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Review

Biological pretreatment of lignocellulosic biomass – An overview

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HIGHLIGHTS

- An eco-friendly process for effective delignification.
- No generation of fermentation inhibitors during the process.
- Major drawbacks include treatment time and sugar consumption.
- No release of toxic compounds to environment.
- No effluent generation during the process.

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ABSTRACT

Pretreatment is an important step in ... in the production of bioethanol from lignocelluosic biomass. Though several pretr... regimes are ...ble, biological pretreatment seems to be promising being an eco-friendly proce... ...no inhibitor generation during the process. In the current scenario there are few limitatio... ...n usi... ...tegy for pilot scale process. The first and foremost one is the long incubation time fo... ...delignification. This can be minimized to an extent by using suitable microbial ...ium. Th... ...an urgent need for research and development activities and fine tuning of the ...ess ...he deve... ...ment of an economically viable process. This review presents an overview of v... ...as aspe... ...of biolog... ...pretreatment, enzymes involved in the process, parameters affecting biolog... ...retre... ...ell as future perspectives.

1. Introduction

Increase in depletio... fossil... ...s and disadvantages of fossil fuel derived transportati... ...e like greenhouse gas emission, pollution, resource depletion ...unbala... ...d supply demand relations leads t... for ...ve energy source from renewable s... ...ce lik... ...gnocelli... ...e biomass (Hamelinck et al., 2005). Eth... ...l from... ...d based materials may lead to "food vs fuel" conflic... ...of world population (Kazi et al., 2010). Corn an... ...ar based ethanol are promising substitute to gasoline productio... transportation sector, are not sufficient to replace global fossil ... consumption each year.

Lignocellulosic biomass serves as a potential source for the production of second generation bioethanol. Since lignocellulosic biomass is composed of cellulose, hemicellulose and lignin, some kind of pretreatment to be carried out for the removal of hemicelluloses and lignin which are bonded by covalent cross linkages and non-covalent forces. The presence of high level of cellulose and hemicelluloses in lignocellulosic biomass is the main advantage for their usage for the production of bioethanol (Cheng et al., 2008). Another main advantage of lignocellulosic biomass is their surplus availability and relatively low cost as well as renewable. It do not compete with food production or animal feed.

Resistance to enzymatic hydrolysis is a major limitation of conversion of lignocellulosic biomass to sugars. Several studies revealed that biological pretreatment especially using white rot fungi can improve the hydrolysis efficiency with the advantage of limited energy consumption (Shi et al., 2009). Many lignocellulosic biomasses like rice straw, sugarcane bagasse, wheat straw, cotton stalk, bamboo, sugarcane tops are some of the abundantly available agro-residue. Many agro-residues are well known for ethanol production.

Fuel ethanol production from lignocellulosic biomass is accompanied by several drawbacks like high production cost, special equipment requirements, large water consumption and complex production technology (Sun and Cheng, 2002). Hence lignocellulosic ethanol production is currently not economically viable.

Most physical and chemical pretreatment using acid, alkali, microwave, steam explosion, ionizing radiation or combined

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processes require special instrument and consume a lot of energy and generate inhibitors which will affect enzymatic hydrolysis and fermentation (Mosier et al., 2005). Biological pretreatment using metabolite of a microorganism in nature for ethanol production from biomass is a promising technology due to its several advantages like eco-friendly and economically viable strategy for enhancing enzymatic saccharification rate. Since no chemicals were used in this process, there is no need for recycling of chemical and does not release toxic compounds to environment.

In biological pretreatment microorganism like brown, white and soft rot fungi were used for degradation of lignin and hemicelluloses from the lignocellulosic biomass. Biological pretreatment using white rot fungi that can degrade lignin seems promising since they consume less energy and less damage to the environment (Chen et al., 2010). Currently several research and developmental activities were going on for detecting the alterations in structure, chemistry and enzymatic hydrolysis of lignocellulosic biomass after biological pretreatment. The byproducts produced during biological pretreatment normally won't inhibit subsequent hydrolysis since the pretreatment is carried out at mild conditions. During biological pretreatment efficient degradation of lignin depends on the lignolytic enzymes produced by basidiomycete like lignin peroxidase, manganese peroxidase and laccase.

