Biological Pretreatment of Biomass to Decrease Energy Consumption in Mechanical Defiberization Process

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It is critical to develop sustainable, effective, and innovative technologies for society, particularly for processing of biomass, so that the green/ sustainable advantages can be extended to the final products. This review examined two-step biological-mechanical defiberization of lignocellulosic biomass to produce fibers. Two biological pretreatment methods of fungi and enzymes were mainly introduced, with particular focus on the energy consumption. Potential application methods, advantages, disadvantages, process economics, and future prospects of two biological pretreatment methods were considered to derive a complete road map for the proposed process. With the help of biological pretreatment, the mechanical pulping production could not only improve the paper strength, but also decrease energy consumption at about 40%. This process fits well with the green/sustainable strategy to produce lignocellulosic fibers with reasonable quality while having minimal environmental impact.

Keywords: Biological pretreatment; Biomass; Energy consumption; Sustainable

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INTRODUCTION

Widely used mechanical pulping processes that incorporate thermal and/or chemical treatments include chemi-mechanical pulping (CMP), thermomechanical pulping (TMP), chemithermomechanical pulping (CTMP), alkaline peroxide mechanical pulping (APMP), and preconditioning refiner chemical APMP (P-RC APMP) (Xu 2001; Sain *et al*. 2002; Kong *et al*. 2009; Hellstroem *et al*. 2012; Li *et al*. 2014; Engstrand *et al*. 2016; Yang *et al.* 2019). Advantages of these processes include higher pulp yield, lower capital costs than chemical pulping, and production of stronger sheets than with either stone groundwood (SGW) pulping or refiner mechanical pulping (RMP). However, the main disadvantage of mechanical pulping is high energy consumption (Zhao *et al*. 2004). A lot of electricity, at great expense, is required to fiberize the wood chips and to subsequently refine the pulps.

Several attempts at thermal treatment and chemical treatment have been made to decrease the energy consumption. However, the resulting lignin-encased fiber has yielded unacceptably poor paper strength and increasing water treatment expense, counteracting the advantages (Liu *et al*. 2011; Miao *et al*. 2014). The disadvantages of chemical and thermal pulping necessitate evaluation of the potential of biological pretreatments. The current biological pretreatment processes are generally based on selective removal of lignin *via* lignin-degrading fungi or their isolated enzymes (Maijala *et al*. 2008). However, such biological pretreatment processes are still in their infancies and experimental stages.

Biological pretreatment is performed by enzyme or fungi during the raw material storage. It removes lignin and prevents cellulose degradation. The pulp and paper industry further explored biological feedstocks that are synthesized, modified, and degraded in nature by a variety of microbes using different enzymes. The biological pretreatment of biomass to minimize energy requirements during pulping is termed "biopulping." Biopulping uses a promising biological method to replace chemical bleaching. During this biological pretreatment, hemicellulose and lignin are partially removed by different kinds of enzymes produced by various fungi. This review presents several aspects of biological pretreatment and implications for trade and industry.

BIOLOGICAL PRETREATMENT

In the pulp and paper literature, there is the technical term "biomechanical pulping," which describes a process of initial biological pretreatment of woody materials followed by mechanical defiberization, so that lignocellulosic fibers of papermaking quality can be produced (Myers *et al.* 1988; Martínez-Iñigo *et al*. 2000; Villalba *et al*. 2006; Vicentim *et al*. 2009; Furukawa *et al*. 2014). This concept originated in the 1950s. In the 1970s, Ander and Eriksson (1977) reported significant energy savings in mechanical pulping with fungal treatment. In fact, there are many patents on the topic, such as Eriksson *et al*. (1976). Bar-Lev *et al*. (1982) also reported decreased energy requirements and improved paper strength properties in TMP when using white-rot fungus treatment prior to secondary refining. Similar results were obtained by Kirk *et al*. (1994) during TMP of fungus (*Ceriporiopsis subvermispora*)-treated pine chips. Moreover, some studies (Shi *et al*. 2002; Ruan *et al*. 2014) on biological pretreatment of recycled fibers (*e.g.*, recycled kraft fibers) have also been reported. It was found that the similar results were observed with recycled fibers, that is the biological pretreatment could not only enhance paper strength, but also reduce energy consumption in mechanical pulping.

Lignin is a natural binder of cellulose fibers in biomass, with the complex three-dimensional heteropolymer consisting primarily of phenyl propane structural units. Therefore, defiberization of cellulose fibers is facilitated when there is partial removal or modification of lignin through lignin oxidation and/or cleavage of the linkages in lignin. For this purpose, there are three key ligninolytic enzymes: manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase (Lacc).

