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Review Article

Microbial Pre-treatment of Lignocellulosic Biomass for Biofuel Production: A Review

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Abstract

Lignocellulosic biomasses (LCB), second-generation biofuels are used as an alternative means to cope with the burning issues of depleting fossil fuels like petroleum products with the added advantage of renewability, lower emission, and lesser pollution. For the increment in the production of LCB biofuels, microbial pre-treatment processes are conducted which accelerates the degradation of organic polymers like lignin and hemicellulose with the activity of potential microorganisms. To increase the efficiency of degradation of hemicellulose, hemicellulolytic fungi including *Trichoderma* and *Aspergillus* and other bacteria produce multi-enzymatic complexes like cellulosomes. Similarly, organisms like *Tinea versicolor*, *Dichomitus squalens*, *Phlebia floridensis*, *Daedalea flavida*, and *Phlebia radiata* contain lignin-degrading auxiliary enzymes and lignin modifying enzymes like laccase and heme-containing peroxidase which aid in delignification process. Several factors are associated with pre-treatment processes like the type of strain, inoculum load, pH, temperature, fatty acids, C/N ratio, time, aeration, grindability, surface area, particle size, and supplements added. To enhance the pretreatment method, the combination of microbial with physical, chemical, and mechanical methods is suggested which leads to a synergistic effect and better yield of the final product. Overall, biofuels should be more employed and this review aims to bring light to the microbial pre-treatment approaches which can aid in the efficient production of biofuels that can directly contribute to environmental sustainability.

Keywords: biofuel; fungi; lignin; ligninase; lignocellulosic biomass; microbial pre-treatment

Introduction

The rising global need for energy, the rapid depletion of natural resources, and the growing issue of CO_2 emissions are the key factors behind the increased interest in non-petroleum-based energy sources, or so-called alternative fuels (Zoghlami and Paes, 2019). As a means of meeting rising global demands and more crucially, as an alternative to fossil fuels, various technologies for producing energy using lignocellulosic biomass have drawn a lot of attention. To reduce the consumption of the available conventional energy resources, intensive research on the usage and implementation of alternative energy resources is being emphasized. The main advantages of biogas compared to conventional fuels are: use of renewable raw materials, low greenhouse gas emissions, production without the need for external or existing natural gas, reduction of pollution level from organic waste and benefits waste biomass management (Mishra, 2018). A relatively recent and popular classification of liquid biofuels includes “first generation” and “second generation” fuels. These terms do not have strict technical definitions. The feedstock they employ is the primary difference between them. A first-generation fuel typically consists of one that utilizes only certain (typically edible) parts of the above-ground plant biomass such as wheat, sugarcane, corn, vegetable oils, etc., and just a small amount of processing is needed to turn it into final fuel. Significant commercial production of first-generation fuels has already begun in a number of countries. Second-generation fuels are typically those produced from lignocellulosic biomass that cannot be used. It is either inedible whole plant biomass (e.g., grasses or trees grown specifically for energy) or inedible crop waste (such as rice husks or corn stalks).

Lignocellulosic biomass (LCB) is the most abundant renewable feedstock from plants mainly composed of polysaccharides (cellulose and hemicellulose) and an aromatic polymer, lignin (Zoghlami and Paes, 2019). Compared to first-generation biofuel (made from grains, seeds and sugar crops) feedstock, lignocellulosic biomass typically: is not edible and does not directly compete with food production, can be bred specifically for energy production, represents more of the above-ground plant material, further increases land-use efficiency, can be produced on a given amount of land (Saritha, 2012). As a result, lignocellulosic biomass is viewed as the sole foreseeable, practical, and sustainable source of renewable fuel. However, lengthy pre-treatment period, relatively low hydrolysis rates, and significant loss of fermentable sugars are some of the drawbacks associated with the use of microbial pre-treatment techniques (Basak *et al.*, 2020). Several studies show that the overall process economics can be enhanced by developing an integrated approach, such as a combined process, as well as by fine-tuning each process variable. For instance, one approach to reducing pre-treatment time is to use a combination of microbial methods with physical and chemical techniques (Ummalyma *et al.*, 2019).

