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RESEARCH ARTICLE

Lignocellulolytic enzymes: Biomass to biofuel.

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Abstract

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Lignocellulosic biomass is an inexhaustible, renewable, and ubiquitous organic material on the Earth. It is present in huge amount as agricultural and forestry residues and wastes generated from different industries including solid municipal wastes. Lignocellulosic biomass is an alternative, economical and eco-friendly source for biofuel production and other bio-based products. Lignocellulose biomass is mainly comprised of cellulose, lignocellulose, and lignin polymers. Each of the structural components of lignocellulose is degraded specifically by a battery of enzymes, such as cellulase, hemicellulase and ligninase enzyme system, and these constituents in turn can be utilized as a sustainable source of energy. In this communication, we are presenting an overview on the enzymes involved in the degradation of most abundant polysaccharide on earth, and other structural components of the lignocellulosic biomass and their application in conversion of biomass to biofuel.

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Introduction:-

Lignocellulose, a renewable organic material, is the chief structural component of all plants from grass to sequoia tree. It is composed of three major components: cellulose, hemicelluloses and lignin. Most often, the composition of these components in plant dry matter vary from a range of 30-50% for cellulose, 20-35% for hemicelluloses, and 5-30% for lignin (Perez et al., 2002; Saini et al., 2015; Sokan-Adeaga et al., 2016). Besides other materials, such as proteins, pectin, extractives (i.e. no- structural sugars, nitrogenous material, chlorophyll and waxes) and ash may also be present in small amounts as lignocellulosic residues (Kumar et al., 2008; Sanchez, 2009; Dashtban et al., 2009). Cellulose, most abundant organic molecule on the Earth, is a linear biopolymer of anhydroglucopyranose molecules linked together by β-1, 4-glycosidic bonds. The cellulose chains are held together by hydrogen bonds to build cellulose fibrils, which are packed in a matrix of hemicelluloses and lignin. Cellulose, hemicelluloses, and lignin are packed closely through covalent and non covalent interactions in a criss-cross network (Isikgor and Becer, 2015; Saini et al., 2015). Each component of lignocellulose have distinct function, such as cellulose provides strength and flexibility, hemicelluloses connects lignin to cellulose fibers, and lignin acts as a cementing material that holds cellulose and hemicellulose fibers together. In addition, lignin provides structural support, resistance, impermeability and protects the internal cellulosic structure from biological and chemical attack (Sorieul et al., 2016; Sajith et al., 2016).

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The industrial production of a variety of chemicals is mainly dependent on fossil resources. The rising demand of fuel supply, increasing depletion of fossil fuel resources, future risk of global warming and environmental concerns have always been challenging to promote new ideas and to expand the biotechnological horizons for sustainable fuel production (Serrano-Ruiz et al., 2011; Isikgor and Becer, 2015). Lately, enzymatic methods have been successfully recognized that can enable use of lignocellulose as a potential alternative for biofuel production. Lignocellulosic biomass, a copious inexhaustible organic source, comprises approximately half of the world biomass (Classen et al 1999). Lignocellulosic biomass is relatively inexpensive feedstock and easily available as residual parts of plant material from forest and agricultural land as well as wastes produced by many industries including paper, agriculture, food and municipal solid waste (Dashtban et al., 2009; Saini et al., 2015). Lignocellulosic biomass, non-fossil carbon source on earth, is a promising alternative to crude oil that can be utilized for the production of biofuels, biomolecules, chemicals and materials (Maki-Arvela et al., 2010; Sun et al., 2011; Moe et al., 2012; Barakat et al., 2013). Additionally, it is a carbon-neutral renewable source that can reduce green house gases (GHG) emission and environmental pollution (Isikgor and Becer, 2015). The enzyme mediated conversion of lignocellulosic biomass includes cellulase, hemicellulase and ligninase enzyme system (Liao et al., 2014). A wide range of microorganisms including fungi, actinomycetes and bacteria have been implicated as potential source of lignocellulosic biomass degrading enzymes. Microorganisms, containing these enzymes, utilize sugar rich fractions of lignocellulose in fermentation processes for growth and production of value added compounds such as ethanol, enzymes, organic acids, food additive etc. (Mussatto and Teixeira, 2010).