In biological pretreatment the white rot fungus helps in delignification which in turn improves the enzymatic saccharification rate. Currently there is a need for unique consortia for biological pretreatment. Effective biodegradation of lignocellulosic biomass takes place by biodegradation by synergistic action of microbial consortium including various bacteria and fungi. There are several advantages of using microbial consortium which include in adaptability, improving productivity, improving enzymatic sa rification efficiency, control of pH during sugar utilization d increasing substrate utilization (Kalyani et al., 2013). Biolog pretreatment is considered as inexpensive proces compar to other pretreatment processes such as AFEX d org solven Large scale operation leads to high operatio osts si pretreatment to be carried out in sterile conditions a hi cost of the process. The process is t slow a s not recommended for industrial purposes (C vedi and V , 2013).

This review discusses polys degrading roorganisms involved in biological pretr atmen ious process parameters affecting the process a ell as future pectives.

2. Biological pretrea nt of nocellulosic biomass

Pretreatment of biom s the fi step in bioethanol process and is the m anging ce t is considered as the critical step and a la impact digestibility of cellulose and it strongl fluenc ownstream costs involving detoxification, enzyme l ment demands and other variables (Zhang, 200 etreatment constitutes for more than 40% of the total processi t. In lignocellulosic biomass the cellulose is protected by hemicelluloses and lignin. Hence it reduces surface area available for enzymatic saccharification. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its sub-microscopic chemical composition and structure so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields. Proper pretreatment may increase the concentration of fermentable sugars after enzymatic saccharification thereby increasing the overall process efficiency. An ideal pretreatment process avoids the needs for size reduction of biomass, makes the lignocellulosic biomass susceptible for quick hydrolysis with increased yields of monomeric sugars and should formation of inhibitory compounds and minimize en y dema nd capital and operational cost requirement (Gu nd Verma, 5).

Hydrolysis of lignocellulosi biom without a pretreatment can yield less than 20% of t sugars, e afte retreatment it can reach up to 0 = 9 with some tment methods (Alizadeh et al., 2005). eral p eatment h thods are currently available with their me merits. The effectiveness of pretreatment depe n the sical st ture, chemical composition of the bi s and the t m conditions.

A numb ocellulosic p eatment technologies are currently unde inves ion in both laboratory as well as pilot scale. Though several pretr ent strategies were available, only a few se be promising. ring the last decades, many pretreat t processes have been developed for decreasing the biomass lcitrance, only a few of them seems to be promising. Pre- ment is pr ably the most energy intensive operation in bioma nversi to fuels or chemicals. Table 1 presents details of differe gical pretreatment strategies involved for pretreatment of lignocellulosic biomass and its advantages.

a et al. (2012) reported selective lignin degrading basidiomycetes and biological pretreatment of bamboo culms for bioethanol production. Fifty-one fungal isolates were obtained and they belong to white rot basidiomycete *Punctularia* sp. TUFC20056 and an unidentified basidiomycete TUFC20057. They showed preferential lignin removal (50%) than *Ceriporiopsis subvermispora* FP90031 and *Phanerochaete sordida* YK624. Pretreatment with *Punctularia* sp. TUFC20056 improved hydrolysis efficiency.

Promising effect of white rot fungus *Irpex lacteus* for biological pretreatment of corn stalks was reported by Du et al. (2011). During biological pretreatment *I. lacteus* can produce varieties of extracellular hydrolytic and oxidative enzymes. Hydrolysis yield of 82% was achieved after 28 days of biological pretreatment. The study revealed that the by-products from biological pretreatment played an important role in enzymatic hydrolysis which may be attributable to hydrolytic enzymes and iron reducing compounds produced by *I. lacteus*.

Taha et al. (2015) reported enhanced straw saccharification through co-culturing of lignocellulose degrading microorganisms. The results indicate that enzyme activities of fungal isolates were two fold higher than those from bacteria. Co-culturing resulted

Table 1
Different biological pretreatment strategies involved for pretreatment of lignocellulosic biomass and its advantages.

