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Review Article

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Bioremediation of Pulp and Paper Mill Wastewater Using Isolated Fungal and Bacterial Strains

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ABSTRACT

Pulp and paper mill wastewater contains high concentrations of toxic pollutants, including lignin and aromatic compounds, which contribute to its dark coloration and pose environmental hazards. Improper discharge of this wastewater can result in severe ecological consequences, such as oxygen depletion, carcinogenic effects, and aquatic toxicity. Various treatment methods, including physicochemical and biological approaches, have been used to address this issue. Among these, biological treatment with microorganisms, particularly bacteria and fungi, has gained attention for its efficiency and sustainability. Bacteria such as Bacillus sp. and Pseudomonas sp. exhibit adaptability across different pH ranges and produce ligninolytic enzymes that aid in pollutant degradation. In contrast, white-rot fungi, including Phanerochaete chrysosporium and Trametes versicolor, secrete potent extracellular enzymes that break down lignin and other complex organic pollutants. This review explores the physicochemical properties of pulp and paper mill wastewater, evaluates bacterial and fungal degradation processes, and compares their efficiency in reducing chemical oxygen demand (COD), biochemical oxygen demand (BOD), and lignin content. It also discusses future strategies for optimizing microbial consortia and enhancing enzymatic activity for large-scale wastewater treatment.

Keywords: Pulp and paper mill wastewater, lignin degradation, bacterial treatment, fungal treatment, bioremediation.

Introduction

The pulp and paper industry ranks third globally in wastewater production [1]. This industry discharges highly polluted black-colored wastewater, characterized by high biochemical oxygen demand (BOD), chemical oxygen demand (COD), and suspended solids (SS), along with toxic compounds [2, 3]. The dark color of this wastewater, primarily caused by lignin, reduces light penetration into shallow water layers, thereby limiting photosynthesis and creating anaerobic conditions [4]. Furthermore, it has been reported that pollutants in this wastewater can induce DNA damage and oxidative stress, potentially contributing to carcinogenic effects in both animals and humans [5].

Moreover, this condition can elevate the mortality rates of marine animals and contribute to water toxicity. Therefore, proper wastewater treatment before discharge is essential to prevent environmental damage [4, 6]. Although various methods, such as physicochemical processes, coagulation, precipitation, adsorption, membrane technologies, and ozonation, are employed for wastewater treatment, they tend to be costly and less preferable compared to biological approaches [7, 8].

Microorganisms, such as fungi and bacteria, offer a promising approach for treating pulp and paper mill effluents. These microorganisms secrete a variety of enzymes, including lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac), that are capable of degrading the complex lignin structure and converting it into valuable compounds [9]. Figure 1 illustrates the microbial lignin biodegradation process. This review examines the physicochemical composition of pulp and paper mill wastewater and evaluates the role of bacterial and fungal biodegradation in reducing COD, BOD, and lignin content.

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Physicochemical Properties Of Pulp And Paper Mill Wastewater

The physicochemical properties of paper mill wastewater are characterized by parameters such as pH, COD, and BOD. COD measures the oxygen needed to oxidize both organic and inorganic substances in the wastewater, while BOD indicates the oxygen consumed by microorganisms during the breakdown of organic compounds [15]. The wastewater composition varies between mills due to differences in the types of solid waste produced [16-17]. One of the primary byproducts is black liquor, an alkaline effluent that contains inorganic substances such as silica, as well as lignin and its derivatives, which are the major pollutants in this waste [18-19].

pH of Pulp and Paper Mill Effluents

The pH of pulp and paper mill wastewater typically ranges from 6.1 to 8.3, depending on the production process. The low pH levels in these effluents may result from acid production by microbial activity [20, 21]. However, Tripathi et al. (2013) reported that pH values vary across sites, with the lowest value (2.1) observed in the chlorination section due to the reaction of water with chlorine. This reaction produces hydrochloric acid and hypochlorous acid, which can significantly lower the pH [22]. In addition, a study by Mohamed et al. (1989) reported that there is a big pH difference between hard and softwood kraft mill as in softwood, the amount of acids and chlorinated phenols are higher than in hardwood. Consequently, the pH in softwood kraft mills is lower than in hardwood kraft mills due to the higher concentrations of acids and chlorinated phenols [23].