Manganese peroxidase is a glycosylated heme-containing enzyme that requires H_2O_2. The action mechanism of MnP is displayed in Fig. 1.

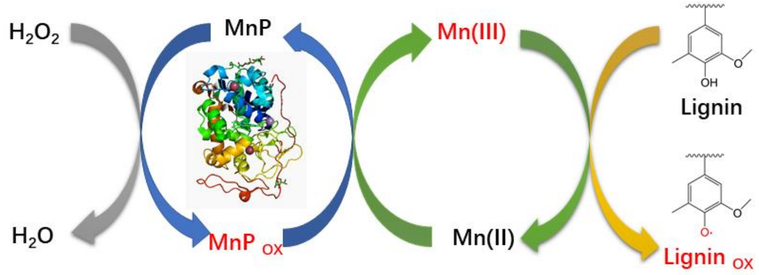

Fig. 1. The action mechanism of MnP

As shown, the MnP peroxidizes unsaturated lipids, leading to the formation of lipoxyradical intermediates. Then, it oxidizes non-phenolic lignin structures. The generation of benzylic fragments from β-O-4 lignin structures by *C. subvermispora* has been attributed to MnP-lipid-mediated peroxidation (Kirk and Cullen 1998).

Similar to MnP, LiP is a heme-containing glycoprotein; it is secreted in the secondary metabolism under limited nitrogen source. The action mechanism of LiP is described in Fig. 2. As a strong oxidizer, LiP can catalyze the oxidation of phenols, aromatic ethers, aromatic amines, and polycyclic aromatic hydrocarbons. LiP is initially oxidized by H_2O_2 to generate a two-electron-deficient intermediate; then, another enzyme intermediate is formed by single-electron oxidization. Finally, the enzyme intermediate returns to its resting state by a single-electron donation. During this process, the lignin is degraded (Collins *et al*. 1997).

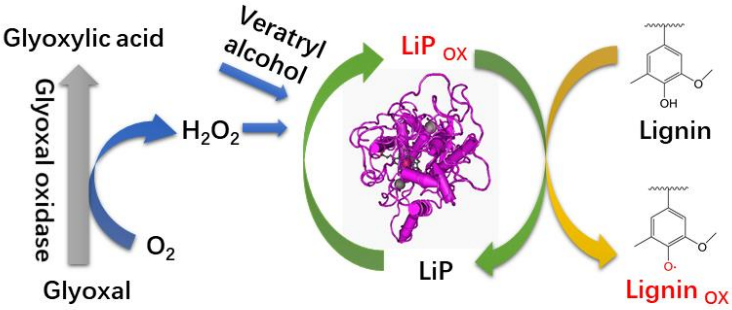

Fig. 2. The action mechanism of LiP

Laccase is four-copper-containing enzyme that is common in fungi. The action mechanism of laccase is shown in Fig. 3. Laccase catalyzes molecular oxygen reduction to water, accompanied by a single-electron oxidation of an aromatic substrate. Laccase can interact directly with phenolic compositions in lignin or in the presence of a mediator. The mediator should have the advantages of the oxidized and reduced forms, being stable, not inhibiting the enzymatic reaction, and the redox conversion being cyclic. Ideally, the low molecular weight mediator could be oxidized into stable high-potential intermediates, which can react with a broad range of substrates. Many mediators have been investigated, including 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), N-hydroxy-phthalimide (HPI), 1-hydroxybenzotriazole (HBT), and violuric acid (VA). Laccase has great biotechnological potential and good application prospects, including phenolic removal and pulp bleaching (Bourbonnais *et al*. 1997).

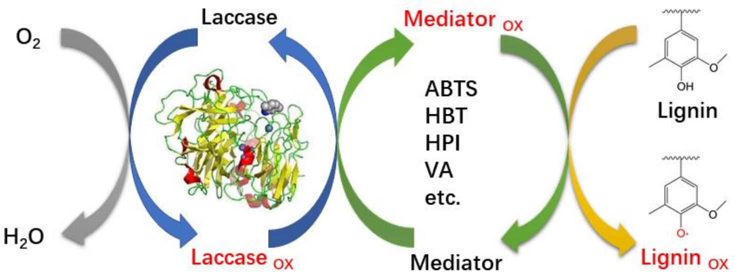

Fig. 3. The action mechanism of laccase

Table 1. Comparison of Process Conditions and Energy Savings Achieved in Studies of Different Biological Pretreatments