Microorganism	Biomass	Major effects	References
Punctualaria sp. TUFC20056	Bamboo culms	50% of lignin removal	Suhara et al. (2012)
Irpex lacteus	Corn stalks	82% of hydrolysis yield	Du et al. (2011)
Fungal consortium	Straw	Seven fold increase in hydrolysis	Taha et al. (2015)
P. ostreatus/*P. pulmonarius*	*Eucalyptus grandis* saw dust	Twenty fold increase in hydrolysis	Castoldi et al. (2014)
P. chrysosporium	Rice husk	–	Potumarthi et al. (2013)
Fungal consortium	Corn stover	43.8% lignin removal/seven fold increase in hydrolysis	Song et al. (2013)
Ceriporiopsis subvermispora	Wheat straw	Minimal cellulose loss	Cianchetta et al. (2014)
Ceriporiopsis subvermispora	Corn stover	2–3-fold increase in reducing sugar yield	Wan and Li (2011)
Fungal consortium	Plant biomass	Complete elimination of use of hazardous chemicals	Dhiman et al. (2015)

in seven fold increase in saccharification rate. Co-culturing significantly increases saccharification which leads to increased commercial potential for the use of microbial consortia.

Biological pretreatment of *Eucalyptus grandis* saw dust degradation patterns and saccharification kinetics with white rot fungi was reported by Castoldi et al. (2014). The treatment produced structural changes in the saw dust fibers and after pretreatment there was a twenty fold increase in reducing sugars. The treatment with *Pleurotus ostreatus* and *Pleurotus pulmonarius* resulted in selective degradation of lignin which is evidenced by FTIR and microscopic analysis.

Potumarthi et al. (2013) studied simultaneous pretreatment and saccharification of rice husk by *Phanerochete chrysosporium*. Effective delignification was carried out by growing the fungus on rice husk and the pretreated biomass were subjected to enzymatic hydrolysis. Enzymes like cellulase, xylanase, lignin peroxidase, glyoxidase and aryl alcohol oxidase were produced during fungal pretreatment. Highest reducing sugar (895 mg/ml) was observed on eighteenth day of fungal treatment. This method avoids operational costs associated with washing and the removal of inhibitors during conventional pretreatment.

Biological pretreatment under non sterile conditions for enzymatic hydrolysis of corn stover was reported by Song et al. (2013). Fungal pretreatment effectively removed lignin and altered biomass structure for enhanced enzymatic hydrolysis. There was 43.8% lignin removal after pretreatment for 42 days with fungi. The saccharification efficiency was seven fold higher when compared to raw corn stover.

Cianchetta et al. (2014) reported effective delignification of wheat straw using *C. subvermispora*. Minimization of cellu losses due to fungal metabolism should be taken into account d ing biological pretreatment. *C. subvermispora* showed minimal c lulose loss and highest sugar yield up to 44% after ten weeks o pretreatment. Various process parameters affecting b al pretreatment like incubation temperature, incubatio e, i ulums concentration were optimized.

Different feed stocks were evaluated for t ffe fungal pretreatment by *C. subvermispor* an al 2011). The results indicate that corn stover, switch ass and woo re effectively delignified by *C. subvermispo* was a two to ee fold increase in reducing sugar yield. E ct of a on of several external carbon sources and enzy nducers we udied and found that addition of glucose malt extract i oved cellulose digestibility of wheat st .

Simultaneous pretre nt saccharification (SPS) using a cocktail of hydrolytic and ing en es from fungal consortium was report imal (2). The novel laccase effectively functi as a toxifyi nt. This is the first report on develop of an friendly simultaneous pretreatment and saccharifica is process completely eliminates the use of haza s chemicals. Conducting pretreatment and saccharification in t me vessel makes the process economically viable, reduces ene consumption and generates a simple process for removal of residual biomass.

3. Polysaccharide degrading microorganisms and enzymes

Lignocellulosic biomass is composed of cellulose, hemicelluloses and lignin. It contains about 20–30% of hemicelluloses and 15–20% of lignin. Earlier studies revealed that presence of hemicellulose in low quantity can also prevent cellulolytic enzymes to degrade cellulose efficiently (Alvira et al., 2011). Different enzymatic activities are involved for the complete hydrolysis of lignocellulosic biomass. Several research and developmental were going on for the development of enzymes with reduced production costs and increased specific activity.