Economic assessments show that the LCB pre-treatment step is the most energy intensive step, potentially accounting for 40% of total processing costs, limiting the commercial use of LCB for biofuel production (Kumar *et al.*, 2022). Furthermore, most physicochemical pre-treatment processes designed to improve the biodegradability of LCBs simultaneously release inhibitory byproducts that adversely affect microbial growth and function during anaerobic bioprocessing (Ravindran and Jaiswal, 2016). In contrast to chemical and physical pre-treatment methods, which exhibit inherent drawbacks such as the production of inhibiting compounds, microbial pre-treatment is increasingly advocated as an ecofriendly process due to their application in milder operating conditions, minimal energy input, low disposal costs and little corrosiveness (Wagner *et al.*, 2018). The current review highlights the use of bioagents, including bacteria, fungi, and/or their enzymes, to trigger the use of lignocellulosic biomass for biofuel production.

Microbial Pre-treatment and its Significance

The major challenge in biofuel production is resistance, mainly due to the presence of crystalline cellulose and lignin. Several factors, such as the degree of lignification, the structural heterogeneity and complexity of cell wall components contribute to biomass resilience that must be overcome for the valuable utilization of lignocellulosic feedstocks (Baruah *et al.*, 2018). During the pre-treatment process, the recalcitrant structure of lignocellulose is disrupted resulting in rupture of lignin sheath, degradation of hemicellulose, and reduction in cellulose crystallinity and degree of polymerization (Chen *et al.*, 2017). Pre-treatment is required to rupture the lignin structure and break down the crystalline structure of cellulose so that the acids or enzymes can easily access and hydrolyze the cellulose. Pre-treatment can be the most expensive process in converting biomass to fuel, but further research and development have great potential to improve the efficiency and reduce costs.

Microbial pre-treatment for increased biofuel production during anaerobic digestion is mainly characterized by fungal (enzymatic) microbial pre-treatment methods (Zheng *et al.*, 2014). Microbial treatments are generally carried out by growing microorganisms directly on raw materials or by using enzyme cocktails. This pre-treatment process is selective: no added chemicals, low energy consumption and low severity (Ummalyma *et al.*, 2019). Microbial pre-treatments mainly rely on enzymes, indirectly indicating the importance of microorganisms producing the respective enzymes involved (Gurung *et al.*, 2011). Using the best microorganisms to convert raw materials into finished products or biofuels can lead to higher productivity in a cost-effective manner. Transformation can be improved by precise knowledge of the microorganisms involved. Microbial pre-treatment is necessary to improve digestibility or fermentation rate performance. Previous studies and reports by various researchers have shown that many bacteria, including bacilli, actinomycetes, and some well-known fungi, can degrade organic matter (Poszytek *et al.*, 2016). Various microbial pre-treatment strategies applied include microaerobic treatment, ensiling or composting, separation of digestion stages, and pre-treatment using various lignocellulolytic bacteria. The net energy output from such approaches is much higher and relatively cheap compared to other established chemical and mechanical approaches.

Lignin and Hemicellulose: Major Components that requires Pre-treatment

The second-most prevalent organic polymer, lignin, makes up 10-30% of lignocellulosic biomass and is abundant in primary plant's cell wall that offers a rigid, effective barrier against oxidative stress and microbial attack meanwhile, inhibits the hydrolysis of cellulose and hemicelluloses (Kawaguchi *et al.,* 2016; Ragauskas *et al.,* 2014). Through multiple ethers and carbon-carbon bonds, 4-hydroxyphenylpropanoid monomers are cross-linked to construct a complex and larger molecular structure of lignin and the most common linkages found in lignin are β-β, β-0-4, and β-5 bonds. The proportion of p-hydroxyphenyl, guaiacyl, and syringyl groups in the monomeric units' phenolic moieties varies depending on the type of plant (Walker, 2010).

