Biodegradation of Lignin Containing Waste

NIKHIL PANDEY[1], RUKHSAR ANSARI[2]
[1]*M.Sc. student, Microbiology department, Bhagwan Mahavir college of Basic and Applied Sciences, Bhagwan Mahavir University, Surat*
[2]*Teaching Assistant, Microbiology department, Bhagwan Mahavir college of Basic and Applied Sciences, Bhagwan Mahavir University, Surat*

Abstract*— Interest in lignocellulosic materials is growing due to their potential as replacements for high-value chemicals derived from petroleum derivatives and their applications in biofuel production, the paper industry, sorbent production, activated carbon, carbon fibers, and bioplastics. Lignin, a complex polymer found in plant cell walls, is a major component of lignocellulosic biomass and poses a formidable challenge for degradation due to its intricate and non-uniform structure. Biological methods, particularly via biotransformation and degradation, have gained attention as economically and environmentally friendly options for lignin utilization. Fungi and bacteria are the primary organisms capable of lignin degradation, with fungi being more efficient but bacteria offering advantages in terms of pH, temperature, and oxygen tolerance. Lignin degradation involves the production of ligninolytic enzymes such as lignin peroxidase, manganese-dependent peroxidase, versatile peroxidase, dye-decolorizing peroxidase, and laccases. The paper industry generates a significant amount of lignin waste, and its treatment using physical and chemical techniques is expensive and results in sludge. Biological approaches offer a more sustainable and cost-effective solution. This review highlights lignin biodegradation by bacteria, ligninolytic enzymes, and the current state of knowledge on lignin degradation from the pulp and paper industry. Further research is needed to explore the potential of bacterial strains and enzymes for effective lignin degradation and wastewater treatment in the paper industry.

***Indexed Terms*— Lignin Degradation, Lignocellulosic Material, Bacterial Lignin Breakdown, Lignin-Modifying Enzymes**

I. INTRODUCTION

Interest in lignocellulosic material is growing because of its potential replacement for high-value chemicals derived from petroleum derivatives, and its potential in the production of biofuels (Alvarez et al., 1991), the paper industry, the production of sorbents, activated carbon, carbon fibers with a very large surface area and pore volume (Arnold et al., 1931), and in bioplastics production. One of the most important mechanisms for lignin utilization is via biotransformation and degradation (Asina et al., 2016). Biodegradation of lignin is an economically and environmentally friendly option (Baldrian et al., 2008), and key research on lignin degradation is based on biological methods, oxygen-dependency, and metabolic methods.

Biological degradation or decomposition of lignin can be carried out by fungi (Arantes et al., 2009) and bacteria. Bacterial lignin breakdown is not as efficient as fungal, and bacterial delignification is more limited and slower than fungal, but bacteria can tolerate a wider range of pH values, temperature, and oxygen availability, and bacteria are easier to manage in comparison with fungus. Ligninolytic bacteria have been found in soil, animals, compost, sediments, insect guts, and sewage. Bacteria have the ability to modify and degrade lignin, including actinomycetes, some Firmicutes, α-proteobacteria, and γ-proteobacteria.

The paper industry is one of the main producers of lignin. The pulp and paper manufacturing industry is responsible for the annual production of 50–70 million m3 of lignin. The dark brown wastewater (the color comes from the lignin and lignin-derived compounds) resulting from the paper industry makes globally an amount of 695.7 million m3. For the treatment of paper mill wastewater and for lignin removal, physical

and chemical techniques can be applied (Singhal et al., 2009). However, in comparison with the biological approach, they are expensive, usually require intense process conditions, and result in sludge (Christopher et al., 2014). The removed lignin from the paper and pulp industry is not intended for application goals (Cotana et al., 2014).

In this review, lignin biodegradation by bacteria, ligninolytic enzymes, as well as some reports of lignin degradation by bacteria, are highlighted. The current state of knowledge on the degradation of lignin from the pulp and paper manufacturing industry is also emphasized.

