in the transport sector is a major challenge that will require a combination of various measures, including improved fuel efficiency, electrification and increased production of conventional and advanced biofuels. According to current assessments, production levels of advanced biofuels are not on track and major efforts are needed in this area in the coming period (IEA, Paris 2017). Advanced biofuels can make a significant contribution to a change from fossil-based energy to renewable energy, especially with regard to long-range road transport, aviation, and maritime transport. The most prevalent biofuel today is ethanol, with both major producers being the USA and Brazil, and the two primary feedstocks are corn grain and cane sugar (Manochio, *et al.* 2017; Chum, H.L. *et al.* 2014)^[59, 22]. Besides being widely used in Otto engine fuels, ethanol, like ED95, is also useful in diesel engines (Chollacoop, N. *et al.* 2011)^[21]. In addition, ethanol in AtJ (alcohol-to-jet) processes can be valued to hydrocarbons (Geleynse, S. *et al.* 2018)^[27]. Processes in which sugars are converted to ethanol, and possibly further to other energy carriers, are therefore likely to

play an important role in the biofuel sector in the future as well. Sugars derived from lignocellulosic biomass can further boost bioethanol production levels and other bio-based energy carriers (Sanderson, *et al.* 2011)^[80]. The main components of dry lignocellulose are cellulose, hemicellulose and lignin. Lignocellulosic feedstocks include energy crops, softwood, hardwood, and agricultural residues (straw, corn stoves, sugarcane bagasse, cassava stems, etc.) The cellulose and the hemicellulose can be hydrolyzed to monosaccharides in sugar-platform processes. The most common concept is based on an initial step of decrease, for example wood chipping, followed by pretreatment and enzymatic saccharification of the remaining cellulose and residual hemicellulose after the pretreatment. As lignocellulose renders biochemical conversion recalcitrant (Meng, X., *et al.* 2014)^[64]. Pretreatment is needed to make lignocellulose more sensitive to enzymatic saccharification. Reducing recalcitrance is key to cost-effective, high-yield bioconversion of lignocellulosic feedstocks. Chemical catalysis or microbial fermentation can refine the sugar to biofuels. Baker's yeast, *Saccharomyces cerevisiae*, is the most common microorganism used for fermenting sugar (Den Haan, R. *et al.* 2013)^[25].