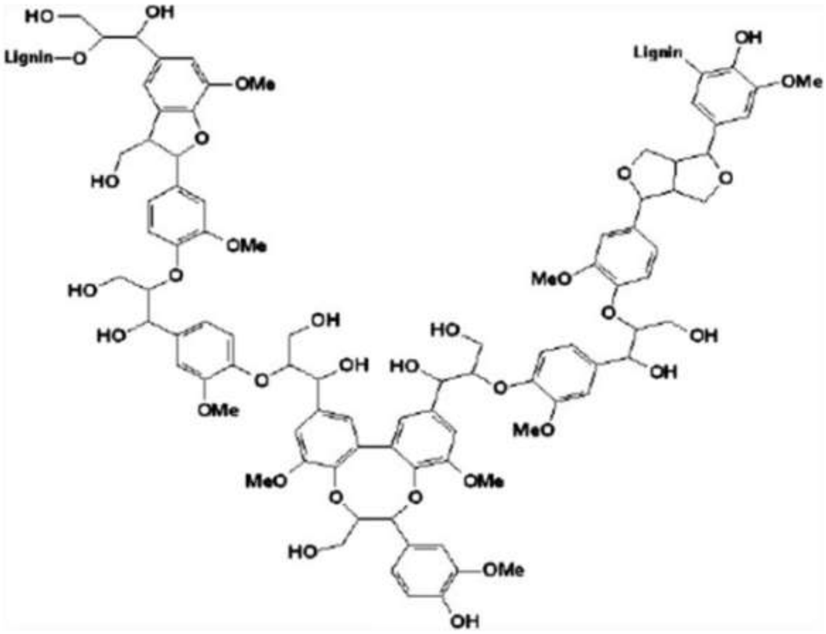

Fig 1: Structure of lignin: Complex cross-linked polymer of aromatic rings (Source: Walker, 2010).

Hemicellulose is ascribed to polysaccharides of heterogeneous composition. Therefore, they require sets of diverse enzymes for complete depolymerization (Kim *et al.*, 2015). The major hemicelluloses include xylans, mannans, and xyloglucans. The structural compositions of these polysaccharides are well known; however, depending on the plant source, the plant age, and the plant tissue, the structural composition of these polymers may greatly vary in their fine structures (Rego *et al.*, 2019). Hemicellulases are a group of enzymes that specifically degrade hemicelluloses which, in addition to their activity on glycosides, are also frequently capable of hydrolyzing the short-chain or monosaccharide chains from the backbone chain of hemicelluloses (Park *et al.,* 2012). Since hemicellulose has considerable side-branch groups, its structure is more open and non-crystalline. Hemicellulose is more hygroscopic than cellulose and attracts more water molecules due to its open structure. The degree of polymerization of hemicellulose in wood is about 100–200, which is very low when compared to that of cellulose, which are 10,000. The difference between wood and non-wood fibers does not appear in the content of cellulose; however, it does appear in the contents of hemicellulose and lignin. Non-wood fibers such as grasses (wheat, corn, rice) contain up to 40% of the major hemicellulose, whereas wood fibers are composed of 25-35% of hemicellulose by dry weight. (Stokke *et al.*, 2013).

The breakdown of hemicellulose requires the coordinated activity of a number of extracellular enzymes that function synergistically to hydrolyze the polysaccharides into small oligosaccharides and finally to convert them to monomers (Alvarez *et al.*, 2019). The complexity of these enzymatic systems is further increased because microorganisms tend to produce different modular enzymes within each class to improve the efficiency of the breakdown of complex and recalcitrant structures such as plant cell walls (Yu *et al.*, 2017). Since these complexes are insoluble in water, their enzymatic hydrolysis poses a great challenge for microorganisms (Qiao *et al.,* 2019). Heteropolymers of hemicellulose do not have crystalline arrangements and are found at random inside the cell wall, forming an interface between lignin and cellulose. Microscopic studies indicate that cellulose microfibrils have a parallel orientation. It has been proposed that due to its cohesion, hemicellulose could act as a lubricant to prevent direct microfibril-microfibril contact both within and on the surface of cellulose (Zhou *et al.,* 2013). Given their complexity, fungi require several hemicellulases to break down plant hemicelluloses. The most widely studied are those involved in the depolymerization of the main xylan backbone, which are classified into two main groups depending on the site upon which they act: endo1,4-β-D-xylanases and exo-1,4-β-D-xylosidase. In addition, α-L-arabinofuranosidases, α-D-glucuronidases acetylxylan esterases, ferulic and pcoumaric esterases and β-galactosidases, called debranching enzymes, are responsible for removing the xylan side chains (Skreiberg *et al.*, 2011). The debranching enzymes work in a synergistic manner in the breakdown of xylan, allowing access of xylanases to the main backbone, while accessory enzymes release the side substituents more easily from xylan fragments (Carrier *et al.,* 2011). However, complete breakdown depends on the participation of α- and β-galactosidases.