Reference	Pretreatment Process Conditions						LRR (%)	BC (% ISO)	ES (%)
	Material	Type of Enzyme or Fungus	BT	T (°C)	RH (%)	Time (d)			
Akhtar (1994)	Aspen wood chips	*Ceriporiopsis subvermispora* CZ-3	SB	27	65	28	---	Decreased 13.2	48.0
Gao *et al*. (1996)	Bagasse	*Phanerochaete chrysosporium*	---	39	80	28	6.05	Decreased 8.70	22.9
	Bagasse	HG-X 03	---	30	80	28	5.20	Decreased 7.70	20.3
Gulsoy and Eroglu (2011)	European black pine	*Ceriporiopsis subvermispora* FP-90031-sp	---	27	75	20	0.92	Decreased 1.58	7.45
Leatham *et al*. (1990a, 1990b)	Aspen chips	*Phanerochaete chrysosporium* BKM-F-1767	RB	39	70	42	0.50	Increased 7.80	38.0
	Aspen chips	*Phlebia brevispora* Nakas HHB-7099	SB	27.5	70	28	39.9	Increased 7.70	47.5
	Aspen chips	*Phlebia subserialis* Donk RLG-6074	SB	27.5	70	28	32.5	Increased 9.50	42.1
	Aspen chips	*Dichomitus squalens* Reid TON-427	RB	27.5	70	28	12.2	Increased 11.6	7.60
	Aspen chips	*Trametes versicolor* Pilát MAD-697	SB	27.5	70	28	15.7	Increased 7.90	2.90
Myers *et al*. (1988)	Aspen wood chips	*Phanerochaete chrysosporium* SC-26	RB	37	70	42	0.32	Decreased 10.7	41.8
	Aspen wood chips	*Dichomitus squalens* TON-429	RB	25	70	42	3.00	Decreased 11.7	54.9
Ramos *et al*. (2004)	Sugarcane bagasse	*Ceriporiopsis subvermispora* L-14807 SS-3	SB	27	70	14	0.17	Decreased 8.65	43.8
	Sugarcane bagasse	*Phanerochaete chrysosporium* BKM-F-1767	SB	27	70	14	0.10	Decreased 4.30	35.2
	Sugarcane bagasse	Enzymatic pretreatment	SB	25-29	70	1.5	0.13	Increased 2.10	41.2

Note: BT: bioreactor type, SB: stationary bioreactor, RB: rotating bioreactor, T: temperature, RH: relative humidity, LRR: lignin removal ratio, BC: brightness change, ES: energy savings

In addition to those enzymes, white rot fungi have attracted much attention due to their simultaneous secretion of hydrolytic and oxidative extracellulase enzymes (Jahan and Farouqui 2000; Mendonça *et al*. 2002; Mardones *et al*. 2006; Afrida *et al*. 2009; Bugg and Rahmanpour 2015). *Trametes versicolor* is a multifunctional white rot basidiomycete (Asgher *et al*. 2012). It can degrade lignin and holo-cellulose simultaneously, resulting in the cells' perforation or thinning of secondary walls (Arica *et al*. 2001). Laboratory studies with *Ceriporiopsis subvermispora* on biomass showed energy savings in the range of 30% to 50%, while decreased brightness was the only drawback.

Biological pretreatment was inspired by the natural rotation process of lignocellulose. Initially, the fungi were colonized on the exposed xylem of lignocellulose. Next, the fungi were expanded and colonized through the parenchyma, thus establishing the network of the organism. With the progression of fungal degradation, the middle lamella was isolated from the cellulose-rich secondary wall structure.

After the biomass biological pretreatment, the penetration and degradation of cell lumens and the softening and swelling of cell walls were visualized by microscopy. In nature, different kinds of bacteria, fungi, and actinomycetes may undertake these reactions, and no toxic or harmful by-products were produced (Qin *et al*. 2009; Talaeipour *et al*. 2010).

Numerous laboratory- or pilot-scale studies of biological pretreatment have been performed. Akhtar (1994) studied the biomechanical pulping of aspen chips using white rot fungus (*Ceriporiopsis subvermispora*), based on its lignin-degrading ability. A decrease of refining energy of 40% to 48% was achieved by 4 weeks' treatment at 27 °C and 65% relative humidity. Akhtar *et al.* (1997) studied the biomechanical pulping of pine chips using white rot fungi. The seed was inoculated onto chips at a ratio of 1:9 in a stationary tray bioreactor. The incubator conditions were 27 °C and 65% relative humidity. It was found that the biological treatment decreased energy use by 42% during post-mechanical refining with only 6% weight loss. Moreover, the strength properties of the synthetic paper sheet were improved, while the brightness and light scattering coefficient of the paper sheet decreased slightly. Gulsoy and Eroglu (2011) studied two-step biological-kraft pulping of pine chips using white rot fungus (*Ceriporiopsis subvermispora*) for 20 d to 100 d at 27 °C and 75% relative humidity. They found that the biological pretreatment could decrease lignin and extractive contents, while viscosity, kappa number, and rejection ratio in the resulting pulps all decreased. As a result, the bio-kraft pulps were better fiberized during refining and decreased energy consumption. More details and comparisons of various biological pretreatments are listed in Table 1.

