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Chapter

Fungal Pretreatment of Lignocellulosic Materials

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Abstract

The biomass must be converted to fermentable carbohydrates through pretreatment process to break down the complex structure to its constituents prior to fermentation. For lignocellulosic materials, lignin moiety is extremely resistant to degradation because of hydrogen bond cross-linking between the cellulose and hemicellulose. Biological pretreatment using white-rot fungi are novel method and environmental-friendly as a method of biomass deconstruction as compared to other conventional means. These fungi can excrete ligninolytic enzymes to degrade lignin although the rate of deconstruction is slow. Hence, this chapter will focus on the fungal pretreatment or delignification process using white-rot fungi as it is an important step to increase the feedstock conversion.

Keywords: biomass conversion, lignocellulose, pretreatment, enzyme, white-rot fungi

1. Introduction

The depletion of fossil fuel energy sources causes much attention on biomass as the source of renewable energy or biofuel. There are three classifications of biofuel according to the feedstock source, which are first-, second- and third-generation biofuels. First-generation biofuels employ an edible biomass as feedstock while second-generation biofuels utilize numerous non-edible feedstock, ranging from lignocellulosic biomass to municipal solid wastes. Third-generation biofuels also exploit non-edible source but different feedstock such as algal biomass and gases [1].

The lignocellulosic biomass mainly composed of cellulose, hemicellulose and lignin. The conversion of lignocellulosic biomass requires a pretreatment process, which is one of the most important and expensive stages in bioenergy production. This process is performed to degrade and remove lignin from the biomass constituents and thus allows further manipulation of the valorizable portion of biomass, that is, increasing the yield of fermentable carbohydrates [2, 3]. Generally, the pretreatment process can be divided into four, that are physical, chemical, physicochemical, and biological methods [2, 4] as in **Table 1**. Subject to the pretreatment strategies, this process can reduce cellulose crystallinity, improve surface accessibility and decrease lignin content [3].

Biological pretreatment methods are performed by biological agents, either the microorganisms or enzymes excreted by the microorganisms. This method usually utilizes mild pressure and/or temperature and does not involve acid, alkali, or any

	Physical	**Chemical**	**Physicochemical**	**Biological**
Objective	Reduce particle size, increase surface area and reduce cellulose crystallinity	Hydrolyze lignin, hemicellulose and cellulose	Breakdown lignin-holocellulose linkages	Degrade lignin from holocellulose components
Type	• Milling • Grinding • Chipping • Freezing • Radiation	• Acid • Alkaline • Ionic liquid • Organosolv	• Steam explosion • Ammonia fiber expansion • CO_2 explosion • Liquid hot water • Wet oxidation	• Microbial consortium • Fungal • Enzymatic
Advantage	• Low environmental impact • Low dangerous chemical requirement • High effectiveness • Short process time • High uniformity and selectivity	• Less dangerous process condition • Lack of by-products degradation	• Less corrosiveness • Higher energy efficiency • Short process time	• Environmental friendly • No chemical requirement • Low energy consumption
Drawback	• High energy requirement • High cost	• Toxicity • Corrosiveness of equipment • Chemical recovery • Production of inhibitors • Long process time	• Chemical recovery and recycling • High operation cost • Formation of inhibitors	• Long process time • Large space requirement • Need continuous monitoring of microorganism growth

Table 1.
Pretreatment strategies of lignocellulosic biomass.

reactive species [2–4]. Since this pretreatment is conducted under mild conditions, it requires much lower energy input and the byproduct(s) would not hamper or inhibit hydrolysis process. Apart from that, there is no need for chemical recovery because no chemicals were employed [3, 5]. Due to these reasons, the biological pretreatment is an environmentally safe process [3–6]. However, the main issue is it consumes a long pretreatment time [4, 7].

This chapter discusses an overview of recent studies on fungal pretreatment using white-rot fungi and important parameters affecting the pretreatment process of lignocellulosic feedstock such as fungal strain, inoculum concentration and moisture content.

2. Fungal pretreatment

The biological pretreatment can be categorized into bacterial consortium, fungal treatments and enzymatic treatments [4, 8]. The commonly utilized microorganisms in this pretreatment of lignocellulosic biomass are filamentous fungi, which can be easily found in the environment such as ground, living plants and lignocellulose wastes [9]. Wood-decay fungi are classified into three main groups, which are white-, brown- and soft-rot fungi [10]. Among them, the most effective are basidiomycetes white-rot fungi because they have the capability to degrade lignin from the holocellulose (cellulose and hemicellulose) surface [2, 7, 9, 11] and cause white-rot on wood or trees, whereas brown- and soft-rot fungi degrade only minimal lignin [6]. Lignin is a polyaromatic polymer that gives rigidity to lignocellulose [7, 11]. Previous studies on three types of rot fungi were presented in **Table 2**.