Lignocellulosic biomass:-

Cellulose is fibrous, insoluble and high molecular weight homopolymer of anhydrous glucose units linked by the β-1, 4 glycosidic linkages and is the primary structural component of plants. The long chain cellulose polymers are linked together by numerous cross linkages, such as hydrogen and vander Waal interactions to pack cellulose into microfibrils. The highly ordered arrangement of cellulose chains result in the development of crystalline region whereas in the amorphous region, it is less ordered arrangement of chains (Gardner and Blackwell, 1974; Saha, 2000; Li et al., 2009). The degree of polymerization of cellulose chain is highly variable ranging from 250 to 10000 and it influences physiological, mechanical and biological properties of the cellulose. Cellulose chain length or degree of polymerization depends on the source of material & treatment methods (Kuhad et al., 1997; Klemm et al., 2005; Sukumaran et al., 2005; Kolakoviu, 2013; Santos et al., 2016). Cellulose is the chief constituent of lignocellulosic biomass and is regarded as the strongest potential candidate for sustainable fuel production due to its environmental friendly characteristics, such as renewability, biocompatibility and biodegradability (Ahn et al., 2012; Isikgor and Becer, 2015).

Table 1:- Lignocellulosic biomass: chemical composition of few different woods

	Cellulose (%)	Hemicelluloses (%)	Lignin (%)
Hardwoods	40-55	24-40	18-25
Softwoods	45-50	25-35	25-35
Grass	25-40	25-40	10-30
Sugarcane bagasse	42	25	20
Rice straw	32.1	24	18
Wheat straw	29-35	26-32	16-21
News paper	40-55	25-40	18-30

Hemicellulose, second most copious component of lignocellulosic biomass, is a heterogeneous polymer of pentoses (including xylose and arabinose), hexoses (mainly mannose, less glucose and galactose), and sugar acids (Saha, 2000; 2003). Typically, it is made up of five different sugars, L-arabinose, D-galactose, D-glucose, D-mannose, and D-xylose along with other components, such as acetic, glucuronic and ferulic acids (Musatto and Teixeira, 2010). Distribution of different sugars in hemicellulose varies with different wood and cultivation conditions (Sorieul et al., 2016). Generally, in soft wood hemicellulosic part is predominantly composed of mannose whereas it is replaced by xylose in the hard woods (Fan et al., 1987; Persson et al., 2006). Usually, hemicelluloses constitute 15-35% of lignocellulosic biomass and the degree of polymerization ranges from 100-200 (Kuhad et al., 1997; Sorieul et al., 2016).

Lignin, third main heterogeneous polymer of lignocellulosic biomass, is a complex molecule formed of three phenyl propionic alcohols including coniferyl, sinapyl and p-coumaryl alcohol. Lignin is embedded in sugar polymer matrix of cellulose, and hemicelluloses, and provides rigidity and structural support to the cell wall. It confers water impermeability to xylem vessel and form a barrier against biological and chemical attack. It is the most recalcitrant component of lignocellulosic biomass (Zaldivar et al., 2001; Hamelinck et al., 2005). Similar to carbohydrate diversity in hemicellulose, distribution of aromatic alcohol is also varies depending on the wood. Soft wood is constituted of more than 90% of coniferyl alcohol, while hardwoods are composed of varying degree of coniferyl and sinapyl alcohols (Harmsen et al., 2010).

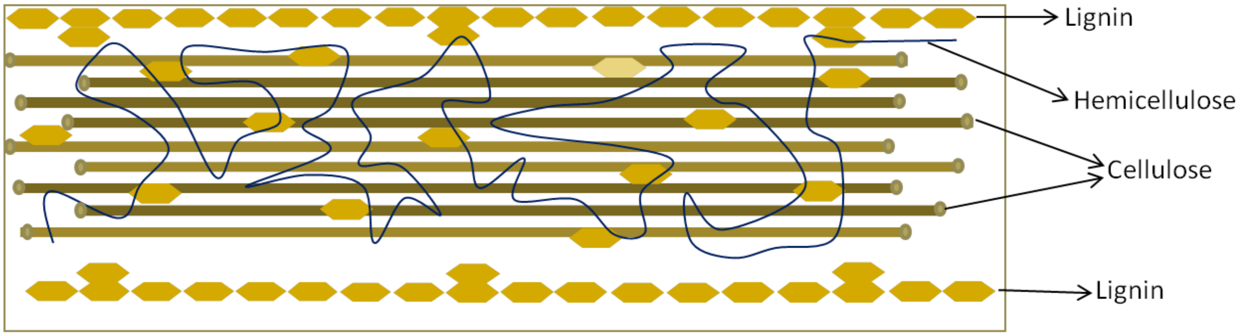

Fig. 1 Representation of lignocellulose structure showing cellulose, hemicelluloses and lignin fractions.