II. LIGNIN DEGRADATION

Lignin degradation plays a pivotal role in mitigating environmental pollution and unlocking novel avenues for the production of valuable chemicals and biofuels. However, due to its intricate and non-uniform structure, coupled with the absence of standard repeating covalent bonds, lignin poses a formidable challenge, resisting degradation by most conventional methods.

While chemical and thermal degradation methods (Figure 1) are viable options, the preference lies with biological approaches for lignin degradation. These methods offer mild reaction conditions and the potential to employ selective ligninolytic enzymes and microorganisms, effectively preventing the formation of undesirable by-products. Moreover, biological processes boast the advantage of no yield loss, unlike thermal lignin decomposition techniques.

Lignin can undergo decomposition through the action of ligninolytic enzymes, resulting in the production of valuable chemicals like vanillin and vanillic acid, which can be utilized in biofuel production. In industries such as cellulose and paper mills, lignin often ends up in wastewater effluent, contributing to environmental pollution by introducing high levels of organic compounds and causing discoloration. Consequently, lignin decomposition plays a crucial role in effective wastewater treatment. Furthermore, microbial degradation of lignin is essential for closing the carbon cycle as it removes the lignin barrier, enabling other microorganisms to access and utilize plant carbohydrates. This process not only promotes the efficient utilization of plant resources but also supports the overall sustainability and balance of the ecosystem (Jiang et al 2019).

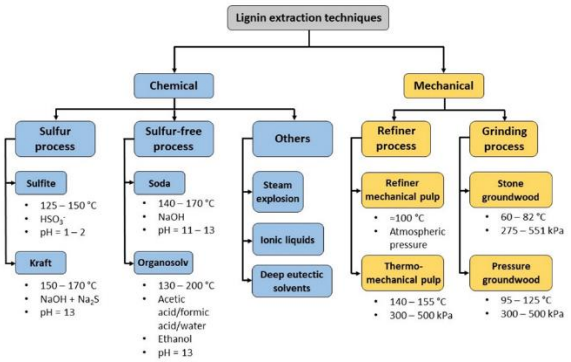

Figure 1 Different extraction methods for lignin (Romaní, A. et., 2020)

III. BIOLOGICAL LIGNIN DEGRADATION

Biological degradation or decomposition of lignin is considered to be a green and environmentally friendly process since fungi and bacteria do not produce secondary pollution (Asina et al 2016). However, hydrolytic enzymes cannot cleave lignin due to its branched three-dimensional structure, as well as the presence of C–C and C–O ether bonds. Additionally, non-phenolic aromatic subunits of lignin cannot be oxidized by low-potential oxidoreductases, such as plant oxidases, which are involved in initiating lignin polymerization. As a result, fungi and bacteria have evolved and developed several groups of enzymes with ligninolytic activity (Boerjan et al 2003).

The biodegradation of lignin is an oxidative process that requires the production of extracellular ligninolytic enzymes, including lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), versatile peroxidase (VP), dye-decolorizing peroxidase (DyP), and laccases (LaC) (figure 2) (Baldrian P et al 2003). The binding affinity of lignin-degrading enzymes is influenced by the type and structure of lignin, with interactions between lignin and amino acids in the enzymes being mediated by three main non-covalent bonds: electrostatic bonds, hydrophobic bonds, and hydrogen bonds.

Since the chemical structure of lignin is highly variable, as well as the enzymes used to degrade lignin, the degradation products also vary (Alvarez P.J.J et al 1991). During multiple biochemical transformations, C–C and C–O monomer bonds are split, and hydroxylation, demethylation, and modification of side chains and other transformations occur. All transformations occur mostly simultaneously (Jiang et al 2019).