2.1 White-rot fungi

White-rot fungi differ significantly in the relative rates at which they attack lignin and carbohydrates in woody or lignocellulosic tissues [6, 17]. They can be differentiated by their delignification mode, named as selective and non-selective delignification as can be seen in **Figure 1**. In selective delignification, mostly lignin and hemicellulose are degraded, while consuming a small amount of cellulose. However, for non-selective delignifiers, all three lignin, hemicellulose and cellulose are degraded almost equally [6, 18]. Even the number of non-selective white-rot fungi is greater than selective white-rot fungi [11], more than 1500 fungi species are selective delignifiers [6]. These fungi are favored for fungal pretreatment in recent researches to ensure a lignin-free and cellulose-rich biomass for next hydrolysis step [3, 7, 17] and enhance the biomass digestibility [3, 18]. Some of the white-rot fungi species were shown in **Figure 2**.

2.2 Enzymatic systems of white-rot fungi

The white-rot fungi play a major role in degrading woods in forest ecosystems [9]. These fungi have the ability to degrade lignocellulosic biomass during their growth in nature owing to the production of two enzymatic systems, which are hydrolytic system and oxidative ligninolytic system [7, 17]. In hydrolytic system, cellulases and hemicellulases are utilized to degrade holocellulose [17]. Non-selective white-rot fungi cause substantial cellulose loss because of their high cellulolytic and hemicellulolytic activity. Conversely, selective white-rot fungi excrete hemicellulolytic enzymes and employ hemicellulose-derived sugars as the main carbon sources [7].

Substrate	Fungi species	Effect	References
Wheat straw	*Ganoderma lobatum* (white-rot)	• Lignin, hemicellulose and cellulose degradation of 50.3, 18.1 and 21.4% • Sugar recovery increased by approximately 27.6%	[12]
	Gloeophyllum trabeum (brown-rot)	• 37.6 and 13.3% of hemicellulose and cellulose removal • Sugar recovery decreased by 10.9%	
Moso bamboo	*Phanerochaete chrysosporium* (white-rot)	• Higher degradability • On lignin over hemicellulose and cellulose	[13]
	G. trabeum (brown-rot)	• Preferential degradability on hemicellulose than lignin and cellulose	
Radiata pine	*Trametes versicolor* (white-rot)	• Loss of lignin at 16%, while both hemicellulose and cellulose at 5% each	[14]
	Stereum hirsutum (white-rot)	• Lignin degradation of 16%, whereas hemicellulose and cellulose of 9% individually	
	G. trabeum (brown-rot)	• Hemicellulose and glucans content reduced approximately to 5 and 3% • Mass loss ranged between 6 and 8% during the first month of biodegradation	[15]
Scots pine	*Daldinia concentrica* (soft-rot)	• 2.5% of weight loss after decayed for 2 months	[16]
	Xylaria acuta (soft-rot)	• Weight reduction of 12.4% after 2 months of incubation	

Table 2.
Previous studies on fungal pretreatment using three different types of rot fungi.

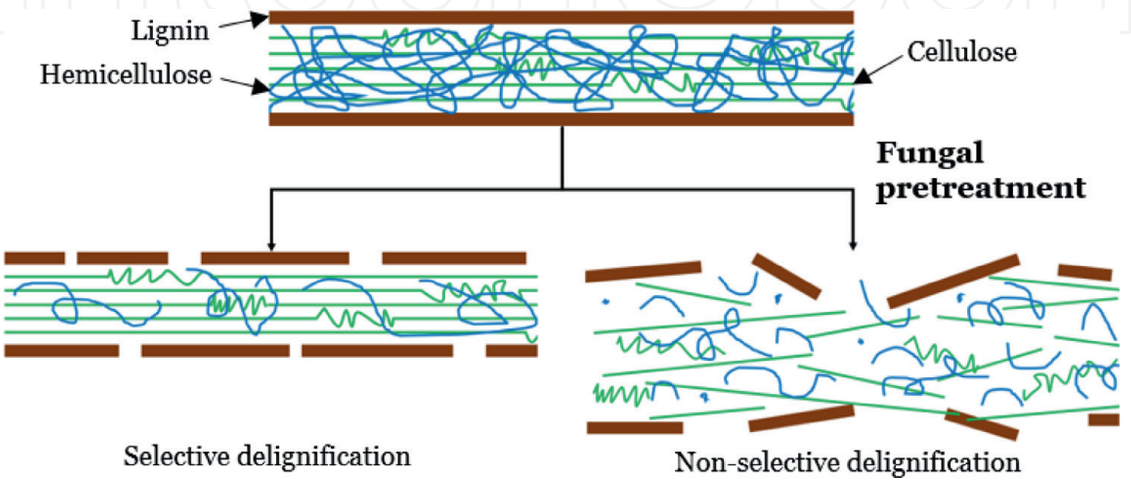


Figure 1.
Mechanism of fungal pretreatment using white-rot fungi on lignocellulosic materials.