Potential Microorganisms and their Enzymes in Pre-treatment

Fungi are among the most active microbes in decomposition of organic matters in general, and thus, these are the most important group of hemicellulolytic microorganisms. Aerobic fungi, such as the fungi *Trichoderma* and *Aspergillus*, secrete large variety of hemicellulases at high concentrations that work synergistically (Wang *et al.,* 2014). Bacteria and some anaerobic fungi produce multi-enzymatic complexes called cellulosomes, which are anchored to the cell surface and allow the microorganisms to bind to lignocellulose substrates and increase the breakdown efficiency of cellulose and hemicellulose (Rajtanen *et al.,* 2020). Most of the enzymes that make up cellulosomes are cellulases, xylanases, other glycosyl hydrolases and in some cases even esterases are also present (Saini *et al.,* 2020). However, in the majority of fungi, hemicellulases are not integrated in cellulosome complexes as in bacteria, and enzymes that act in a synergistic manner are self-induced depending on the substrate that is present, which leads to the breakdown of the plant cell wall and the internalization of the hydrolysis products into the cell (Gonzalez *et al.,* 2020). Many bio refineries industries are inclined towards a powerful oxidative agent, ligninase which is highly selective approach in lignin degradation and comparably inexpensive (Vanholme *et al.,* 2010). Lignin-degrading enzymes are broadly classified into two types: lignin-degrading auxiliary enzymes which are incapable of degrading lignin on their own, necessitating the involvement of additional enzymes for complete degradation and remaining is lignin modifying enzymes (Janusz *et al.,* 2017). Laccases, which contain copper, work together with lignin peroxidase and manganese peroxidase to completely degrade lignin whose ability is boosted by the phenolic components such as 3-hydroxyanthranilic acid and 3-ethylthiazoline-6-sulfonate as it catalyzes the radicalization of phenolic units found in lignin and phenolic compounds, as well as aromatic amines (Andberg *et al.,* 2015). There are numerous enzymes that significantly assist in the degradation of lignin and they are aryl alcohol dehydrogenase, cellobiose, aromatic acid reductase, vanillate hydroxylase, dioxygenase and catalase. The non-productive adsorption of cellulolytic enzymes onto lignin caused by hydrogen bonding, hydrophobic, and electrostatic interactions are thought to be the main reasons for lignocellulose's low saccharification efficiency (Yarbrough *et al.,* 2015).

Different microorganisms produce different enzyme combinations with different lignin degradation mechanisms (Sahadevan *et al.,* 2013). Corn Stover pre-treated with a fungal consortium for 42 days resulted with 43.8% lignin removal and 7-fold increase in hydrolysis. (Song *et al.,* 2013). White rot basidiomycete *Punctularia sp*. TUFC20056 and an unknown basidiomycete TUFC20057 removed lignin more efficiently (50%) than *Ceriporiopsis subvermispora* FP90031 and *Phanerochaete sordida* YK624 (Suhara *et al.,* 2012). Actinobacteria *Steptomyces cyaneus* pre-treated with Barley straw substrate was found to be 29-52 % lignin degradation when incubated for 21 days (Isroi *et al.,* 2011). The treatment of eucalyptus grandis saw dust with *P. ostreatus* and *Pleurotus pulmonarius* is reported to be selective degradation of lignin (Castoldi *et al.,* 2014).