Lignin degradation takes place in two phases. During the first phase, homocyclic aromatic compounds are converted into protocatechuic acid and catechol. The first phase of lignin degradation is mainly cleavage of the β-O-4 aryl ether bond in the phenylen unit. During the second phase, a series of intermediates are formed due to the cleavage of the central ring. The produced aromatic compounds, catechol, and protocatechuic acid, are the predominant intermediates during the lignin biochemical conversion (Bugg et al. 2011)described catabolic pathways for the breakdown of lignin components: by β-aryl ether degradation pathways (bacteria and fungi), biphenyl degradation pathways (bacteria), diarylpropane degradation pathways (bacteria and fungi), degradation of phenylcoumarane and pinoresinol lignin components (bacteria and fungi), bacterial degradation of ferulic acid, and oxidative cleavage of protocatechuic acid (bacteria).

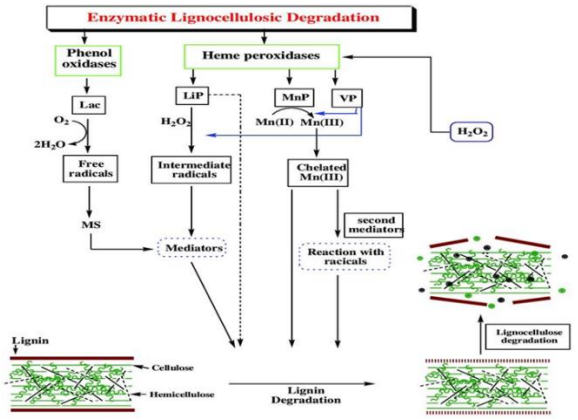

Figure 2 Microbial lignin degradation (Javaid et al 2019)

One of the processes in which biodegradation of lignin takes place is composting. During the composting, the mixed microbial community present in the compost pile is active and microorganisms convert organic material into compost (humus), carbon dioxide, water, and heat. It is assumed that humus is formed mainly from lignin, polysaccharides, and nitrogenous compounds, so that the complete mineralization of lignin does not occur during the composting process. During composting, thermophilic microfungi and actinomycetes are responsible for lignin degradation (Kansal et al. 2008).

Lignin degradation and modification has been mostly studied in basidiomycetes. White and brown rotting fungi play an important role in the degradation of lignocellulosic biomass due to the secretion of extracellular ligninolytic enzymes. Different white-rot fungi produce different combinations of enzymes, for example LiP and MnP, MnP and LaC, and LiP and LaC. Brown-rot fungi are able to successfully degrade cellulose and hemicellulose, but lignin only to a limited extent. Aerobic white-rot fungi basidiomycetes can carry out complete degradation of lignin. Due to the strict fungi growth conditions, their industrial application is limited, since high yields and productivity are required (Baldrian P 2006).

IV. LIGNIN DEGRADATION BY BACTERIA

A. Lignin degradation by Bacillus ligniniphilus L1, Halotolerant bacterium

Zhu et al. 2017 investigated lignin degradation and colour removal from alkaline lignin using the alkaline halotolerant bacterium Bacillus ligniniphilus L1 during a 7-day experiment. Degradation metabolites were monitored using the Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The experiments were performed at 50 °C as the optimal temperature for the investigated strain for lignin degradation, with lignin as the only carbon source and with the combination of glucose-lignin as the carbon source. After 7 days of incubation of the investigated strain with lignin as the only carbon source, they recorded 38.9% lignin degradation and 30% colour removal. As manganese peroxidase and laccase are hypothesized to decolourize lignin (Zhu et al. 2017) suggested that Bacillus ligniniphilus L1 can secrete laccase or manganese peroxidase to degrade lignin. GC-MS analysis showed that 15 aromatic compounds were identified during 7 days of incubation of the investigated strain with lignin, and nine aromatic compounds were identified in the control sample (non-

inoculated sample). The lignin metabolite that was detected the most was vanillic acid, accounting for 44.2% of all produced aromatic metabolites. This is followed by 4'-hydroxyacetophenone with 14.5%, vanillin with 8.7%, and 4-hydroxyphenylacetic acid with 7.2%. The authors believe that the L1 strain degraded lignin, but also that it degraded aromatic compounds from lignin or used it as a source of carbon or energy. In addition to the detected 15 aromatic compounds with one phenyl ring, the authors assume that there can be many other aromatic compounds that were not detected by GC-MS because they were present in low concentrations below the detection limit. In the whole genome of the L1 strain there were no observed LiP or MnP genes. The results obtained by the combination of GC-MS and genome data suggest that there can be three pathways of lignin degradation in the L1 strain: the gentisate pathway, the benzoic acid pathway, and the β-ketodiapate pathway.