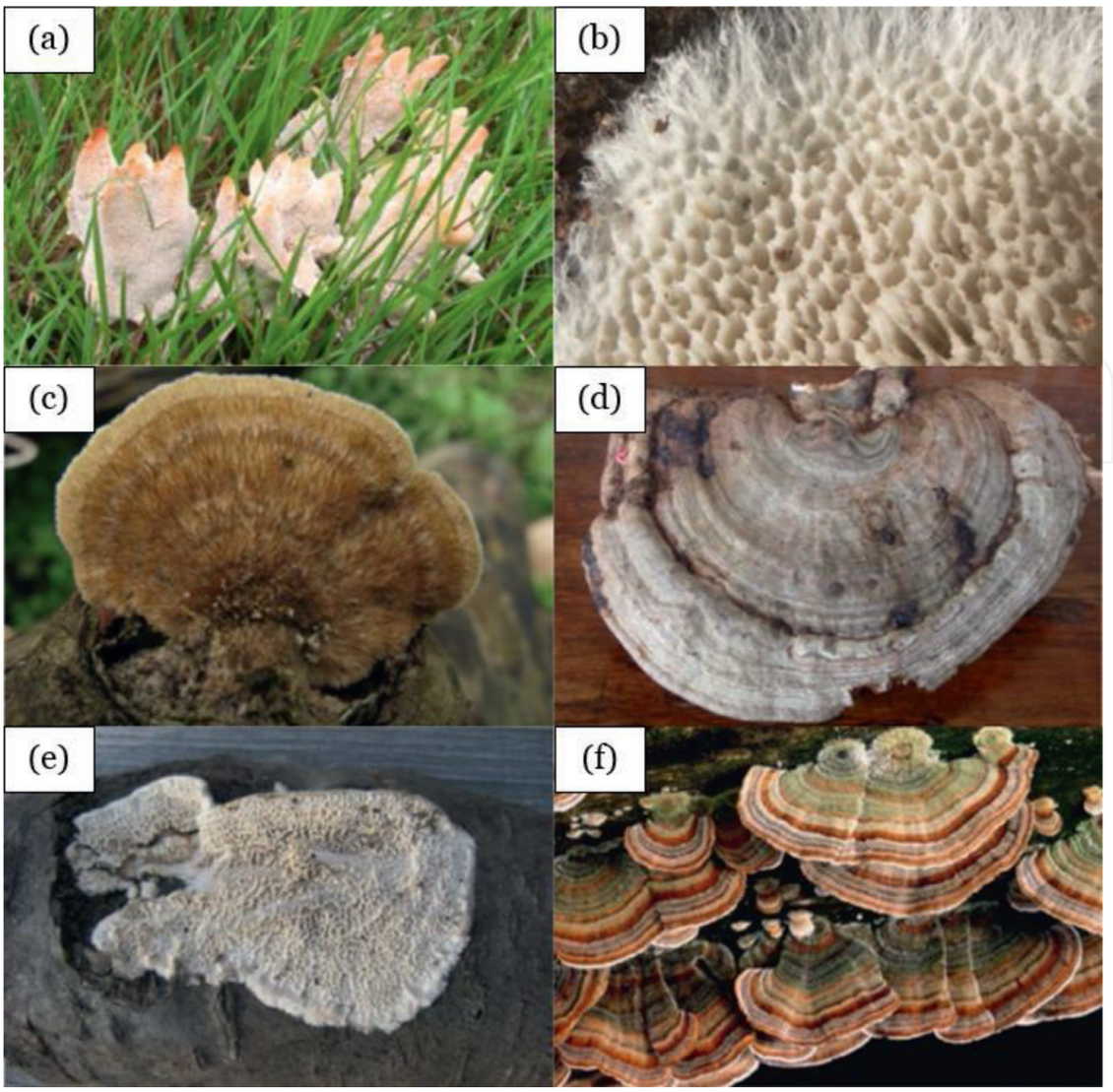


Figure 2.
White-rot fungi of species (a) Abortiporus biennis*, (b)* Ceriporiopsis subvermispora*, (c)* Coriolopsis trogii*, (d)* Ganoderma applanatum*, (e)* Irpex lacteus *and (f)* Trametes versicolor *[19].*