Chemical Oxygen Demand of Wastewaters

High COD levels often indicate a toxic state in wastewater, indicating the presence of substantial organic compounds, such as lignin, carbohydrates, and chlorinated organic compounds [24]. Kumar et al. (2015) reported that the concentration of dissolved substances in this wastewater ranges from 395 to 2500 mg/L, with COD values varying between 480 and 4450 mg/L [25, 26]. Some researchers recorded a COD level of 2664.2 mg/L in a mill, attributed to the presence of weak black liquor in wastewater [27].

Biological Oxygen Demand of Effluents

Ram et al. (2019) reported BOD values in various pulp and paper wastewater samples, ranging from 118 ppm to approximately 959 ppm [28]. Devi et al. (2011) reported a BOD concentration of 203 mg/L in wastewater from an Indian paper mill [29]. Beyond lignin, this wastewater also contains xenobiotic compounds, such as chlorinated phenols, polyaromatic hydrocarbons (PAHs), and high sodium levels [20, 30]. Furthermore, color serves as a key indicator of wastewater quality, with its intensity increasing due to the presence of low- and high-molecular-weight organic compounds resulting from lignin breakdown [22].

Treatment Of Wastewater By Bacteria

The complex organic composition of pulp and paper mill wastewater requires efficient treatment strategies. Bioremediation using bacteria presents a promising and sustainable solution [27]. Numerous bacteria have been isolated from various sources to evaluate their enzymatic activity in lignin biodegradation. Zainith et al. [31] isolated Bacillus aryabhattai capable of producing manganese peroxidase from pulp and paper industry wastewater. Their findings revealed that this bacterium can reduce color by 67% and lignin content by 54%. Moreover, FT-IR analysis indicated that some new chemical groups, such as carboxyl (–COOH), alkene (C=C) emerged after wastewater treatment due to the lignin degradation. Chandra et al. [33] utilized Pseudomonas putida, Citrobacter sp., and Enterobacter sp. to degrade various substances, including phenols, while also reducing BOD and COD levels in wastewater with these microorganisms. In another study, Abhishek et al. [6] screened Citrobacter freundii and Serratia marcescens for their ability to degrade kraft lignin in wastewater under both co-culture and axenic conditions. Their findings revealed that these bacteria, when cultured together, degraded 70% (2000 mg/L) of kraft lignin. In contrast, under axenic conditions, Citrobacter freundii and Serratia marcescens broke down 50% and 55% of the lignin, respectively . Furthermore, researchers discovered that the bacterial strain RGM2262, in both biofilm and planktonic forms, can completely remove color and phenolic compounds from effluent. This strain achieved reductions in total organic carbon (TOC) by 70% and COD by 80% [34]. In a study by Raj et al. [35], Paenibacillus sp., Aneurinibacillus aneurinilyticus, and Bacillus sp. bacteria were isolated from pulp and paper mill wastewater. The experiments demonstrated that these bacteria were capable of reducing the wastewater color range by 39% to 81% and lignin content by 28% to 53%. Furthermore, these bacteria successfully decreased BOD and COD levels by approximately 70% and 65%, respectively. Among these bacteria, Bacillus sp. exhibited the highest lignin removal rate at 53%, while Paenibacillus sp. showed the greatest phenol reduction at 77%. Chandra et al. [36] also isolated bacteria, including Paenibacillus sp. (AY952466), Aneurinibacillus aneurinilyticus (AY856831), and Bacillus sp. (AY952465), for wastewater treatment. Their results indicated that Bacillus sp. demonstrated the maximum ability for color and lignin reduction, achieving 65% and 37%, respectively.