POTENTIAL APPLICATION METHODS

Spraying

A fungus-containing solution may be sprayed onto wetted biomass. As shown in Fig. 4, a similar concept is the application of xylanase- or cellulase-containing solutions to dissolving pulp to enhance its reactivity or accessibility (Chen *et al*. 2016a,b). A high biomass concentration is beneficial to the adsorption of the fungal solution. In the proposed process, the spraying can be implemented on the biomass storage piles or in a belt conveyer (improving contact/accessibility). The biomass can then be stored for 2 to 12 hours, so that the biological reactions sufficiently occur. In a laboratory study, the spraying technology successfully upgraded dissolving pulp, increasing its Fock reactivity by approximately 160% (Li *et al*. 2018).

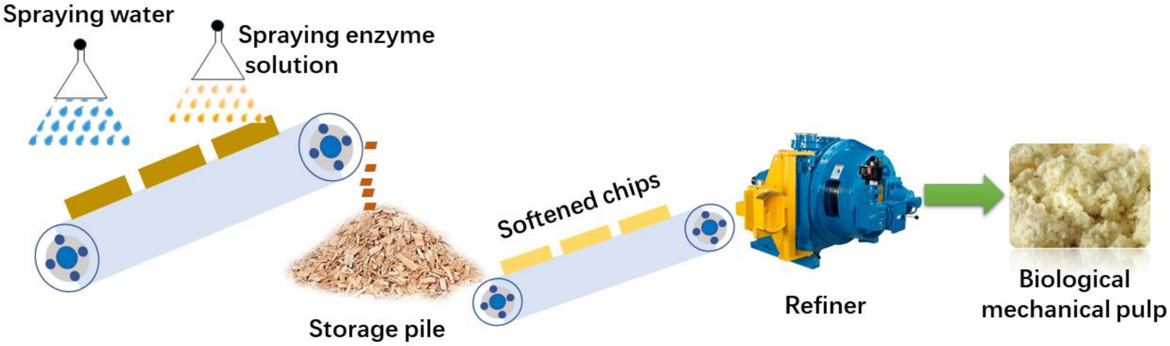

Fig. 4. Process flowsheet of biological mechanical pulping

Field/Outdoor Storage

Typically, the biological pretreatment would take a long time (weeks, or even months), which would not be compatible with the usual industrial manufacturing process. However, if this pretreatment could occur concurrently with the harvesting, collection, transportation, and storage of the raw material, the long reaction times required may no longer be a limiting step, as shown in Fig. 4.

Cui *et al*. (2012) discussed the effect of wet storage of corn stover using fungal assistance (*C. subvermispora*) on raw material digestibility. They found that the fungal pretreatment caused extensive degradation of lignin, while the effect on carbohydrates was much less. Approximately 40% of the lignin was degraded during 90 d of wet storage, and most of the delignification (35.8%) occurred in the first 35 d. In the 90 d of wet storage, the degradation rate of cellulose was less than 11%, and a majority of the loss occurred after 35 d. At 35 d and 90 d, the degradation rates of xylan were 26.3% and 36.5%, respectively. The total loss of dry matter reached 20% at 90 d. Finally, the enzymatic degradability of the corn stover was improved 2 to 3 times.

Biological pretreatment uses lignin-degrading microorganisms, such as white-rot fungi, to degrade lignocellulose. They secrete lignin-degrading enzymes, making the resultant substrates more susceptible to subsequent mechanical defiberization. Therefore, the integration of biological pretreatment into the harvesting, collecting, transportation, and storage of raw material is a promising approach for the proposed two-step biological-mechanical defiberization process.