The main enzymes in ligninolytic system to degrade lignin and open the phenyl rings are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase [5, 7, 17, 20]. Nevertheless, not all of these enzymes are secreted by fungal cultures [7]. Lignin peroxidase (EC 1.11.1.14), also known as ligninase, is a heme-protein involves in oxidizing and/or cleaving of non-phenolic aromatic lignin moieties and similar molecules. Manganese peroxidase (EC 1.11.1.13) is a heme-containing glycoprotein, aids delignification by catalyzing reaction that oxidizes phenolic compounds in the presence of Mn^{2+}. Laccases (EC 1.10.3.2) are copper-containing oxidase enzymes that act on phenols and similar molecules by executing one-electron oxidations [5–7]. Versatile peroxidase (VP) is regarded as the third peroxidase, a LiP-MnP hybrid as it is capable of degrading both phenolic and non-phenolic lignin compounds and Mn^{2+} [6, 7].

2.3 Pretreatment of lignocelluloses with white-rot fungi

The sugarcane bagasse was subjected to fungal pretreatment by *P. ostreatus* and *C. subvermispora* for a period of 60 days [18]. At the end of pretreatment, *P. ostreatus* homogeneously degraded all the lignocellulose components of lignin, xylan and glucan up to 11.1, 15.7 and 8.4%, respectively. *C. subvermispora* yielded

obvious lignin and xylan removal while consuming minimal glucan at 48, 47 and 13.6%, correspondingly. With sugarcane bagasse as the biomass, *P. ostreatus* behaves non-selectively due to the fact that the digestibility is not enhanced even when it degrades both lignin and polysaccharides. In contrast, *C. subvermispora* shows selective behavior as it removes lignin and xylan while sustaining glucan, which further improved the digestibility.

The biodegradability of wheat straw and oak wood chips treated with the white-rot fungi *C. subvermispora* and *L. edodes* was observed for 56 days [21]. Using wheat straw as the feedstock, *C. subvermispora* reached higher lignin, hemicellulose and cellulose degradation of 83.3, 80.5 and 20.2% than *L. edodes* with the values of 71.7, 69.3 and 12.2%, respectively. Different observation was found when choosing oak wood chips as the biomass. *C. subvermispora* achieved lower lignin, hemicellulose and cellulose removal of 53.5, 50.6 and 17.4% than *L. edodes* with the values of 60.6, 56.3 and 37.3%, correspondingly. Both fungi selectively degraded lignin in wheat straw and wood chips but with different strategy. *C. subvermispora* colonizes the biomass predominantly during the first 7 days and breaks lignin and hemicelluloses without growing, whereas *L. edodes* constantly grows and removes lignin during the growth. The relative lower lignin removal of wood chips compared to wheat straw indicates that the fungi had more difficulty to penetrate the wood chips due to its dense structure.

In a research done on pretreatment of willow sawdust via the white-rot fungi *A. biennis* and *Leiotrametes menziesii*, it was revealed that *A. biennis* was more preferable for fungal pretreatment even though it has lower delignification than *L. menziesii*, because it consumed a very low amount of cellulose [22]. After 30 days of treatment, the lignin, hemicellulose and cellulose loss attained by *A. biennis* were 17.1, 19.3 and 7.4%, respectively. On the other hand, higher lignin, hemicellulose and cellulose removal was achieved by *L. menziesii*, with the corresponding values of 30.5, 42.4 and 26.6%.

Xu et al. [23] reported that within 12 days of pretreatment, the highest lignin loss achieved by medicinal mushroom, *Inonotus obliquus*, using wheat straw as substrate is at 72%, with cellulose loss of 55%. However, lower delignification was observed for corn stover and rice straw of 47 and 39% with cellulose reduction of 55 and 45%. The hemicellulose content of wheat straw, corn stover and rice straw were decreased to 46, 39 and 44%, respectively. From these results, *I. obliquus* shows its potential as a delignifier of agricultural biomass as it can produce high-activity-level ligninolytic and hydrolytic enzymes.

T. versicolor and *S. hirsutum* showed selective delignification characteristics during the pretreatment of radiata pine wood chips [14]. Both fungi have the largest selectivity value on 21 days of treatment, with *T. versicolor* exhibited better selectivity than *S. hirsutum*. Both of *T. versicolor* and *S. hirsutum* delignified the chips by 16%. The hemicellulose and cellulose was reduced at 5% each for *T. versicolor* whereas 9% each for *S. hirsutum*. As the treatment period was increased, the selectivity values of both fungi decreases because cellulose was degraded together with lignin.