One of the major factors that limit commercialization of the bioethanol production from lignocellulosic biomass is the cost as well as hydrolytic efficiency of the enzymes. Accessory enzymes are those enzymes which act on less abundant linkages found in plant cell walls. These include arabinases, lyases, pectinases, galactanases and several types of esterases.

Several studies revealed that supplementation of accessory enzymes will improve hydrolysis efficiency. Significant research has been made to improve the economic viability of enzymatic hydrolysis. Efficient and cost effective p isolation and purification of accessory enzymes nee o be es ished. Development of a reliable hydrolysis kin helps in t design and operation of hydrolysis reactors w ich w elp to inc se specific activities and economic via y of en atic rolysis for bioethanol production.

Enzymatic hydrolysis ludes th rocessing eps that convert the carbohydrate polym omeric ugars. Cellulose crystallinity, accessible ace ar d prot on by lignin and cellulose sheathing b micelluloses e various potential factors that contribut sistance o nass to enzymatic hydrolysis. It is carri d out cellulases at mild conditions of pH and temperature 4.5 and 50 respectively. Some proteins like swollenin important r m non-hydrolytically loosening the cell sic fibril network and do not act on β-1, 4 glycosidic bonds in ulose. Swo in increases the accessibility of cellulases to cell e chains dispersion of cellulose aggregations and there xposi individual cellulose chains to the enzyme. Enzyme actors which affects hydrolysis includes enzyme entration, enzyme adsorption, end-product inhibition, ther- ivation and unproductive binding to lignin. The rate of zymatic hydrolysis is mainly affected by structural features of cellulose which include cellulose crystallinity, degree of polymer- ization, accessible surface area, particle size as well as presence of associated materials like hemicelluloses and lignin (Binod et al., 2011).

Enzymatic hydrolysis is affected by cellulase accessibility to cellulose and cellulase effectiveness. Studies revealed that there is a strong correlation between rate of hydrolysis and enzyme adsorption. Cellulase accessibility to cellulose is affected more by xylan removal than lignin removal. Though xylan or lignin removal enhances saccharification rate, the xylan removal directly impacts glucan chain accessibility. Hence removal of xylan is more advantageous than removal of lignin. Xylan removal helps in reduced enzyme inhibition by xylo-oligomers as well as reduced requirements of accessory enzymes.

The enzymatic digestibility of native biomass is very low unless a very large excess of enzyme is used because of the structural characteristics of the biomass. Lignocellulosic biomass is a heterogeneous complex of carbohydrate polymers and lignin which typically contains 55–75% carbohydrates by dry weight. Cellulose is a polymer of glucose and the specific structure of cellulose favors the ordering of polymer chains into tightly packed, highly crystalline structures that are water insoluble and resistant to depolymerisation (Mosier et al., 2005). The other component of lignocellulosic biomass includes hemicelluloses which is a branched polymer of glucose or xylose substituted with arabinose, xylose, galactose, fucose, mannose, glucose or glucuronic acid or with some side chains containing acetyl groups of ferulate (Carpita and Gibeaut, 1993). Hemicellulose will form hydrogen bonds with cellulose microfibrils and provides the structural backbone to plant cell wall. Lignin present in the cell wall imparts further strength. Recalcitrance of lignocellulosic biomass for hydrolysis is due to crystallinity of cellulose, accessible surface area and heterogeneous

nature of biomass as well as protection of cellulose by lignin (Chang and Holtzapple, 2000).