ADVANTAGES AND DISADVANTAGES OF BIOLOGICAL PRETREATMENT

Biological pretreatment mainly included fungal and enzymatic pretreatment. Fungal pretreatment is a relatively simple process and could be incorporated into any existing mills with minimal cost. Moreover, fungal pretreatment could save a lot of electric energy and increase the throughput of a mill considerably. Compared with traditional RMP, it also increases paper strength, and studies suggest that fungal pretreatment is also effective in removing wood extractives from chips. It can reduce by approximately 30% the dichloromethane extractable resin. Fischer *et al*. (1994) reported that triglycerides were

60% removed, which could resolve the sticky deposits on paper machines (Fischer and Messner 1992). Compared with fungal pretreatment, enzymatic pretreatment needed only hours to enhance pulping and paper properties, greatly decreasing the processing time and improved the pulp yield. Furthermore, it was found that enzymatic pretreatment reduced the energy consumption in a proportion similar to that of *C. subvermispora* fungal pretreatment and increased the pulp tensile index. Also, an advantage of enzymatic pretreatment was that brightness was increased, whereas fungal pretreatment reduced the brightness (Ramos *et al*. 2004). Additionally, biological pretreatment can decrease consumption of cooking and bleaching chemicals and increase production capacity. Improving the delignification efficiency can indirectly decrease pulping energy consumption and pollution (Kirk *et al*. 1994). The amount of waste generated by biological pretreatment should be considerably lower than that generated by current CTMP processes (Kong *et al*. 2009). Biological pretreatment increased the pulp tensile index compared with the normal CTMP pulps (Sain *et al*. 2002; Ramos *et al*. 2004). Actually, the wastewater from a biological pretreatment mechanical process is less toxic, although it may have slightly greater biochemical oxygen demand and chemical oxygen demand than untreated pulp wastewater (Sykes 1994). These findings indicate that bio-pulping has environmental compatibility. Consequently, biological pretreatment technology has developed rapidly in recent years, and pilot scale tests have been carried out in the world (Heitner *et al*. 2010).

At present, there are still some disadvantages in biological pretreatment. For instance, the processing conditions (*e.g.*, temperature and relative humidity) of fungal treatment are harsh (Akhtar *et al.* 1997). Also, it takes a long time (more than 10 days) to treat pulp with fungi, which greatly affects the production speed of pulp, so it is difficult to meet the demand of industrial production (Akhtar 1994; Gao *et al.* 1996). Moreover, white rot fungi are mostly used in fungal treatment, which is difficult to expand culture. In addition, a small amount of cellulose was usually degraded in the process of biological pretreatment (Ramos *et al*. 2004; Gulsoy and Eroglu 2011).

PROCESS ECONOMICS

The economic potential of biomechanical pulping is promising. An early economic assessment of a thermomechanical pulping mill with a daily output of 250 tons was performed (Scott *et al*. 1998). The capital costs of integrating biomechanical pulping technology into the thermomechanical pulping mill are estimated at approximately $6 million to $8 million. This early estimation is influenced by appropriate conditions, as there is some variability in capital costs, particularly those associated with integrating new facilities into existing sites. Based on 33% energy savings and a 5% decrease in the kraft final product, approximately $5 million could be saved each year. The cost of additional bleaching chemicals has been quantified and included in the estimation. The other benefits of bio-pulping, such as environmental benefits and pitch decrease, have not yet been considered.

FUTURE PROSPECTS

Biomechanical pulping has great potential in reducing energy consumption and pollution problems, as well as increasing pulp physical strength. However, the relatively long processing period (20 d to 28 d) hampers real application (Ferraz *et al*. 2007; Saritha *et al*. 2012). Moreover, it would require very large handling containers and decrease production efficiency. Therefore, screening for high-efficiency fungal strains would be the future focus. The energy consumption reduction is related not only to the lignin removal ratio but also to the defibration area of the cell wall (Flores *et al*. 2009). Hence, the screening standard for fungal strains should be reconsidered. Enzymatic pretreatment has great advantages, being a rather fast reaction (a couple of hours), easy controlled, and compatible with the ongoing process. It has great potential in future commercial applications. But, the fiber quality should receive more attention when using enzyme cocktail pretreatment. The optimal enzyme should minimize hemicellulose degradation; otherwise the resulted paper would have poor inter-fiber bonding. Genetic engineering technologies can play an important role in fungal strain and enzyme production. The integration of biological pretreatment into the raw material transport and storage processes is an alternative and feasible method to decrease the production costs. Environmental influence data should be obtained from systematic studies to compare biomechanical pulping with other mechanical pulping processes, thus achieving equivalent quality and yield of pulp. Advancement of technology and optimizing the production process could further facilitate commercial-scale application of biomechanical pulping.

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CONCLUSIONS

1. Biological pretreatment is judged to be a promising approach in decreasing energy consumption of mechanical pulping. The fungi and enzymes (*i.e.* MnP, LiP, and laccase) have showed application potential.
2. Incorporating the biological pretreatment with the harvesting, collection, transportation and storage of raw material is an alternative way to solve the long treatment time.
3. The economic evaluation of biological pretreatment should comprehensively consider the capital cost, energy consumption, and pulp properties; it should also be environmentally friendly.

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