B. Lignin degradation by Pseudomonas sp.

Yang et al. 2006 investigated the biodegradation of lignin, namely alkaline lignin and raw lignocellulosic material (switchgrass, corn stalk, and wheat straw), using the bacterium Pseudomonas sp., isolated from rotten wood in China. The decomposition of alkaline lignin was monitored after 3 and 7 days of incubation. The process of lignin degradation was monitored by Gel-Permeation Chromatography (GPC), Field-Emission Scanning Electron Microscope (FE-SEM), and GC-MS. In raw lignin samples, after treatment with Pseudomonas sp. Q18, the amount of residual lignin was reduced, with the amount of residual material in relation to total lignin being lower for switchgrass than for corn stalk and wheat straw. The treatment of switchgrass resulted in the greatest loss of mass from dry biomass, almost 25%, compared with corn stalks and wheat straw. FE-SEM analysis of wheat straw after treatment with Pseudomonas sp. showed a damaged stalk structure and numerous small fragments on the surface compared with the appearance of wheat straw before decomposition. FE-SEM analysis of alkaline lignin showed that after treatment, the smooth surface of lignin was completely eroded. GPC analysis of alkaline lignin showed a decrease in molecular weight after treatment with the investigated strain, which coincides with the lignin content after treatment. The GPC results suggest that the high molecular weight alkaline lignin particles are depolymerized into smaller particles after treatment. Analysis using GC-MS showed that after the incubation of lignin with Pseudomonas sp. the concentration of aromatic compounds with a phenolic ring significantly increased, which indicates the degradation of lignin. The strain Q18 can use the compounds of low molecular weight as energy or carbon source, as shown by substantial consumption and catabolism of aromatic compound. Depolymerisation of lignin, aromatic catabolism, and production of co-products occurred simultaneously. During the alkaline lignin decomposition process, the number of organic acids and esters increased, such as oxalic acid, ethyl acetate, and 3-acetyloxybutanoic acid ethyl ester. This increase possibly reflects chemical reactions among primary microbial metabolites or cleavage of lignin intermediates after degradation. The authors of the research assume that the Q18 strain possesses DyP peroxidase (PmDyP) based on the analysis of lignin-derived metabolites, which belongs to the B-type subfamily of DyP. This strain has the potential for use in a refinery for lignocellulose biodegradation.

C. Lignin degradation by Brevibacillus thermoruber

Niu et al. 2021 investigated the degradation of lignin using *Brevibacillus thermoruber*, at temperatures of 37 °C and 55 °C. The bacterium *Brevibacillus thermoruber* has the ability to secrete MnP, LaC, and LiP, and was isolated from aerobic corn stalk and food factory sludge compost. Bacillus possess broad physiological characteristics, high adaptability, a short cycle of proliferation, and can produce thermostable enzymes. Atomic Force Microscopy (AFM), FTIR, and UHPLC-QTOF/MS were used to analyse lignin before and after decomposition. During the biodegradation of lignin, 81.97% of lignin degradation was achieved during 7 days using the bacterium *Brevibacillus thermoruber*, similar to what is achieved by lignin degradation using fungi. At 37 °C, the lignin degradation pathway (G and H monomers) took place via the β-ketodiapate pathway. At 55 °C, the product of lignin degradation (S monomer) was mainly benzoic acid, that is, the path of lignin degradation took place via the path of benzoic acid. Extracellular enzymes secreted by *Brevibacillus thermoruber* were adsorbed on the lignin surface, which disrupted the lignin structure, increased the surface roughness, decreased the surface size, increased the specific

surface area, and increased the number of active sites, which helped the lignin degradation. The degradation products of lignin were analysed and compared with the Metlin database, where 40 compounds were identified. The amount of lignin decomposition products changed over time and depending on the temperature at which the decomposition took place. The efficiency of lignin degradation increased with an increase in temperature.