Lignocellulolytic enzymes:-

The lignocelluolytic enzymes are required for pretreatment of lignocellulosic biomass to access the potential of cellulose and hemicelluloses for a wide range of industries including biofuel, paper, textile etc. These enzymes catalyze degradation of lignocellulosic biomass to use as a source of carbon and energy and for ecological recycling. The lignocellulosic biomass is a complex structure and its degradation is carried by a complex array of microbial enzymes which are divided into lignolytic, cellulolytic and hemicellulolytic enzymes (Malherbe and Cloete, 2000; Sukumaran et al., 2010).

Lignolytic enzymes:-

Liginin is a highly insoluble complex heterogenous polymer that is composed of three phenyl propionic alcohols monomers: p-coumaryl, sinapyl and coniferyl alcohol. The crosslinking between different polymers confers the rigidity and complexity of the plant cell wall (de-Souza, 2013). Lignin composition in the lignocellulosic biomass varies among plant sources and it may be in the range of 5-30% of the biomass. The lignolytic enzymes, implicated in degradation of lignin into low molecular weight compounds, generally comprise a group a three principal enzymes: lignin peroxidase, manganese peroxidase and laccase (Saini et al., 2015; Sokan-Adeaga et al., 2016). Lignolyic enzymes are produced by a wide range of microorganisms including bacteria, fungi and actinomycetes. White rot fungi are particularly associated with the production of extracellular enzymes responsible mainly for lignolytic function. Basidiomycetes family members such *as Coriolus versicolor*, *Phanerochaete chrysosporium and Trametes versicolor* are the most extensively studied microorganisms for lignolytic activites (Wang et al., 2003; Sokan-Adeaga et al., 2016).

Laccase (EC 1.10.3.2, p-diphenol:dioxygen oxidoreductase) is an oxidase, containing four copper atoms per molecules at their active site. These multicopper glycoproteins catalyze oxidation of phenolic compounds with concomitant reduction of molecular oxygen to water. Laccases, extracelluar inducible metalloenzyme that utilize simple oxygen as an oxidizing agent as well as cofactor, are involved in efficient oxidation, cleavage and polymerization of a number of biological as well as synthetic phenolic and non-phenolic compounds (Fisher and Fong, 2014; Saini et al., 2016; Singh et al., 2016a). Laccase catalyses the oxidation of ortho- and para-diphenols, aminophenols, polyphenols, polyamines, lignins and aryldiamines as well as some inorganic ions coupled to the reduction of molecular oxygen to water (Yaropolov et al., 1994; Solomon et al., 1996; Unal et al., 2011). In lignocellulose, laccase catalyze the oxidation of most recalcitrant component i.e. polyphenol of the biomass to yield phenoxy radicals and quinines. Though they are produced by plants, insects bacterial and fungal microorganisms but basiodiomycetes fungi are considered to be the main producers of potential laccases for biofuel production from lignocellulosic biomass. *Aspegillus nidulans*, *Phanerochaete chrysosporium*, *Pleurotus pulmorious*, *Lentinula edodes* and *Phellnus ribis* are the known producers of laccases (Kunamneni et al., 2007; Arora and Sharma, 2010; Sajith et al., 2016). Many molecular studies have suggested that the catalytic site of laccases is conserved in different fungal species, but remaining structures of the protein show high diversity (Maciel et al., 2010; Gochev and Krastanov 2007).

Lignin peroxidase (EC 1.11.1.4), a member of oxidoreductases family, can specifically degrade high redox potential compounds in the presence of hydrogen peroxide. Lignin peroxidases catalyze the oxidation of a wide range of phenolic as well as nonphenolic aromatic substrates that make up around 90% of the lignin polymer. They are extracellular heme protein and produced by several species of basidiomycetes and actinomycetes. Due to wide range of substrate specificity and high redox potential, they are utilized in various industrial applications (Maciel et al., 2010).