3.1. Lignin degrading enzymes

Lignin is the most abundant aromatic polymer consisting of non-phenolic and phenolic structures. Lignin forms an integral part of secondary walls in plants and it plays an important role in enhancing the efficiency of water conduction in vascular plants. Some fungi, bacteria and insects are capable of producing enzymes which can digest lignin. This includes lignin peroxidases and laccases. Peroxidases enzyme includes lignin peroxidase (E.C. 1.11.1.7) and manganese peroxidase (E.C. 1.11.1.7) are the two major components of lignolytic enzyme system. These are heme-containing glycoprotein which requires hydrogen peroxide as oxidant. Lignin peroxidase degrades non-phenolic lignin units. Manganese peroxidase acts on phenolic and non-phenolic lignin units through lipid peroxidation reactions (Binod et al., 2011). It oxidizes Mn^{2+} to Mn^{3+} which oxidizes phenol rings to phenoxy radicals leading to decomposition of compounds. Lignin degrading enzymes are produced by *P. chrysosporium*, *Ceriporiala cerata*, *Cyathus stercolerus*, *C. subvermispora*, *Pycnoporus cinnarbarinus*, *Pleurotus ostreaus* and *P. chrysosporium* (Kumar and Wyman, 2009).

Laccases (E.C. 1.10.3.2.) are copper containing enzymes which are involved in lignin degradation. Laccases acts along with lignin peroxidase and manganese peroxidase leading to complete degradation of lignin. It catalyzes the oxidation of phenolic units in lignin and phenolic compounds and aromatic amines to radicals. The potential of laccase to degrade lignocelluloses is increased by phenolic compounds like 3-hydroxyanthranilic acid, 2,2 P-azi (3-ethylthiazoline-6-sulfonate) which will act as redox medi rs. Without the role of redox mediators laccases have a limited e t (Saloheimo et al., 2002).

3.2. Cellulose degrading enzymes

Cellulases catalyze the hydrolysis of , 4 s in cellulose by two different catalytic mechanisms retaini d the inverting mechanisms. For the conve of cellulose glucose requires the action of three enz es – glucanase, ellobiohydrolase and β-glucosidase. glucanases drolyze β-1, 4 glycosidic linkages in the cell e chain; cellobi rolase cleaves off cellobiose units from end of e chain and β-glucosidase converts cellobiose to glu (Hi et al., 1996). Several fungal species have the ability uce ex ellular fungal cellulose degrading enz Cellu have carbohydrate binding module which i ne d to th ic domain by a flexible linker. These m es play important role in binding the enzyme to the crystalli llu cing cellulase activity (Bayer et al., 1998).

3.3. Hemicellulose rading enzymes

Hemicellulose is a branch polymer consisting of a mixture of energy rich glucose and sugar monomers. The most abundant hemicelluloses are xylan which is composed of pentoses like xylose. Xylanases are enzymes which catalyzes the hydrolysis of xylan. Hemicelluloses create a cross linked network for the structural integrity of cell walls. The complete hydrolysis of xylan requires the action of multiple xylanases with different specificities and action (Binod et al., 2011). Softwood hemicelluloses are primarily composed of glucomannans, arabinoglucuronoxylans, arabinogalactans and xyloglucans while the hard woods are primarily composed of xylans and glucomannans (Zhang et al., 2012). Microbial source for commercial production of xylanase include *Aspergillus niger*, *Trichoderma reesei*, *Bacillus* and *Humicola insolens*.

Hemicellulases and other accessory enzymes have become crucial for the improved hydrolysis efficiency of lignocellulosic biomass. Hence efficient hydrolysis of hemicellulose fraction becomes crucial and supplementation of accessory enzymes increase hydrolysis yields and thereby reduces enzyme costs and dosages (Alvira et al., 2011). Endoxylanases and exoxylanases are needed to initiate break up of cross linked hemicelluloses. B-xylosidases convert xylo-oligosaccharides to xylose with xylose of varying length of oligomers formed as intermediate. α-arabinofuranidase converts arabino to furanose and pyranose forms.

3.4. Other factors

Reactive oxygen sp s (ROS ay an i rtant role in wood decay by fungi (Hamm 02). Th e are three major types of wood decay b gi an s have i ved in all of them. Studies revealed t ignocellulo e mes are too large to penetrate lignifi walls in woo d ROS act as an agent which initiates de ay in s dary wood cell wall. ROS like hydroxyl radicals, hydroperoxyl ra ls and peroxyl radicals produced by fungi att wood polyme additives like copper, manganese, lino- acid, dirhamnolipid, veratryl alcohol can enhance the produc- of peroxi by fungi (Kuijk et al., 2015).