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Additionally, A. aneurinilyticus and Paenibacillus sp. resulted in lignin degradation of 33% and 30%, respectively. Notably, when these bacteria were cultured together, the co-culture significantly enhanced both color reduction, reaching 69%, and lignin degradation, increasing to 40%. Islam Khan et al. [37] successfully isolated and identified Bacillus altitudinis SL7 from paper mill effluent. They assessed its degradation ability on a medium enriched with alkali lignin (3 g/L) using FT-IR and GC-MS analyses. The results revealed that alkali lignin was degraded into low-molecular-weight compounds, including vanillin, oxalic acid, and ferulic acid. An et al. [38] isolated Serratia sp. AXJ-M from soil near paper factory wastewater and analyzed its ability to biodegrade lignin. They hypothesized that DyP (dye decolorization peroxidase) was responsible for breaking down ether bonds, such as β-O-4 and Cα-Cβ. Subsequent assessment revealed that this bacterium is capable of removing up to 71% of COD and 86% of phenols, effectively reducing wastewater toxicity. This bacterium has proven to be highly beneficial in wastewater treatment due to its thermotolerance and resilience to lignin aromatic compounds. In another study [39], researchers isolated novel bacteria, including K. pneumoniae strains NITW715076, NITW715076_1, NITW715076_2, and NITW715076_3, for their lignin degradation capabilities. The researchers then analyzed their enzymes and the concentration of phenolic compounds using various tests, including germination assays and GC-MS analysis. The findings indicated that these strains possessed laccase and MnP enzymes, crucial for lignin degradation. Notably, a consortium of strains NITW715076_2 and NITW715076_1 demonstrated a significant lignin degradation rate of 82.31%. Conversely, the lignin degradation rate for strain NITW715076_2 in axenic culture was lower, at 74.1%. Subsequently, Ojha and Tiwari [40] screened Bacillus subtilis, Bacillus endophyticus, and Bacillus sp. from marine sediments for lignin degradation in pulp and paper mill effluent. Various conditions were optimized to achieve the maximum level of lignin degradation. Finally, the researchers found that at 33 °C and pH 7.92, with the presence of glucose and yeast extract, these bacteria are capable of decolorizing wastewater up to 68.29%. Verma & Chandra [41] introduced Acinetobacter baumannii PC4 and Bacillus cereus PC10 into the native bacterial community of pulp and paper mill wastewater. Under normal conditions, the removal rates for lignin, color, and COD were 53%, 46%, and 76%, respectively. However, co-culturing these bacteria with the native microbiota significantly enhanced these removal rates. Specifically, lignin removal increased by 10%, color removal by 28%, and COD removal by 10%. Furthermore, GC-MS analysis revealed a decrease in the peaks of certain compounds and the emergence of new products, including 1-(Trimethylsilylmethyl1) dimethylsilyloxy tetradecane and 1-ethyl-5,6-dimethoxy-2,3-dihydroindole. Table 1 presents a summary of bacterial isolates for the refinement of pulp and paper wastewater purpose.

Among the bacterial strains studied, Bacillus sp. and Planococcus sp. demonstrated the highest lignin degradation efficiency, while co-culturing strains further enhanced biodegradation. This effect is likely due to the ligninolytic enzymes produced by these bacteria. Future research should optimize bacterial consortia for large-scale wastewater treatment applications.

Lignin Biodegradation In Effluent By Fungi

Fungi are widely studied for their ability to degrade lignin due to their production of potent extracellular enzymes. However, since the pH of pulp and paper mill wastewater typically ranges from 7 to 9, adjusting it to favor fungal activity can be costly. In contrast, bacteria can thrive in a broader pH range, making them a cost-effective alternative without requiring pH modification [7,10-12]. Wu et al. [47] investigated the treatment of pulp and paper mill wastewater using several white-rot fungi, including Phanerochaete chrysosporium, Pleurotus ostreatus, Lentinus edodes, Trametes versicolor, and strain S22. The results demonstrated a successful reduction of lignin by over 71% and COD by up to 48%. White-rot fungi, including B. adusta and P. chrysosporium were employed to treat synthetic pulp and paper mill effluent. These fungi achieved a degradation rate ranging from 74% to 97% in synthetic effluent. When applied to industrial wastewater, these fungi achieved up to 100% lignin degradation [11].