Factors affecting Microbial Pre-treatment process

pH

When pre-treating biomass biologically, the pH is regarded as a crucial parameter. Given that it affects the growth of bacteria and their metabolic processes, pH is a relatively important element. Studies show that several white-rot fungi, in addition to the bacterial and archaeal communities, exhibit ligninolytic activity at pH between 4.0 and 5.0, which aids in lowering the medium acidity during digestion. The structure and synthesis of laccase are impacted by pH changes that are outside of the optimum range, which ultimately results in a decrease in the activity of the enzymes (Patel *et al.,* 2009; Sindhu *et al.,* 2015). A pH adjustment will have an impact on the laccase's three-dimensional structure, which will therefore result in less laccase activity (Sindhu *et al.,* 2016). In the case of laccase production, pH has a significant impact, and even a modest shift in intracellular pH will cause a reduction in the synthesis of macromolecules. *Tricholoma giganteum* AGHP produced the most laccase (1.27 105 Ug1 of dry substrate) at pH 5.0. Higher pH levels did not result in an increase in the synthesis of enzymes (Patel and Gupte, 2016).

Temperature

During the pre-treatment, it is essential to maintain the incubation temperature, which can vary depending on the microorganisms involved. While Basidiomycetes are active between 25 and 30 degrees Celsius, the majority of Ascomycetes grow at 39 degrees Celsius. In addition to regulating the ideal temperature for biomass pre-treatment, fungal physiology also aids in heat generation, raising the media's temperature (Isroi *et al.*, 2011).

Fatty acids

Various volatile fatty acids, such as acetic, propionic, or butyric acids, are thought to be crucial intermediates in the biofuel production process. Ideally, the processes that lead to methanogenesis are suppressed at increased concentrations of volatile fatty acids (Weiland, 2010). Volatile fatty acids (VFA) can suddenly increase or build up, which slows down the growth of bacteria that produce acids, reducing acidogenesis and methane production (Mishra *et al.*, 2018).

Carbon/Nitrogen (C/N) ratio of Biomass

Energy crops, forest remnants, home waste, and agricultural waste are the main sources of renewable energy derived from lignocellulosic biomass (Mishra *et al.*, 2018). An immediate indicator of a substrate's biodegradability is its C/N ratio, which is particularly important for lignocellulose. When choosing either a single microbial species or a consortium for biological pre-treatment, consideration must be given to the proximate of lignocelluloses, which vary among biomass varieties, species, growth circumstances, and feedstock maturation (Sindhu *et al.*, 2015).

Pre-treatment time

The incubation time for pre-treatment is determined by the microbial strains and the biomass composition (Mishra *et al.*, 2018). Low delignification rates have an impact on large scale biological pre-treatment of lignocellulose since the process takes longer to complete. According to reports, corn stalk treatment with the wood-decaying Basidiomycetes *Irpex lacteus* lost a substantial amount of glucan (37.5%) and xylan (59%) as well as 37.6% of its lignin in 42 days (Du *et al.*, 2011; Sindhu *et al.*, 2015). Thus, it was found that *Irpex lacteus* pre-treatment had a negative effect on the generation of sugar while helping to lessen the resistant nature of maize stalk. In order to achieve sugar recovery and microbial activity the strain type and incubation period must be turned before pre-treatment (Du *et al.*, 2011; Sindhu *et al.*, 2015).

Inoculum load

In the process of microbial pre-treatment, the inoculum's concentration is crucial. The time factor is essential for successful substrate colonization since it depends on the kind and quantity of inoculum. The kind and quantity of inoculums have an impact on how long it takes for the substrate to become colonized (Kuijik *et al.*, 2015). A shorter period of time for the substrate to colonize will result from a higher inoculum concentration (Madadi *et al.*, 2017). To guarantee good fungal growth and substrate colonization, sufficient amounts of inoculum must be specified (Singh PK *et al.*, 2018).

Aeration

In order to produce and activate ligninolytic enzymes, aeration is thought to be crucial in the biological pre-treatment of biomass utilizing live cells (Mishra *et al.*, 2018). According to reports on the oxidative processes involving microorganisms, oxygen plays a significant role in the breakdown of lignin. Aeration performs a variety of tasks, such as gaseous exchange, CO removal, heat regulation, and uniform dispersion of volatile substances produced by microbial metabolic activities, which contribute to the production of biogas (Isroi *et al.*, 2011).