The delignification properties of two white-rot fungi, rainbow fungus (*T. versicolor*) and edible oyster fungus (*P. ostreatus*), on solid oriental beech wood (*Fagus orientalis* Lipsky) was studied for 120 days [10]. For both fungi, there is no substantial difference observed on lignin and cellulose degradation, with lignin degradation was more effective in the first 30 days of exposure. After 120 days of incubation, *T. versicolor* and *P. ostreatus* decayed lignin by 57.4 and 56.5%, and cellulose by 16.7 and 13.9%, respectively. Meanwhile, the decrease in total carbohydrate content was significantly higher for the first 30 days using *T. versicolor* as compared to *P. ostreatus*. At the end of the exposure period, the total carbohydrate

content was almost the same, 7.3 and 6.7%, correspondingly. Both fungi are selective delignification, since the degradation of cellulose starts only after 60 days of incubation.

Four agricultural residues (wheat straw, corn stover, barley straw, and corncob) were pretreated for 21 days using the white-rot fungus *Irpex lacteus* [24]. The highest lignin removal was detected using corn stover (45.8%) as the feedstock, followed by wheat straw (42.3%), barley straw (31.0%) and corncob (17.1%). For glucan digestibility, the increment was significant for corn stover (up to 59.2%), wheat straw (up to 54.8%) and barley straw (up to 53.9%), except for corncob (reduced to 30.3%). The increase in xylan digestibility was observed in corn stover (up to 82.1%), wheat straw (up to 78.0%) and barley straw (up to 58.2%), but not for corncob (decreased to 22.4). Generally, all residues showed a reduction in lignin content. In the case of glucan and xylan digestibility, only corncob yielded lower digestibility after treatment. However, to be specific, *I. lacteus* behaves differently when subjected to different types of raw materials.

The lignin, hemicellulose and cellulose biodegradation of oil palm empty fruit bunches was investigated by exploiting two white-rot fungi, *P. ostreatus* and *P. chrysosporium* [25]. The lignin degradation was higher with *P. ostreatus* (51.9%) than with *P. chrysosporium* (42.1%) after treating for 21 days. In contrast, lower hemicellulose and cellulose degradation rates were noted for *P. ostreatus* (13.8 and 7.6%) compared to *P. chrysosporium* (27.7 and 28.2%). Since only a small amount of cellulose was degraded, fungal pretreatment using *P. ostreatus* is acceptable for palm residues. The fungus *P. ostreatus* can be considered as a selective delignifier because the cellulose degradation happens only after the 21 days of treatment, whereas *P. chrysosporium* is a non-selective delignifier as it concurrently breaks down lignin and structural carbohydrates.

Ishola et al. [26] found that fungal pretreatment improved the digestibility of oil palm empty fruit bunches by 4.5 times. The digestibility of untreated bunches was only 3.4%. This value was raised to 15.4% after the bunches were pretreated by *Pleurotus floridanus* fungus. After the pretreatment, the percentage of total lignin removal was very low, which is reduced by 0.03%. The hemicellulose content was increased by 4.4%, whereas the cellulose was decreased by 5.0% due to fungal attack on the linkage between lignin and carbohydrate.

Enhancement of hemicellulose accessibility was reported when fresh poplar wood (*Populus tomentosa*) was treated for 56 days with a common white-rot fungus on angiosperm wood, *Trametes velutina* [27]. Comparison between untreated and fungi-pretreated material revealed that lignin degradation can positively impact hemicellulose conversion. This was proven with the reduction in lignin content by 7.2% has resulted to an increase in both hemicellulose and cellulose content by 1.0 and 6.4%, consecutively. These findings suggested that lignin degradation rendered xylan more susceptible to xylanase and that in turn rendered cellulose more susceptible to cellulase.

For woody materials and agricultural residues feedstock, the ligninolytic systems and the appropriate fungal strains for the delignification may be different as they have different structure and chemical composition. Hence, it is important to discover the most significant white-rot fungal strain by assessing the strains for the highest degradation ability with the lowest holocellulose utilization as fungal selection subjects to the lignocellulosic biomass chosen for processing [11, 18]. Moreover, one fungus yields a very large difference of the decayed lignin-hemicellulose-cellulose ratio from another fungus, even when using different strains of the same species [6]. Some of recent researches on fungal pretreatment were tabulated in **Table 3**.