4. Mo echanisms and regulation of enzymes involved biological pretreatment

Molecular techniques can be employed to improve lignin degradation potential of fungi. Earlier studies revealed that expression of white rot fungal genes encoding lignolytic enzymes is differentially regulated at the transcriptional level based on the conditions used in biological pretreatment. Expression of *P. chrysosporium* genes are strongly influenced by nitrogen and carbon limitation. Regulatory elements present in the promoter regions of genes encoding lignolytic enzymes play an important role in transcriptional activation. Studies conducted by Cohen et al. (2001) revealed that transcription levels are collinear to enzyme activities in culture media. Heterologous expression studies revealed that in most case although the properties and activities of heterologous expressed genes are similar to that of native enzymes, yields obtained are too low. Ogawa et al. (1998) introduced *mnp* cDNA of *P. ostreatus* in *Coprinus cinereus* combined the high MnP production of *P. ostreatus* and fast growth of *C. cinereus* resulting in higher lignin degradation after 16 days. Salame et al. (2010) reported influence of substrate on production of isozymes by *C. subvermispora* and carbon and nitrogen play an important role in expression of genes involved in lignin degradation.

5. Parameters affecting biological pretreatment

Though biological pretreatment does not generate any inhibitors and eco-friendly process, it is a relatively time consuming process. Optimization by selecting the most effective strain and culture conditions can make the process more efficient by reducing the treatment time and carbohydrate loss (Kuijk et al., 2015). Important process parameters affecting biological pretreatment include the nature as well as composition of biomass and other parameters like type of microorganism involved, incubation temperature, pH, incubation time, inoculums concentration, moisture content and aeration rate.

5.1. Biomass type

Lignocellulosic biomass is abundantly available bioresource which includes agricultural and forest residues and energy crops. Lignocellulose is mainly composed of cellulose, hemicelluloses and lignin and small amount of other organic and non-organic components like proteins, lipids and extractives. Composition of the feed stocks varies with species and variety. It can also vary due to growth conditions and maturation. Composition of the feed stock greatly affects the type of biological pretreatment to be involved. Hence a compositional analysis to be carried out and biological pretreatment can be carried out using microbial consortium producing the desirable enzymes for hydrolysis of the lignocellulosic biomass. Biomass should be harvested at the suitable stage of maturity which would not only provide good biomass yield but could produce reducing sugars with biological pretreatment.

5.2. Incubation temperature

It is important to maintain optimum incubation temperature during biological pretreatment. The optimum temperature varies with the type of microorganism employed. Most of the white rot ascomycetes fungi grow optimally around 39 °C while the white rot basidiomycetes grow optimally around 25 and 30 °C. The metabolism of these fungi generates heat and develops temperature gradients in solid state media. The accumulated heat can destroy or inhibit fungal growth and metabolism. One of the major challenges in scale up of solid state cultivation is to design and develop a suitable bioreactor with minimal heat generation. Different optimal temperature for biological pretreatment of biomass is due to fungal physiology, fungal strain and type of substrate (Millati et al., 2011).

5.3. Incubation time

Incubation time required for biological pretreatment varies depending upon the composition of the biomass and [illegible] used for pretreatment. Long incubation time due to low delignification rate is one of the major barriers for large scale application of biological pretreatment. The pretreatment of corn stalks with *I. lacteus* showed maximum lignin degradation yield of 37.6% after 42 h of pretreatment. Losses of glucan and xylan were 37.5% and 59.7% respectively. In this process the lignin and xylan removal though decreased the recalcitrance of corn stalks for enzymatic saccharification there was sufficient glucose loss during biological pretreatment. Hence equilibrium conditions to be used to achieve a balance between increase in enzymatic saccharification efficiency and the consumption of polysaccharides during biological pretreatment ([illegible] et al., 2011). Incubation time of biological pretreatment should [illegible] for the efficient conversion of biomass.