Grindability

Another factor influencing the LCB pre-treatment, which involves particle size reduction, is grindability. Cellulose and lignin are the parts of LCB that are fibrous and difficult to process. Several pieces of literature claim that the Hardgrove Grindability Index (HGI) test has been used to determine how grindable the coal is in LCB. The HGI analysis requires pre grinding to obtain biomass with particle sizes ranging from 0.6 to 12 mm before HGI analysis, which is insufficient for characterizing the grindability of LCB. The energy used for grinding is not considered in HGI evaluation (Capareda, 2013).

Types of microbial strains

In the pre-treatment process, fungal strains like *Gloeophyllum trabeum* release certain enzymes that aid in the depolymerization of hemicellulose and cellulose as well as the modified form of lignin as brown residues (Gao *et al.*, 2012; Sindhu *et al.*, 2015). Interestingly, compared to using a single microbe during pre-treatment, the utilization of a microbial consortium considerably improves delignification, xylan breakdown, and increased accessibility of cellulose surface in biomass (Sindhu *et al.*, 2015).

Accessible surface area

The Accessible surface area is another significant component that has a great impact on the processing of lignocellulosic biomass. Specific surface area and pore volume are two structural properties that are related to accessible surface area (Liu *et al.*, 2015). Accessible surface area might grow as a result of pore volume expansion and particle size decrease. Accessible surface area might grow as a result of reduced particle size or increased pore capacity. Research hypothesized that the enzymatic hydrolysis of fragmented pine wood could result in an increase in the accessible surface area (Torr *et al.*, 2016). Breakdown of aspen wood may also increase the accessible surface area, which in turn enhances the accessibility of fibers to future enzymatic hydrolysis. On the other hand, it is highly challenging to analyze accessible surfaces (Goshadrous *et al.,* 2013). The specific surface area will grow when the particle size is decreased (Octavia *et al.,* 2017). The reduction in particle size during the ball milling pre-treatment of lignocellulosic biomass results in an increase in the specific surface area of the cellulosic component. This results in increased cellulose accessibility and increased glucose yield (Lu *et al.,* 2019).

Particle size of biomass

The biomass's particle size plays a significant role in the biological pre-treatment process. More water diffusion, microbial surface colonization, and metabolite transport inside the substrate are prevented the larger the particle size. Appropriate particle size is necessary for increased biomass hydrolysis since small particle size negatively impacts the gaseous exchange (Kuijik *et al.,* 2015). Particle size reduction through milling, grinding, and extrusion can be done during enzymatic hydrolysis to increase surface area interaction with cellulosic fibers and enzymes, deconstruct compact lignocellulosic biomass structure and increase hydrolysis rate (Silva *et al.,* 2012; Pang *et al.,* 2019; Yu *et al.,* 2019). Numerous studies have demonstrated that mechanical pre-treatment reduces the particle size of LCB, such as woody chips (Jiang *et al.,* 2017), corn stover (Yu *et al.,* 2019), miscanthus and wheat straw (Kim *et al.,* 2018), facilitating the subsequent enzymatic hydrolysis (Kavitha *et al.,* 2017; Banu *et al.,* 2018). Wheat straw particle size threshold limit is 270 m (Silva *et al.,* 2012).

Supplements

Several factors, such as different additives (Cu2+, Mn2+, ferulic acid, xylidine, veratric acid, vanillic acid, cinnamic acid, etc) have been previously reported in research studies on their impact on ligninolytic enzyme production (Meehnian *et al.,* 2016; Liu Y *et al.,* 2013). The formation of laccase in *S. ostrea*, *T. pubescens*, *P. eryngii*, and *P. ostreatus* is reportedly influenced by copper at different concentrations, according to numerous research (Usha KY *et al.,* 2014).

Synergistic Microbial Pre-treatment Approach

Microbial Pre-treatment coupled with Physical, Chemical, or Mechanical pre-treatment methods is found to be an effective and efficient solution for biomass bioconversion to bioproducts. Mild physical or chemical pre-treatment before microbial pre-treatment can make the retreatment times quicker. Also, combined pre-treatments can potentially solve the problems related to physical and chemical treatments. The combined pre-treatment process leads to a synergistic effect, enhancing the final products.