D. Lignin degradation by Streptomyces sp. and Mycobacterium sp., White-Rot Fungi Coriolus versicolor and Trametes gallica, and purified laccase enzyme

Asina et al. conducted experiments on the degradation of a high lignin concentration (industrial lignin) of 13.3 g/L during 54 days using the white-rot fungi *Coriolus versicolor* and *Trametes gallica*, the bacteria Streptomyces sp. and *Mycobacterium* sp., and a purified laccase enzyme. The expression of lignin-modifying enzymes depends on the growth phase of the microorganism and on the occurrence of a secondary metabolism, so subtle changes in the balance between actively growing and dying biomass can lead to variations in the extent of activity of extracellular enzymes over time. Laccase activity in both fungal strains was significantly higher compared with bacterial strains. Most bacterial laccases are expressed intracellularly, but some strains of *Streptomyces* sp. produce extracellular laccases. The activity of extracellular laccase varies significantly depending on the pH value of the solution and the composition as a result of the interference of electrostatic interactions and hydrogen bonds within the tertiary structure of the protein. During the entire experiment, all four investigated strains showed significant MnP activity, and LiP activity was not recorded in any strain (Baldrian P. 2006). Contrarily, Adhi et a 2016 noticed significant LiP activity in numerous *Streptomyces* strains. The *Streptomyces* strain did not show apparent laccase activity. The extent of lignin decomposition and observed enzyme activity are not directly correlated (Asina et al. 2016) suggested that some other enzymes besides the investigated ones might be engaged in the biodegradation of lignin. Mineralization of kraft lignin was more significant by fungi, and bacteria partially decomposed and modified lignin. The highest mass loss was obtained with fungal treatment, especially with C. versicolor, 45 ± 8% at the end of the experiment, or 25 ± 15% when taking into consideration lignin mass loss in the control. The slight weight loss was observed with bacterial and laccase treatment. The extent of repolymerization is more pronounced during the decomposition of lignin by laccase and fungi. Fungi have shown the ability to cleave highly cross-linked fractions of lignin, with a fine balance between cross-linking by polymerization and degradation. After bacterial degradation of lignin, the accumulation of phenolic monomers was recorded without their further catabolism.

Effective degradation of kraft lignin (the most numerous forms of lignin, obtained by acid dissolution of black liquid) which is formed as a by-product of alkaline sulphide treatment of lignocellulose characteristic of the paper industry during a short incubation time was achieved mainly by bacterial strains (30–81.4%) because the treatment with fungi is often unstable when carried out by rough industrial treatments.

V. ENZYMES INVOLVED IN LIGNIN DEGRADATION

Lignin degradation primarily relies on two distinct categories of enzymes: lignin-modifying enzymes (LME) and lignin-degrading auxiliary (LDA) enzymes. LME encompasses phenol oxidases like laccases and heme-containing peroxidases such as lignin peroxidase, manganese peroxidase, and versatile peroxidase. Recently, heme-thiolate haloperoxidases have also shown potential involvement in lignin degradation, although their classification is yet to be confirmed. In contrast, LDA enzymes do not possess the ability to degrade lignin independently but play a vital role in completing the degradation process. This group includes glyoxal oxidase, aryl alcohol oxidases, pyranose 2-oxidase, cellobiose dehydrogenase, and glucose oxidase (De Gonzalo et al., 2016).

In nature, only Basidiomycota fungi belonging to the aerobic white rot group have the capability to completely degrade lignin. The enzymatic reaction responsible for breaking down the aromatic ring in lignin requires oxygen or reactive oxygen species, making it unsuitable for anaerobic environments.