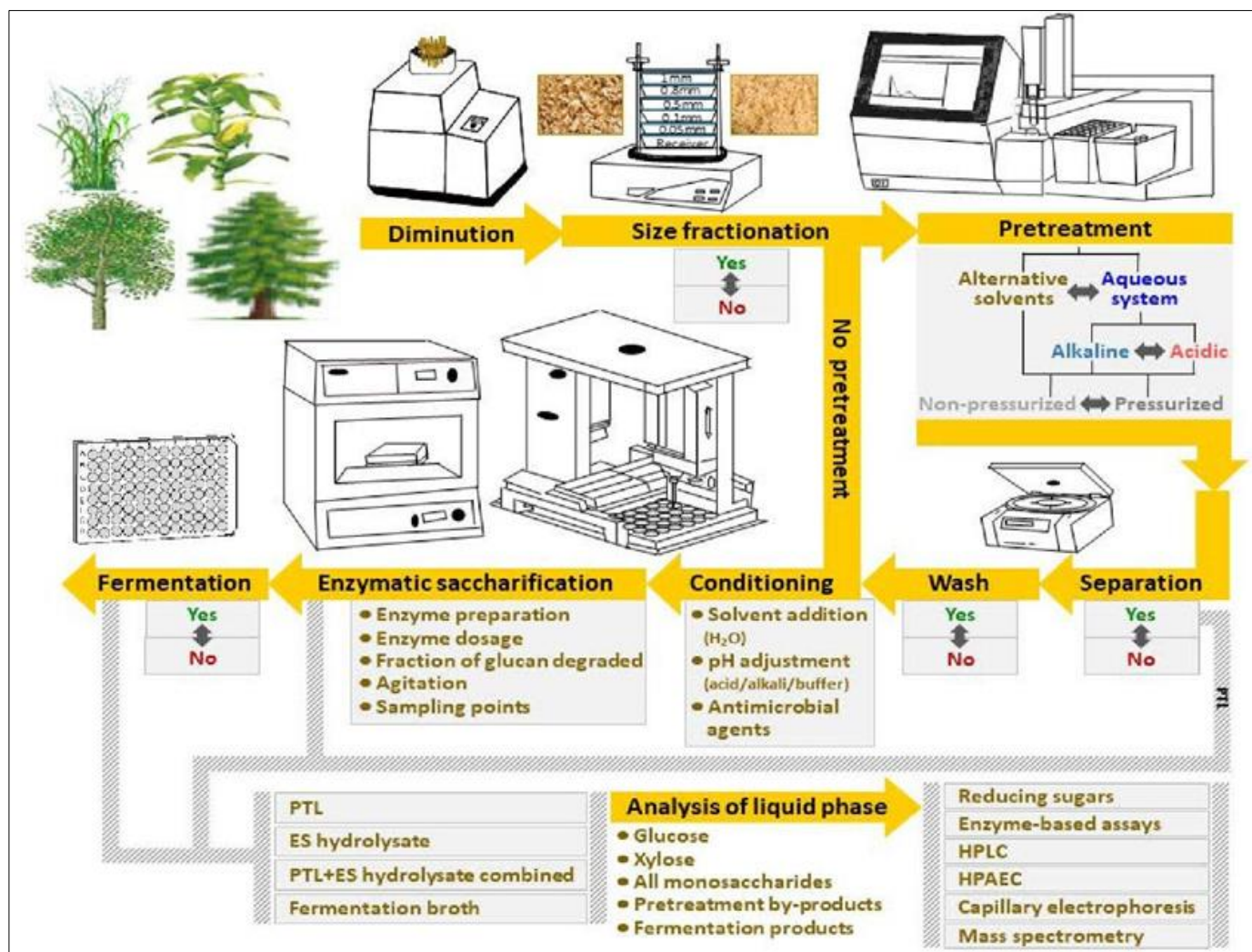


Fig 4: Schematic depiction of typical workflow of analytical enzymatic saccharification of lignocellulosic feedstocks (Meng, X., *et al.* 2014) [64].

Combined simultaneous enzymatic saccharification and comminution (SESC) and anaerobic digestion for sustainable biomethane generation from wood lignocellulose

The extensive use of fossil fuel has environmental, social, and political implications that compel different countries to explore alternative and renewable sources of energy. Reducing reliance on oil-producing nations increases protection against declining fossil fuel supply in the midst of rising demand. Methane rich biogas has long been used as a renewable fuel and is an attractive way to the decline of fossil natural gas energy reserves (Weiland, 2010) [95]. Wood is the richest natural resource for processing biogas. It is however resistant to digestion by anaerobic means. The rigid framework through which the major components, cellulose, hemicellulose, and lignin, are arranged within the cell wall limits susceptibility to microbial attacks. The highly recalcitrant lignin (Ko, *et al.*, 2009) protects holocellulose which is more degradable. Although anaerobic lignocellulose digestion has been well-studied, large-scale pretreatments for wood biomass have yet to be done under mild conditions. In general, vigorous thermochemical pretreatments are required under severe pH conditions. (Hendriks and Zeeman, 2009) [31]. The capacity to enhance anaerobic digestion of wood biomass has been investigated. Wood comminution beyond the cell wall dimension could increase both enzymatic and microbial access to the holocellulose portion, (Menardo, *et al.*, 2012) [63] with a faster rate of hydrolysis and greater efficiency in methane production (Speda, *et al.*, 2017) [85].