5.4. Moisture content

High substrate concentrations have to be used for biological pretreatment to make the process economically viable. Using high dry matter leads to generation of increased concentration of inhibitory compounds which will adversely affect the reducing sugar yield. Hence pretreatment to be carried out with a compromised condition to minimize the generation as well as accumulation of inhibitory compounds (Kuijk et al., 2015). Initial moisture content is essential for the establishment of microbial growth in the biomass. Initial moisture content critically affects the fungal growth and enzyme production and significantly affects lignin degradation. Earlier studies conducted by Reid (1989b) revealed that an initial moisture content of 70–80% was optimal for lignin degradation and ligninase activities of most white rot fungi. Xu et al. (2001) reported that lower solid liquid ratio is more beneficial for the production of manganese peroxidase and lignin peroxidase enzyme. Shi et al. (2008) reported biological pretreatment of cotton stalks using *Penicillium chrysogenum* where higher moisture content (75–80%) resulted in more lignin degradation than lower moisture content (65%). Optimum moisture content varies with the biomass type and microorganism involved in the process.

5.5. Type of microorganism

Fungal pretreatment using wood rot fungi is one of the effective methods for enzymatic saccharification. Brown rot fungi, *Gloeophyllum trabeum* produce enzymes which can depolymerize cellulose and hemicelluloses in wood with modified lignin in the brown residue (Gao et al., 2012). Pretreatment with fungi could increase the enzymatic hydrolysis through lignin degradation. The results indicate that this pretreatment caused partial defibrating effect on corn stover as well as partial removal of xylan and modification of the structure of lignin resulted in disrupting the structure of the cell wall thereby increasing the accessibility of cellulase to lignocellulose. Several studies revealed that use of fungal consortium strains perform better and faster degradability of biomass when compared to single culture. Asiegbu et al. (1996) performed delignification of spruce saw dust using *P. chrysogenum*, *Tinea versicolor* and *Pleurotus sajor-kaju*. When pure cultures were used the delignification rate 0–5% while the consortium showed 16% of lignin removal.

5.6. Aeration

Aeration is an important factor affecting biological pretreatment. It affects the production and activity of lignolytic enzymes. The functions of aeration include oxygenation, CO_2 removal, heat dissipation, humidity maintenance as well as distribution of volatile compounds produced during metabolism (Millati et al., 2011). Since lignin degradation is an oxidative process, oxygen availability is important for ligninase activity of white rot fungi. Studies conducted by Reid (1989a) revealed that active aeration is necessary to provide uniform air diffusion if the biological pretreatment is carried out in packed reactors. High aeration could improve delignification rate and hence controlled aeration is essential for improvement of biological pretreatment. Productivity of manganese peroxidase is not significantly affected by aeration (Millati et al., 2011). Some studies revealed that lignin peroxidase productivity using *P. chrysosporium* can be increased with increasing the aeration rate (Couto et al., 2002).

5.7. pH

pH plays an important role in fungal cultivation and its control in solid culture is difficult. Lignolytic enzyme production is affected by the initial pH of the medium. Most white rot fungi grow well at pH range 4.0–5.0 and they reduce the acidity of the substrate during their growth (Agosin and Odier, 1985). Patel et al. (2009) reported that laccase production is significantly affected by change in pH of the medium. Optimum pH for *P. ostreatus* is 5.0. Change in pH will affect the three dimensional structure of laccase which in turn leads to decrease of laccase activity.

5.8. Inoculum concentration

Inoculum concentration plays an important role in biological pretreatment. The time required for the colonization of the substrate is influenced by the type and amount of inoculums (Kuijk et al., 2015). Spores are the commonly used inoculum. Larger quan-

tity of inoculum leads to shorter time for colonization of the substrate.

5.9. Particle size

Particle size is another important factor which affects biological pretreatment (Kuijk et al., 2015). Usage of large particle size limits penetration of fungi into biomass and prevents diffusion of air, water and metabolite intermediates into the particles. Small particle reduce size of inter particular channel which will adversely affect the inter particle gas circulation. Hence an optimum size particle has to be used for effective biological pretreatment.

6. Combination pretreatment

Studies revealed that combination pretreatment was found to be more effective when compared to pretreatment with chemical or biological method alone. Table 2 shows different combinations of biological pretreatment strategies adopted for pretreatment of lignocellulosic biomass.