Microbial Pre-treatment coupled with Liquid Hot Water

A combined hot water pre-treatment and microbial pre-treatment has been improved by enzymatic saccharification of *Populus tormentosa*, and the combination reported 92.33% removal of hemicellulose along with 2.6- a fold increase in glucose yield as compared to a single process of hot water pre-treatment (Wang *et al.,* 2012). Similarly, another research reported that the glucose yield increases about 65% when the collaborative hot water pre-treatment (170°C for 3 min at 0.75Mpa) and fungal pre-treatment (*Ceriporiopsis subvermispora*) were carried out. This is because the fungal biodegradation of soybean straw was enhanced due to hot water pre-treatment (Wan and Li, 2011).

Microbial Pre-treatment coupled with Mild Acid (0.25% H_2SO_4)

The formation of inhibitory compounds and pre-treatment time can be reduced by the combination of mild acids and fungal pre-treatment. This type of pre-treatment can be found in *Echnidontium taxodii*. The combination pre-treatment results in a high yield of reducing sugars, nearly doubled (improved from 1.13 to 2.11) on water hyacinth as compared to single-step acid pre-treatment (Ma *et al.,* 2010).

Microbial Pre-treatment coupled with alkali (NaOH) and Hydrogen Peroxide (H_2O_2)

Biomass (*Bambusa balcooa*) pre-treated with Alkaline peroxidase was separately pre-treated with active *Lenzites elegans* culture and Laccase enzyme. The result showed the cellulose content increased to 76.6% and 64.8% with lignin content decreasing to 14.5% and 20.5% respectively. The combined effect showed a decrease in recalcitrant material i.e., Lignin content along with the higher release of cellulosic fibers (Praveen *et al.,* 2021).

Microbial Pre-treatment coupled with Alkali (NaOH)

The combined pre-treatment of alkali (NaOH) with *Lenzites elegans* whole cell culture increases the cellulosic content to 70.2% with a decreased lignin content of 20.1%. Similarly, the combined enzymatic pre-treatment of laccase enzyme and NaOH resulted in a virtue effect in declassifying cellulose content of 74.8% (Praveen *et al.,* 2021). Similarly, under mild alkaline environment (1.5% NaOH, 30-75°C for 15-20 min) pre-treatment of cornstalks with fungi *Irpex lacteus* might modify the lignin structure and enhance lignin biodegradation and xylan elimination (Yu *et al.,* 2010).

Microbial pre-treatment coupled with ultrasound

Biomass from birch and pine dust was treated with ultrasound resulting in an increase in delignification to some extent. This might be because ultrasound loosened the wood structure by weakening of bonds between polysaccharide and lignin molecules, therefore, increasing their accessibility for fungal growth leading to high sugar yield in comparison to fungal treatment on its own (Jatoi *et al.*, 2021).

Conclusion and Future Perspective

The path towards sustainability is challenging. The overconsumption of non-renewable resources and to switch towards a renewable source contrariwise, might just be the solution. Refining the use of existing biomass through different approaches of microbial pre-treatments has been employed. A review by Jatoi *et al.*, 2021 demonstrated different methods employed for production of LCB like gasification, pyrolysis, liquefaction and transition metal catalysis. Second generation biofuels like LCB made from agricultural and forestry residues are abundant and a great option against the fossil fuels but it is the matter of how these substrates are utilized in an optimum condition for a maximum yield of final products. LCB is efficient but there are certain drawbacks associated with this approach such as lengthy process, relatively low hydrolysis rates, and significant loss of fermentable sugars. To overcome the drawback, combination method approach is advisable such as microbial pre-treatment coupled with physical, chemical and mechanical pre-treatment methods. The intrinsic structure of biomass, the nature of the microbes used, the types and number of enzymes used, addition of substrates etc. are some of the fundamental steps that can be adjusted and modified in the treatment process to make a desirable input for a better output.

Author's Contribution

All of the authors worked on the concept and design of the study, data acquisition and drafting of the manuscript. Bibek Rana Chhetri did the analysis and interpretation of the data. Final form of manuscript was approved by all of the authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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