Manganese peroxidases (EC 1.11.1.3), also a member of oxidoreductases family, are low redox potential heme peroxidases and require hydrogen peroxide for their activity. They can be manganese dependent or versatile peroxidases (Fisher and Fong, 2014; Placedo and Capareda, 2015). Manganese peroxidases oxidize Mn^{2+} to highly reactive Mn^{3+} that catalyzes the oxidation of phenolic structure to phenoxy radicals. It is mainly produced by white rot fungi (Hofrichter, 2002; Hammel and Cullen, 2008).

Cellulolytic enzymes:-

Cellulose, most copious constituent of plant cell wall and a renewable resource, is of considerable economic importance due to its potential applications for deriving bioenergy and biobased products. Cellulose is used as food source by a wide array of microorganisms and animals. The cellulolytic enzymes are important and essential for hydrolyzing cellulose into fermentable sugars that can be used for further applications. The efficient hydrolysis of cellulose is primarily carried out synergistically by a complex enzyme system comprising endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) (Kuhad et al., 2011).

Endoglucanase (endo-β-1,4-D-glucanase, endo-β-1,4-D-glucan-4-glucanohydrolase) acts in a random manner on amorphous sites in the cellulose fibers and hydrolyze internal β-1,4 linkages to generate oligosaccharides of free chain ends. Endoglucanase also act on cellodextrin and convert them to cellobiose and glucose (Beguin and Aubert 1994; Lynd et al., 2002). Exoglucanase (exo-β-1,4-D-glucanase, cellobiohydrolase) acts on microcrystalline region in the cellulose polysaccharide chain to release cellobiose residues generally from the non-reducing ends (Beguin and Aubert 1994). β-glucosidase catalyze hydrolysis of the soluble cellodextrins and cellobiose to glucose residues (Beguin and Aubert 1994; Bhat and Bhat, 1997).

These three proteins work synergistically and catalyze appropriate hydrolysis for obtaining glucose residues which are used for various applications including production of biofuel, feed stock, single cell protein, and chemicals etc. (Lynd et al., 2002; Li et al., 2009). Among the microbes, fungi are dynamic cellulase producers and responsible for approximately 80% of the cellulose hydrolysis on the Earth (Moore, 1996). Primarily, ascomycota, basidiomycota and deuteromycota members of the fungi are possessed with efficient cellulolytic activities. The aerobic fungal cellulolytic enzyme are preferred widely for industrial application as these are extracellular and secreted in bulk during growth (Mathew et al., 2008). Primarily, species of *Aspergillus, Trichoderma, Penicillum* and *Sclerotium* are considered potential candidates for industrial production of cellulolytic enzymes (Pointing, 1999; Sajith et al., 2016).

Hemicellulases:-

Hemicellulases, most often glycoside hydrolases, are generally produced together with cellulases. Xylan and mannan are most copious component of the hemicelluloses. Xylan forms the major part of hardwood hemicellulose whereas mannan is twice the amount of xylan in softwood hemicelluloses. The disparities in the distribution of the carbohydrate in the wood fibers are dependent on the wood species and cultivation conditions.

Hemicellulases are represented by xylanases (EC 3.2.1.8), β-mannanases (EC 3.2.1.78), β-xylosidase (EC 3.2.1.37) and arabinofuranosidases (EC 3.2.1.55) (Sajith et al., 2016). The cellulose fibers in plant are held together by lignin and xylan. The synergistic action of xylanase is required for optimal hydrolysis of lignocellulosic residues (Ghose and Bisaria, 1979). Xylanses catalyze the hydrolysis of internal β-1,4 xylosidic linkages in xylan to oligomers. β-xylosidase hydrolyze xylobiose and small xylooligosacchaides to xylose and facilitate the hydrolysis of xylan.
Additional hemicelluolytic enzymes, such as β-mannanases and arabinofuranosidases are required for mannan or arabinofuranosyl containing hemicelluloses. Arabinofuranosidase catalyze the removal of arabinosyl substituents and facilitate increase in the access points of xylanase to the xylan (Dekker, 1985; Biswas, 2014).

Mannanases hydrolyze β-D-1,4 mannopyranosyl linkages in mannose containing polysaccharides such as glucamannans, galactomannas and galactomannans. The side group connected to xylan and glucomannan chains can be cleaved by α-glucuronidase (EC 3.2.1.131), α-arabinosidase and α-D-galactosidase (EC 3.2.1.22). Many fungal species especially *Aspergillus*, *Penicillum* and *Trichoderma* are associated with the simultaneous extracellular production of cellulases and hemicellulases (Dashtban et al., 2009; Anwar et al., 2014; Sajith et al., 2016).