Combination of biological and liquid hot water treatment for the improved enzymatic saccharification of *Populus tomentosa* was reported by Wang et al. (2012). The study revealed that highest hemicellulose removal of 92.33% was observed by combination of fungal treatment with liquid hot water. This resulted in a 2.66-fold increase in glucose yield than liquid hot water pretreatment. Combinational pretreatment presents a promising strategy to develop advanced biomass pretreatment systems.

Combination of mild physical or chemical with biologic treatment of rice hull was reported by Yu et al. (2009). The revealed that this novel two step pretreatment showed l severity requirement of fungal pretreatment time. Physical chemical pretreatment were carried out with und a H_2O_2 while biological treatment was carried with *streatu*. The combined treatment showed better li remov than one step treatment. The combined treatment us and *P. ostreatus* for 18 days was more ective sole pretreatment of rice hulls using *P. ostreatu* 60 days.

Ma et al. (2010) reported mil treatment a combination of biological pretreatme using *inodontium taxodii* or *Antrodia* sp. 5898. The co ned pretrea nt using *E. taxodii* and 0.25% H_2SO_4 was fo to more effective m one step treatment. The reducing s r yield reased to 1.13–2.11 folds than acid pretreated wate cin The study revealed that combination of biological and m d pretr ent is a promising strategy for im nt o zym c hydrolysis and ethanol productio m w r hyacin th low lignin content.

Saw et al. 95) reported consecutive treatment by *P. chrysospo* osion for the enzymatic saccharification of plan ass. The study revealed treatment of wood meal prior to steam losion enhanced the saccharification of wood meal. Maximum reducing sugar yield was observed when consecutive treatments such as fungal treatment for 28 days followed by steam explosion at 215 °C for 65 min.

Studies conducted by Kadimaliev et al. (2003) revealed that treatment of birch and pine saw dusts with ultrasound increased the intensity of lignin degradation. Ultrasound treatment loosened the wood structure by weakening the bonds between and within polysaccharide and lignin molecules and thereby increasing the accessibility of fungal enzymes. The results indicate that pretreatment of lignocellulosic substrate with alkali or ultrasound is essential for intensification of bioconversion.

Balan et al. (2008) studied the effe l conditioning of rice straw followed by AFEX pretrea nt and matic hydrolysis. The study revealed that biolo pretreatm of rice straw with white rot fungus *P. ostreatus* fo ed by AFE pretreatment gave high glucan and xyla nversion n pre tment of rice straw with one stage AFE

7. Kinetics and m elin es on b gical pretreatment

For impro the convers iency for sugar production from ligno ulo iomass it is ssential to understand the fundamentals of what f ctors affect sugar production. This can be achi by compari xperimental and the simulated data to el to identify prob ms associated with the lignocellulosic anol process. Biological pretreatment involves degradation of substrate he action of extracellular enzymes produced by t icrobes.

8. Conclusions and future perspectives

B ethanol from lignocellulosic biomass serves as an alternative source of renewable energy. Fine tuning of pretreatment technologies for different biomass types and development of an economically viable process are still needed. Biological pretreatment have several advantages over conventional chemical pretreatment strategies, several challenges need to be addressed before implementing at the commercial scale. To address these drawbacks significant research and developmental activities are needed for reducing the cost of pretreatment and enzymatic saccharification systems, reactor configuration to minimize heat generation during biological pretreatment and identification of efficient lignin hydrolyzing microbes using advanced molecular techniques.

Table 2
Different combination of biological pretreatment strategies adopted for pretreatment of lignocellulosic biomass.

Strategy	Biomass	References
Biological + liquid hot water pretreatment	*Populus tomentosa*	Wang et al. (2012)
Biological + ultrasound + H_2O_2	Rice hull	Yu et al. (2009)
Biological + 0.25% H_2SO_4	Water hyacinth	Ma et al. (2010)
Biological + steam explosion	Plant Biomass	Sawada et al. (1995)
Biological + alkali/ultrasound	Birch saw dust/pine saw dust	Kadimaliev et al. (2003)
Biological + AFEX	Rice straw	Balan et al. (2008)

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