In Thailand, researchers isolated thermotolerant white-rot fungi, among which Daedaleopsis sp., Schizophyllum commune PT, and S. commune SL exhibited growth at 40 °C. These species were then assessed in two different effluents with COD levels of 4000 mg/L and 4347 mg/L. Only Daedaleopsis sp. and P. chrysosporium exhibited wastewater decolorization, achieving an average of 65% in the former and 75% in the latter effluent [48]. The wood-rot fungus Fibrodontia sp. RCK783S was used due to its potent ligninolytic enzymes. This fungus demonstrated significant color removal (61.58%) and total organic carbon reduction (48.32%) [49].

Moreover, Fibrodontia sp. RCK783S proved to be an effective degrader of lignin waste and phenolic compounds [50]. Aspergillus flavus strain F10 is another fungus that proved to be effective in lignin and color reduction in pulp and paper effluent. The results indicated that this fungus reduced color and lignin by up to 51% and 61%, respectively, after a 10-day treatment period [51]. Leppänen et al. [52] isolated several white-rot fungi, including Polyporushirsutus, Daedaleoflavida, and Phellinus sp., and investigated their ability to remove color and COD from wastewater at both laboratory and pilot scales.

The results showed that, at the laboratory scale, Phellinus sp. achieved color and COD removal rates of 62.2% and 42.1%, respectively. However, at the pilot scale, Polyporushirsutus demonstrated 66.2% color removal and 37.3% COD reduction. Singhal et al. [53] conducted research on Emericella nidulans var. nidulans, isolated from the sediments of pulp and paper mill effluent. This fungus achieved decolorization and delignification rates of up to 66.66% and 37%, respectively. It was found that pH was a significant factor in the performance of fungus, while increasing the temperature to 30 or 35 °C did not significantly affect the process. Subsequently, researchers investigated Pleurotus ostreatus, which effectively removed 60% of COD, 76.8% of BOD, and 80% of color. In a separate study, researchers screened a number of white-rot fungi, including Trametes versicolor, Phanerochaete chrysosporium, and Pleurotus sajor-caju, for the treatment of paper wastewater. The results indicated a reduction in lignin absorbance rates ranging from 25% to 46%, and a 74% color reduction [54]. Liu et al. [55] utilized Aspergillus niger for the biodegradation of alkali lignin in the effluent. This fungus achieved reductions in color, turbidity, and COD by 60%, 43%, and 77%, respectively. A study by Prabu and Udayasoorian [56] investigated the white-rot fungus Phanerochaete chrysosporium, screened from soil, for its ability to biodegrade phenolic compounds and decolorize effluent. The study reported that this fungus achieved removal of 79% of COD, 84% of color, and 91% of chlorinated substances. Schizophyllum commune at a pH range of 4-5 was reported effectively remove 90% of color, 70% of BOD, and 72% of COD [57]. Other researchers investigated Coriolus versicolor, Tyromyces albidus, Trametes gallica, and Pleurotus ostreatus in submerged fermentation conditions using paper mill sludge. Furthermore, the researchers measured the ligninolytic enzyme activities of these fungi and found that T. albidus exhibited the highest laccase activity (202 U/L), while C. versicolor had the highest MnP activity (50 U/L), and the maximum cellulase activity was recorded for P. ostreatus (0.26 µmol). The results showed that all four fungi reduced the lignin content in sludge samples, and FT-IR analysis confirmed these findings [58]. Table 2 summarizes fungi that have been reported for their lignin degradation potential.

Based on the reports, it is perceived that while white-rot fungi such as Phanerochaete chrysosporium and Trametes versicolor exhibited the highest lignin degradation efficiencies, Aspergillus species demonstrated superior adaptability to variable environmental conditions. Future research should focus on optimizing fungal enzyme production for industrial applications.