Substrate	Fungi species	Inoculum conc.	Moisture content (%)	T (°C)	pH	Time (days)	Nutrient	Effect	References
Sugarcane bagasse	*P. ostreatus*	0.05 w/w %	N.S	27	N.S	60	+	• Lignin, xylan and glucan degradation up to 11.1, 15.7 and 8.4% • Glucan and xylan digestibility reached 35 and 19%	[18]
	C. subvermispora							• Lignin, xylan and glucan removal at 48, 47 and 13.6% • Glucan and xylan digestibility increased up to 55 and 27%	
Wheat straw	*C. subvermispora*	10 w/w%	70%	24	N.S	56	N.S	• Decrease in lignin, hemicellulose and cellulose were 83.3, 80.5 and 20.2%	[21]
	L. edodes							• Lignin, hemicellulose and cellulose biodegradation of 71.7, 69.3 and 12.2%	
Oak wood chips	*C. subvermispora*	10 w/w%	70%	24	N.S	56	N.S	• Reduction of lignin, hemicellulose and cellulose content at 53.5, 50.6 and 17.4%	[21]
	L. edodes							• Lignin, hemicellulose and cellulose removal increased to 60.6, 56.3 and 37.3%	
Willow sawdust	*A. biennis*	0.32 w/w %	80%	27	N.S	30	—	• Degradation of lignin, hemicellulose and cellulose reached 17.1, 19.3 and 7.4%	[22]
	L. menziesii	0.48 w/w %						• Lignin, hemicellulose and cellulose loss of 30.5, 42.4 and 26.6%	
Wheat straw	*I. obliquus*	8%	N.S	28	6	12	+	• Decrease in lignin, hemicellulose and cellulose up to 72, 46 and 55%	[23]
Corn stover									

Substrate	Fungi species	Inoculum conc.	Moisture content (%)	T (°C)	pH	Time (days)	Nutrient	Effect	References
								• Lignin, hemicellulose and cellulose reduction reached 47, 39 and 55%	
Rice straw								• Removal of lignin, hemicellulose and cellulose increased up to 39, 44 and 45%	
Radiata pine	*T. versicolor*	N.S	70%	25	N.S	21	N.S	• Loss of lignin at 16%, while both hemicellulose and cellulose at 5% each	[14]
	S. hirsutum							• Lignin degradation of 16%, whereas hemicellulose and cellulose of 9% individually	
Beech wood	*T. versicolor*	N.S	65%	22	N.S	120	N.S	• Reduction of lignin and cellulose up to 57.4 and 16.7%	[10]
	P. ostreatus							• Lignin and cellulose biodegradation of 56.5 and 13.9%	
Corn stover	*I. lacteus*	50 v/w%	7.3–8.5%	30	N.S	21	N.S	• Removal of lignin reached 45.8% • Glucan and xylan digestibility increased up to 59.2 and 82.1%	[24]
Wheat straw								• Lignin loss was 42.3% • Digestibility of glucan and xylan reached 54.8 and 78.0%	
Barley straw								• Lignin removal up to 31.0% • Digestibility of glucan and xylan enhanced to 53.9 and 58.2%	
Corncob								• Degradation of lignin at 17.1% • Glucan and xylan digestibility reduced to 30.3 and 22.4%	

Substrate	Fungi species	Inoculum conc.	Moisture content (%)	T (°C)	pH	Time (days)	Nutrient	Effect	References
Oil palm empty fruit bunches	*P. ostreatus*	N.S	67%	30	N.S	21	N.S	• Lignin, hemicellulose and cellulose degradation at 51.9, 13.8 and 7.6%	[25]
	P. chrysosporium							• Reduction of lignin, hemicellulose and cellulose were 42.1, 27.7 and 28.2%	
Oil palm empty fruit bunches	*P. floridanus*	N.S	59.40%	31	N.S	28	+	• Lignin and cellulose were reduced by 0.03 and 5.0%, while hemicellulose was increased by 4.4% • Digestibility was improved by 4.5 times	[26]
Poplar wood	*T. velutina*	100 v/w%	N.S	28	N.S	56	N.S	• Delignification by 7.2%, whereas both hemicellulose and cellulose were increased by 1.0 and 6.4%	[27]

N.S, not specified.

Table 3.
Summary of recent publications on fungal pretreatment.