Enzymatic hydrolysis of biomass from wood

Woody biomass may be harvested sustainably in significant amounts from forests or even intensively managed plantations worldwide (Perlack, *et al.*, 2005) [74]. In the case of cultivated areas, woody crops such as *Populus*, *Salix*, *Eucalyptus*, *Alnus*, *Loblolly*, *Lodgepole*, *Ponderosa* among other hardwoods and softwood strains or varieties are typically produced (Johnson, *et al.*, 2007). In addition, short-rotation woody crops and other intensively managed tree crops are one of the most sustainable sources of biomass, given that they are strategically positioned on the landscape and handled with good practice for soil and water conservation, recycling of nutrients and proper preservation of genetic diversity (Hall, 2008) [30]. Such woody biomass sources also offer secondary benefits such as carbon sequestration, which stands out as an environment for the stabilization of biodiversity and soil quality (Moser, *et al.*, 2002) [67]. Hundreds of millions of tons of woody biomass, comprising 30% of the total biomass estimated to be usable for biofuels, can be renewable in different parts of the world, including the United States, Scandinavia, New Zealand, Canada, Japan and South America. (Zhu, and Pan, 2010; Da Silva, *et al.*, 2013) [33].

Enzymatic hydrolysis performance of woody biomass at high substrate loadings

The method of lignocellulose-to-sugars using cellulolytic enzymes consists of pretreatment for the removal of lignin and hemicellulose, enzymatic hydrolysis of polysaccharides to sugar monomers, fermentation of sugars to ethanol and

recovery of ethanol from the process stream despite substantial progress towards the commercialization of second-generation bioethanol or other bio-based agriculture products (Peplow, 2014) [73]. Two substrates, unbleached hardwood pulp (UBHW), and pretreated poplar organosolv (OPP) were used. UBHW has approximately 80 per cent cellulose content with 19.6 per cent xylene. The UBHW is an ideal pretreated wood substratum, as it contains a minimum amount of lignin and other contaminants. OPP contains 87 per cent small xylene cellulose. OPP's lignin content is only marginally higher than UBHW's. A mixture of cellulases (Cell clast 1.5L) and beta-glucosidase (Novozym 188) at an enzyme load of about 35 mg Cell clast g⁻¹ glucan and 20 mg Novozym 188 g⁻¹ glucan was used. UBHW reached a conversion rate of cellulose-to-glucose of 84 per cent after 96 h of incubation at 20 per cent substratum consistency. At a consistency of 2 percent, it achieved 100 percent conversion of cellulose to glucose after 24 h of incubation. The lower conversion rate at 20% consistency compared to 2% is mainly due to the inhibition effects from the high glucose content in the hydrolysate (Xiao, *et al.*, 2009) [99]. The most enzymatic hydrolysis studies are conducted at 2–20% total solid to prevent enzyme inhibition. However, this enzymatic hydrolysis was performed at 25% total solid; the highest sugar yield was achieved from the pretreated LP2 sample with 96% for cellulose and about 100% for hemicellulose conversion. This is the highest sugar yield reported for loblolly pinewood. (Rana, *et al.*, 2012) [76].

Wood characteristics and enzymatic saccharification of cotton wood

Energy scarcity and global climate changes caused by unsustainable use of traditional energy sources like gasoline and coal have sparked renewed interest in generating sustainable and clean biofuels from bio resources. Among various biofuels, bioethanol from the fermentation of sugar or starch-based plant materials such as sugar cane and maize are one of the most significant in terms of production costs, gasoline replaceability and market value (Brown and Brown 2012) [17]. Lignocellulosic material contains primarily cellulose, hemicellulose, and lignin. In lignocellulose cells, cellulose aggregates are interlaced into highly crystallized cellulose microfibrils, (Salmen, 2004) [78] and the amorphous hemicelluloses and lignin form a soft matrix around the cellulose microfibrils (Fratzl, *et al.* 2004) [84]. The compactness of the microfibrils and the defense against the matrix of lignin-hemicelluloses prevent the hydrolysis of cellulose by enzymes or other chemical reagents (Taherzadeh and Karimi 2007b) [89]. The usual way to increase the rate of sugar conversion is to perform chemical, thermal or mechanical pretreatments on the raw materials to split the matrix of lignin hemicelluloses, to increase the surface area of cellulose or to decrease the crystallinity of cellulose (Hendriks and Zeeman 2009; Zhu and Pan 2010) [31] with the assistance of modern bio-technology, raw lignocellulosic materials with desired chemical composition can be obtained to ease the processing of bioethanol. By down-regulating the 4-coumarate: Coenzyme A Ligase (4CL) gene, poplar (Hu, *et al.* 1999) [98] and pine (Wagner, *et al.* 2009) [94] trees with reduced lignin content and increased cellulose content were developed, and increased enzymatic saccharification efficiency was observed (Min, *et al.* 2012).