Conversion of lignocellulosic biomass into ethanol:-

In the light of fossil fuel crisis and continuously rising demand of fuel supply, the application of naturally occurring starch and cellulose have been strongly considered as potential source of alternative biofuels. The conversion of starch from corn and other crops into fuel is one of the largest worldwide applications in biotechnology. In nature, starch is the most abundant polysaccharide food reserve after cellulose and the primary source of carbon and energy on the Earth (Buleon et al 1988; Sharma and Satyanaryana 2013; Singh et al., 2016b). It is deposited in plant cells in the form of granules as reserve material. The predominant sources of starch for industrial purposes are cultivated crops such as maize, tapioca, potato, wheat, oats, barley etc. The application of starch containing crops for continuous production of ethanol can lead to the competition with starch based food supply (Cherubini, 2010; Li et al., 2009; Isikgor and Becer, 2015).

On the other side, lignocellulosic biomass is the most abundant inexhaustible renewable organic material that may be available as non-edible portion of the plants, woody residual parts of plants, agricultural wastes and wastes generated from various industries including paper, food and municipal solid waste. The wastes generated every year in huge amount can be utilized for the production of value added products. The production of lignocellulosic biomass is more economic and faster than other agricultural feedstock such as corn, sugar cane and soybeans (Perez et al., 2002; Huber, 2008; Taherzadeh and Karimi, 2008).

The bioconversion of the lignocellulosic biomass to alternative fuel includes hydrolysis of constituents of biomass to fermentable reducing sugars followed by sugar fermentation using yeast to produce ethanol and other fuels. The first step involves degradation of the lignocellulosic polymer, delignification to release cellulose and hemicelluloses contents from their complex with lignin, and depolymerization of carbohydrate polymer to produce free sugars (Lee, 1997; Perez et al., 2002).

In conventional methods hydrolysis is performed by the chemical treatment of cellulose with an acid. Different temperature and pressure conditions are required according to the acid strengths. In the presence of water, reaction between decrystallized cellulosic mixture of sugar and acid leads to the liberation of individual sugar molecules. The dilute-acid process is a harsh process that results in the formation of toxic products that may interfere with the sugar fermentation. Cellulose chain can also be enzymatically hydrolyzed under relatively mild conditions (50°C and pH ~5) without formation of byproducts that would otherwise inhibit enzyme activity. An anaerobic thermophilic bacterium, *Clostridium thermocellum*, has significant potential to hydrolyze cellulose and, at the same time, fermentation of resulting sugars to ethanol. This microbe offers advantages such as higher growth rate & metabolic activities, and improved enzyme stability. Besides, the recovery of products is comparatively easier (Shoham et al., 1999; Perez et al., 2002; Kumar et al., 2009; Bogati, 2011). In the second step, free sugars, such as glucose, galactose, and mannose are readily fermented to alcohol. *Saccharomyces cerevisiae* has been conventionally utilized for the production of ethanol from six carbon sugars (Jeffries and Jin, 2004; Mosier et al., 2005). The five carbon sugars, such as xylose and arabinose require additional efforts for their hydrolysis to achieve higher production of cellulosic biofuels and other products. In recent years, metabolic engineering of microorganisms has shown significant progress in the efficiently fermentation of hydrolysates containing xylose and arabinose (Becker and Boles 2003; Ohgren et al., 2006; Kumar et al., 2009).

Conclusion:-

Lignocellulosic biomass, a potential source for the production of biofuel and other chemicals, is primarily beneficial for the areas where huge amount of waste materials are easily available. Non-edible and residual parts of plants from forest and agriculture, municipal solid waste and waste generated from food and paper industries are generated every year in huge amount that can be utilized for the production of value added products. The production of lignocellulosic biomass is more economical than other agricultural feedstock such as corn, sugar cane and soybeans. The constituents of the lignocellulosic biomass are degraded by an array of enzymes that work synergistically, and turn biomass into biofuels and bio-based products. Though a wide array of microorganisms, primarily fungi and bacteria have been reported as lignocellulolytic microorganisms but only few of them have been studied extensively. Despite the progress achieved, more efforts are required to improve catalytic activities and production efficiency of lignocellulolytic enzymes through genetic engineering and molecular modeling that can have significant industrial impact.

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