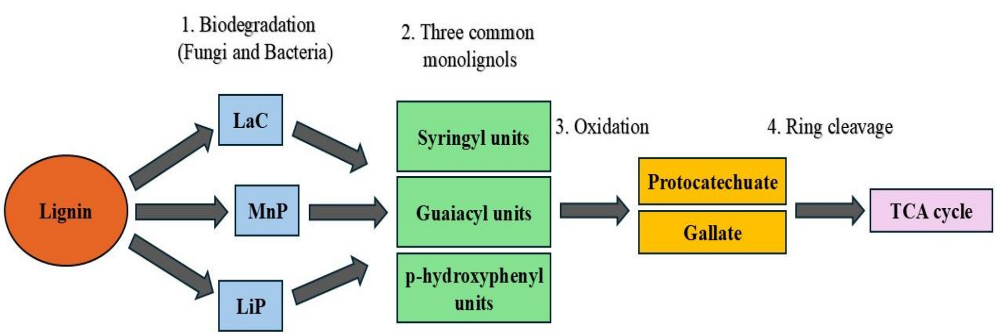

Fig. 1. Lignin biodegradation by microorganisms.

Table 1. Reported isolated bacteria for the reduction of lignin, COD, and BOD from pulp and paper wastewater.

Bacteria	Lignin	COD	BOD	Reference
Paenibacillus sp. strain LD-1	54%	78%	83%	[17]
Serratia liquefaciens	58%	85%	-	[2]
Pseudomonas aeruginosa, P. aeruginosa, and Bacillus megaterium	-	250 mg/L	30 mg/L	[18]
Bacillus flexus RMWW II	100%	84%	-	[42]
Planococcus sp. TRC1	74%	85%	-	[43]
Serratiamarcescens NITDPER1	59%	-	-	[44]
Bacillus magnetrium and Pseudomonas plecoglossicida	816.52 mg/L	3915.36 mg/L	1232.63 mg/L	[45]
Bacillus subtilis and Klebsiella pneumonia	80%	73%	62%	[46]

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Table 2. A summary of reported fungi with lignin biodegradation ability.

Fungi	Lignin	Reference
Merulius aureus syn. Phlebia sp., Fusarium sambucinum Fuckel MTCC 3788	79%	[59]
Cryptococcus sp. isolate FIST3	24%	[7]
Aspergillus flavus, Emericella nidulans	19–41.6%	[60]
Trametes versicolor	50%	[61]
Neurospora discreta	70%	[62]
Aspergillus fumigatus	17.58%	[63]
Ceriporiopsis subvermispora CZ-3	62%	[64]
Trametes pubescens	83%	[65]
Aspergillus foetidus	90%	[66]
Tinea versicolor	54%	[67]
Phanerochaete crysosporium	95.8%	[68]

Conclusion

Lignin, a complex and resistant natural polymer, poses significant challenges for degradation due to its robust structure. The biodegradation of lignin in wastewater, particularly from industries like pulp and paper mills, presents a promising alternative to costly and energy-intensive physicochemical methods. Diverse bacteria and fungi, including those from the *Gammaproteobacteria* and *Firmicutes phyla*, as well as white-rot fungi such as *Phanerochaete chrysosporium* and *Trametes versicolor*, have demonstrated significant ligninolytic enzyme activity, including lignin peroxidase, manganese peroxidase, and laccase. These microorganisms show considerable potential for lignin biodegradation, offering a more sustainable approach to wastewater treatment.

While bacterial species like *Bacillus sp.* and *Pseudomonas sp.* are adaptable across a broad pH range and are effective in reducing COD and BOD, white-rot fungi produce potent extracellular enzymes that break down lignin, although their activity is often pH-dependent. Despite their potential, challenges such as enzyme stability, process scalability, and efficiency remain, particularly in industrial applications.Future research should focus on optimizing microbial consortia, improving enzyme production through genetic modifications, and exploring the integration of biological treatments with physicochemical processes to enhance treatment efficiency. Overcoming these challenges could make microbial bioremediation a more viable large-scale solution for treating pulp and paper mill wastewater, thus supporting sustainable industrial practices and reducing environmental impact.

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