The cellulase-mediated saccharification on wood derived from transgenic low-lignin lines of black cottonwood (*Populus trichocarpa*)

Most of the first-generation bioethanol is derived from sugar cane and corn, which causes some social problems as it competes with our food supply chain. Bioethanol of second generation is derived from lignocellulose and is already being tested in pilot plants. The fundamentally complicated structure of lignocellulose makes it immune to enzymatic hydrolysis of fermentable sugars. Some pretreatment methods, includes physical, physicochemical, chemical and biological, and are therefore necessary to make lignocellulosic alterable to enzymatic hydrolysis. Energy plantations of fast-growing hardwoods may be a way of ensuring a sufficient supply of bioethanol feedstock. For an energy plantation, transgenic trees with the desired characteristics, such as down-regulated lignin or down-regulated xylene, may be desirable candidates. In this study, the effect of lignin content on enzymatic saccharification was evaluated with and without pretreatment using three down-regulated transgenic lignin samples and a wild type as a guide. Four pretreatments, including diluted sulphuric acid, ozone delignification, autohydrolysis and green liquor, were used. These pretreatments have different degrees of delignification abilities. The green liquor pretreatment is an alkaline process developed recently in our laboratory and shows great potential (Taherzadeh, *et al.* 2007).

Future perspectives in enzymatic saccharification of woody and lignocellulosic biomass

Considering the fast depleting fossil sources, there has been a major impetus worldwide to develop alternate source of fuels and bio-based chemicals in this context, woody and lignocellulosic feedstocks have been perceived as most potential and sustainable source for producing alternate fuels and platform chemicals (Lynd, L.R. *et al.*, 1991; Sheldon, R.A. *et al.*, 2014) [55, 83], these include agricultural by-products such as wheat and rice straw, husks, corn stover, corn cobs, bagasse, oilseed cakes, wood, grasses and dedicated energy crops such as miscanthus and switchgrass (Kurian, J.K. *et al.*; Kocar, G. 2013) [47]. Extensive researches are underway for developing efficient processes to utilize them for viable production of fuels and chemicals. The basic woody and lignocellulosic utilization strategy involve three essential and inter-dependent steps namely (i) Pretreatment of the biomass, necessary for disrupting lignocellulosic interactions to make cellulose and hemicellulose, and other carbohydrate polymers better accessible for enzymatic hydrolysis in the next step commonly called as saccharification; (ii) Saccharification of pretreated material by hydrolases such as cellulases, xylanases, and other carbohydrases; and (iii) Appropriate fermentation of monosaccharides, generated out of the saccharification, for production of desirable products *viz.*, biofuels or other platform chemicals. All three processes have been extensively reviewed in the recent year (Taherzadeh, M.J. *et al.* 2007; Kumar, R. *et al.* 2008; Brodeur, G. *et al.* 2011) [45, 16]. It has been generally agreed that effectiveness of pretreatment and saccharification determines the viability and yield of the fuel or other products in the fermentation step. Number of excellent treatment methods such as acid or alkali treatment, hot water washing, steam explosion and ammonia fibre expansion have been developed in the past (Mosier, *et*

al. 2005)^[68]. Since composition and structure of biomass varies from source to source, the efficacy of the pretreatment method also varies for each biomass and no single method can be said to be suitable in generic manner. Nonetheless, optimum pretreatment has been standardized for most of the commonly available biomass, which can be recommended and used with required variation. The saccharification step still remains as one of the critical bottlenecks. An ideal process should generate stoichiometric amount of fermentable monomeric sugars out of the lignocellulosic complex and yield attainable so far has been less than satisfactory and rather poor.

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