3. Parameters affecting pretreatment process

High lignin degradation can be achieved by having high activities of white-rot fungi and production of ligninolytic enzymes. This is influenced by several pretreatment parameters such as fungal strain, inoculum concentration, moisture content, aeration, pH, temperature, supplements and incubation time [28–30]. Moreno et al. [29] reviewed that for solid state fermentations (SSF), depending on the strain used, the usual conditions that have been used are at moisture content 45–85%, pH 4–5, with an inoculum level of 1–10 mg/g substrate (dry weight), at temperatures ranging from 15 to 40°C and over 1–12 weeks. The optimization of these parameters is important to increase the efficiency of the pretreatment by reducing the carbohydrate loss and pretreatment time [31]. However, for most of these factors, the optimal conditions are depended on the substrate and fungal strain [28]. Temperature and pH are reported to affect fungal metabolism, spore germination and growth. Low moisture content can reduce nutrients availability and growth, while higher moisture content can boost contamination, reduce heat and oxygen transmission, and affect enzyme production [32]. Adekunle et al. [33] reported that the pH and temperature of the SSF play a vital role in the production of laccase by *T. versicolor*.

3.1 Fungal strain

In order for the white-rot fungi to be used in the pretreatment process, screening of a large number of fungal isolates is important in order to have the right isolates for the process. Screening step allows the selection of isolates with the highest ligninolytic enzymes production and activity as well as high lignin degradation on the specific substrates. In order to limit matter losses, selective delignification is crucial and high fermentable sugar losses must be avoided. White-rot fungi strains should therefore be carefully selected based on these important parameters. **Table 4** summarizes some of white-rot fungi species that have been studied for several lignocellulosic biomass pretreatment.

3.2 Inoculum concentration

Inoculum concentration is an important factor in biological pretreatment. Sufficient amounts of inoculum must be defined to ensure good fungal growth and substrate colonization. The time required for the colonization of the substrate is affected by the type and amount of inoculums [31]. Higher concentration of inoculum will lead to shorter time of colonization of the substrate [49].

3.3 Moisture content

Moisture content of the solid state fermentation is a critical aspect for fungal growth and activities. Lignin degradation is significantly influenced by this factor as it affects the growth and activities of the fungal. Increasing the moisture content enhances the nutrient transfer but reduces the porosity of the substrate and limits oxygen transfer [28]. However, insufficient water content in the substrates may cause deactivation of the fungi. Optimum moisture content depends upon the organism and the substrate used for SSF [30]. The range of moisture content of substrate for SSF using white-rot fungi is usually between 45 and 85% [29]. A study on the effect of moisture content for delignification of cotton stalks by *Daedalea flavida* MTCC 145 (DF-2) in SSF found that the highest ligninolytic enzyme

Fungi species	Substrate(s)	Enzymes	References
Ceriporiopsis subvermispora	Hazel branches	Laccase and MnP	[34]
	Albizia chips		[35]
	Miscanthus sinensis		[36]
Echinodontium taxodii	Bamboo	Laccase and MnP	[37]
Trametes versicolor	Pine wood chips	Laccase	[14]
	Corn stalk		[33]
	Corn silage		[38]
Tricholoma giganteum	Wheat straw	Laccase	[39]
Schizophyllum commune	Banana stalk Corn cobs Sugarcane bagasse Wheat straw	Laccase, LiP and MnP	[40, 41]
Pseudolagarobasidium acaciicola	*Parthenium* biomass	Laccase	[42]
Dichomitus squalens	Chestnut shell	Laccase	[43]
Daedalea flavida	Cotton stalks	Laccase and LiP	[44]
Tremetes villosa	Coconut shell Sugarcane bagasse Sisal fiber	MnP	[45]
Stereum ostrea	Wheat bran	MnP	[46]
Pleurotus ostreatus	Rice straw	N.S	[47]
Polyporus brumalis	Wheat straw	N.S	[48]

N.S,not specified.

Table 4.
Lignocellulosic biomass pretreatments with different white-rot fungi species and their isolated enzymes.

activities, optimal lignin degradation 29.88 ± 0.97% (w/w) with cellulose loss 11.70 ± 1.30% (w/w) were observed at 75% moisture content [44]. It was reported that the lignin degradation increased with increase in moisture content. Cellulose and hemicellulose degradation were found to be increased at higher moisture content and small particle size. The selectivity value, SV also influenced by the moisture content. Increase in moisture content decreased the SV, and this may be due to the decreasing of lignin degradation compared to cellulose loss caused by oxygen diffusion declining and ligninolytic enzymes inhibition. Similar condition was also reported for SSF of steam-exploded cornstalk by *T. versicolor* where the highest laccase activity achieved in this study was 2765.81 Ug^{-1} at 75% moisture content [33]. A study on laccase production by a novel white-rot fungus *Pseudolagarobasidium acaciicola* LA 1 through SSF of *Parthenium* biomass reported that the highest laccase activities, 16,388 Ug^{-1} of substrate was found at liquid to solid ratio of 5 with an incubation period of 7 days [42].