Consequently, anaerobic fungi lack the necessary enzymatic machinery to mineralize lignin.

A. Lignin peroxidase (LiP)

Lignin peroxidase (LiP), initially discovered in the white rot fungus Phanerochaete chrysosporium, is an enzyme renowned for its ability to break down lignin, a complex polymer present in plant cell walls. LiP exhibits a relatively broad substrate specificity, capable of oxidizing various organic molecules. It is secreted as a family of isozymes with diverse compositions and isoelectric points (pI), which can be influenced by growth conditions (Barr DP et al., 1992). Structurally, LiP consists of two domains that form an active center cavity enclosing a single ferric ion chelated by a heme group. The enzyme contains glycosylation sites, calcium binding sites, and disulfide bridges that contribute to its stability. LiP exhibits a molecular mass ranging from 35 to 48 kDa and a pI between 3.1 and 4.7. Notably, LiP possesses a high redox potential of approximately 1.2 V at pH 3, enabling the oxidation of substrates unreactive with other peroxidases (Barcelo AR et al., 2004).

LiP's catalytic cycle involves oxidation by H_2O_2, resulting in the formation of compound I, a ferryl oxo porphyrin radical cation intermediate. The enzyme undergoes two single-electron reduction steps facilitated by an electron donor substrate like veratryl alcohol (VA), forming compound II and a VA radical cation. Compound II then oxidizes another VA molecule, simultaneously regenerating the native state of LiP and initiating a new catalytic cycle. VA serves as a small molecular weight redox mediator facilitating electron transfer between the enzyme and its polymeric substrate.

VI. DEGRADATION OF LIGNIN FROM PULP AND PAPER MANUFACTURING INDUSTRY

Lignin degradation and decolorization in paper mill wastewater can be accomplished through various biological approaches, including the use of microorganisms and enzymes. Bacteria and fungi have been extensively studied for their lignin-degrading capabilities in biological wastewater treatment processes, demonstrating effective removal of lignin and color from paper and pulp industry effluent (Jing, H. et al., 2020).

Table 1 Various ligninolytic bacterial strains isolated from lignocellulosic waste containing sites

Bacterial Strain	Source of isolation	Lignocellulose substrates supporting Growth	Relevant characteristics	Growth conditions	References
Psudomonas sp.	Soil	Effluents	Enhanced degradation of benzene and p-xylene in the presence of toluene	aerobic	Alvarez and Vogel (1991)
Aneurinibacillusaneurinilyticus (AY856831)	Pulp paper mill effluent	Kraft lignin	Decolorize kraft lignin, produce low-molecular-weight compounds	Facultative anaerobe/microaerophilic	Chandra et al. (2007); Raj et al. (2007a)
Azotobacter	Soil		Decolorize and solubilize lignin	Aerobic	Morii et al. (1995)
Bacillus cereus	Pulp paper mill effluent	Phenol (with glucose)	Degrade phenol and pentacholorophenol pollutants	Aerobic	Singh et al. (2009)
Bacillus megaterium	Soil		Decolorize and solubilize lignin		Morii et al. (1995)
Bacillus sp. (AY952465)	Pulp and paper sludge	Kraft lignin	Decolorize kraft lignin, produce low-molecular-weight compounds	Facultative anaerobe, 10 % NaCl	Chandra et al. (2007); Raj et al. (2007a)
Citrobacterfreundii	Pulp paper mill effluent	10 % Black liquor	Decolorize lignin	Microaerophilic	Chandra and Abhishek (2011)
Citrobacter sp.	Rayon grade pulp black liquor	10 % Black liquor		Requires oxygen	Chandra et al. (2011)
Enterobacter	Soil	Lignin model compounds	Oxidative ligninolytic enzymes	Requires ABTS	Yadav et al. (2014)
Escherichia coli	Soil	Lignin model compounds	Oxidative ligninolytic enzymes	Requires ABTS	Yadav et al. (2014)