3.4 Temperature

Temperature is another very critical factor in the pretreatment using white-rot fungi. However, different genus has different tolerant to temperature. Fungal physiology, fungal strain and types of substrate also resulted in different optimal temperature for biological pretreatment [30]. This statement is in agreement with several studies which showed the production of ligninolytic enzyme using white-rot

fungi has various optimal temperature [33, 50]. White-rot basidiomycetes grow optimally at temperature between 25 and 30°C while most of white-rot ascomycetes fungi grow optimally at 39°C [5]. The metabolism of these fungi produces heat and develops temperature gradients in SSF media. The accumulated heat can lead to adverse effect on the fungal growth and their metabolic activity which leading to the denaturation of the key enzymes. From the studies on pretreatment of rice straw, ligninolytic activities by *S. commune* was found to be peaked at 30 and 35°C [40, 41]. Meanwhile the highest ligninases production by *T. versicolor* was reported at 40°C [51]. Adekunle et al. [33] reported in their study on the SSF of steam-exploded cornstalk with *T. versicolor* that there was a direct correlation between the temperature and laccase production, with the highest laccase activity of 2677.16 U g^{-1} was produced at 28°C. The maximum production of laccase by *T. giganteum* AGHP (1.53×10^5 Ug^{-1} of dry substrate) was obtained at 30°C [39]. This study also showed that lower temperature of 10 and 20°C are not suitable for the growth of fungi due to lower enzyme production. Similar result was obtained from the study on laccase production by *Pseudolagarobasidium acaciicola* LA 1, the optimum production (19,944 Ug^{-1} dry substrate), was found at 30°C [42].

3.5 pH

pH is one of the prominent parameters in the cultivation of fungi and it is very problematic to control in SSF [52]. The initial pH of the medium influences the microbial growth and the production of ligninolytic enzyme. White-rot fungi grow well at pH 4–5, while substrate acidity decreases their growth. A study conducted on the isolation of laccase from a novel white-rot fungus *Pseudolagarobasidium acaciicola* LA 1 through SSF of *Parthenium* biomass showed that the isolated laccase was found to perform optimally at pH 4.5 and highly stable within the range of pH 4–7 for 24 h [42]. The effect of pH is important in the case of laccase production, and a small change in intracellular pH will result in a decrease of macromolecules synthesis. Patel and Gupte [39] reported that the maximal laccase production (1.27×10^5 Ug^{-1} of dry substrate) by *Tricholoma giganteum* AGHP was achieved at pH 5.0. No increment in the production of enzyme was found at higher pH. This may be attributed to the poor mycelial growth at an elevated pH which may restrict the laccase production. It was reported that the maximum ligninolytic activities by *T. versicolor* were found at pH 4.0 and 5.0 [33]. Asgher et al. [40] showed that the optimum enzymes production by *S. commune* IBL-06 was found to be at pH 5 while pH 4.43 and 4.46 for *S. commune* NI-07 [41]. Change in pH will affect the three dimensional structure of laccase which in turn leads to the decrease in laccase activity [5].

3.6 Aeration

Production and activity of ligninolytic enzymes are also influenced by aeration. There are several purposes of aeration such as to supply oxygen into the media, for the removal of CO_2, heat dissipation, distribution of water vapor to regulate humidity, and circulation of volatile compounds produced during metabolism. Thus, this factor should be optimized to improve rate of delignification [49].

3.7 Supplements

Other factor such as various supplements (Cu^{2+}, Mn^{2+}, ferulic acid, xylidine, veratric acid, vanillic acid, cinnamic acid, guaiacol, etc.) for the SSF media have previously been reported in studying their effect on production of ligninolytic

enzymes [44, 53]. Many studies reported that the copper at various concentrations influences laccase production in *S. ostrea*, *T. pubescens*, *P. eryngii*, and *P. ostreatus* [46, 54]. This is related to the role of Cu^{2+} that controls the transcription of laccase gene and also enhances the stability of this enzyme. Meanwhile, the concentration on Mn^{2+} influenced both MnP and laccase production by different *Pleurotus* species [55].

4. Conclusion

Pretreatment is very crucial in the conversion of lignocellulosic materials to other value-added products as lignin acts as the barrier for enzyme penetration. Comparing various pretreatment strategies, fungal pretreatment is more favorable because it is an environmental-friendly process. White-rot fungi with high selectivity of delignification than cellulose removal are more desirable compared to other microorganisms as cellulose is the feedstock for the subsequent hydrolysis process. Fungal strain, inoculum concentration, moisture content, temperature, pH, aeration and supplements are crucial parameters for fungal growth and metabolism to achieve good pretreatment outcome.

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