Klebsiella pneumonia	Rayon grade pulp black liquor	10 % Black liquor		Requires oxygen	Chandra et al. (2011)
Paenibacillus sp. (AY952466)	Pulp and paper sludge	Kraft lignin, phenol (with glucose)	Decolorize kraft lignin produce low-molecular-weight compounds	Facultative anaerobe, 3 % NaCl	Chandra et al. (2007); Raj et al. (2007a)
Pantoea sp.	Pulp paper mill effluent		Decolorize, reduce COD and BOD degrade lignin and chlorophenol, ligninolytic enzymes	Aerobic, pH 7	Chandra et al. (2012)
Pseudochrobactrum glaciale	Pulp paper mill effluent		Decolorize, reduce COD and BOD, degrade lignin and chlorophenol, ligninolytic enzymes	Aerobic, pH 9	Chandra et al. (2012)
Pseudomonas putida	Pulp paper mill effluent	Effluent	Remove color, phenolics, and sulfide	Aerobic	Chandra et al. (2001)
Serratia marcescens	Soil; rayon grade pulp black Liquor	10 % Black liquor	Decolorize and solubilize lignin	Requires oxygen	Morri et al. (1995); Chandra et al. (2011)

While fungi, particularly white-rot fungi, have shown promise in lignin degradation, bacteria offer advantages in terms of their adaptability to different environmental conditions. Bacteria can tolerate a wider range of pH values, temperature, and oxygen availability, making them more versatile for treating paper and pulp mill effluent. Some bacteria harbor a high proportion of lignin-degrading genes, hinting at the potential discovery of novel enzymes and pathways (Kamali, M et al., 2015).

Several fungal species, including *Schizophyllum commune*, *Tinctoria borbonica*, and *Phanerochaete chrysosporium*, have demonstrated lignin degradation from paper and pulp mill effluent. Similarly, bacterial strains such as *Bacillus* sp., *Bacillus endophyticus*, and *Bacillus subtilis* have been effective in degrading lignin and reducing color in wastewater. Microbial consortia and certain protozoa have also exhibited lignin-degrading capabilities (Khan, S.I. et al., 2022). The use of microbial consortia, including strains like *Bacillus sp*., *Bacillus subtilis*, *Bacillus megaterium*,

and *Pseudomonas aeruginosa*, has shown promise in lignin degradation and color reduction. Notably, bacterial strain RGM2262 achieved significant removal of color and phenolic structure, along with reductions in total organic carbon and chemical oxygen demand. Moreover, *Trabulsiella guamensis*, *Aspergillus flavus*, *Emericella nidulans*, and other bacterial strains have exhibited lignin degradation capabilities in paper mill wastewater (Luo, H. et al., 2017).

Conventional wastewater treatment methods are often inadequate for paper mill wastewater treatment, emphasizing the importance of utilizing lignin-degrading bacteria. The role of bacteria in lignin degradation is a key focus in this article, highlighting the diverse range of bacterial strains and microbial consortia that have demonstrated efficient lignin degradation from paper industry wastewater (Luo, H. et al., 2017).

CONCLUSION

Lignin, a resilient and abundant compound in nature, presents challenges in terms of its degradation and decomposition. However, microorganisms and enzymes offer a promising and sustainable approach for lignin degradation. Numerous bacteria and their associated enzymes have shown potential in the breakdown of lignin, decolorization, and reduction of toxicity. Further exploration is needed to identify bacterial strains that are efficient in treating pulp and paper mill wastewater. Lignin-degrading bacteria primarily belong to α-proteobacteria, γ-proteobacteria, certain Firmicutes, and actinomycetes. The group of ligninolytic enzymes, including laccases, lignin peroxidase, manganese-dependent peroxidase, versatile peroxidase, and dye-decolorizing peroxidase, play a vital role in the sequential degradation and transformation of lignin. The discovery of a significant proportion of lignin-degrading genes in actinobacteria and proteobacteria suggests the existence of unexplored pathways and enzymes, providing exciting opportunities for future lignin degradation research.

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