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Expanding the scope of water analysis: Employing multi-parameter evaluation to enhance understanding of wastewater assessment in the paper industry

Bhuvnesh Swami, Ram Kumar
Department of Microbiology, School of Applied Sciences, Shri Venkateshwara University, Uttar Pradesh, India
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Abstract
The pulp and paper industry produces substantial volumes of wastewater infused with hazardous organic compounds and heavy metals, presenting significant environmental and health risks. This research offers an extensive physicochemical analysis and GC-MS identification of organic contaminants present in the effluent from K.R. Pulp and Papers Limited, India. Wastewater samples underwent analysis in accordance with APHA standard methods, uncovering significantly elevated pollution loads. The Biochemical Oxygen Demand was found to range from 3,653 to 4,180 mg L^{-1}, while the Chemical Oxygen Demand ranged from 17,890 to 19,100 mg L^{-1}. The BOD/COD ratio of less than 0.2 substantiates the resistant characteristics of the organic matter. Lignin concentrations ranging from 38,950 to 39,000 mg L^{-1}, alongside total phenol levels of 389 to 432 mg L^{-1} were identified as contributors to toxicity. GC-MS analysis revealed the presence of eight predominant organic compounds: Nonacosane (18.2%), Heptacosane (15.6%), Fatty acids (15.2%), Octadecanoic Acid (12.8%), Hexadecane (10.4%), a complex nitrogen compound (8.7%), Phthalic acid esters (6.3%), along with a variety of other organic compounds (12.8%). The research clearly demonstrates that the effluent presents significant toxicological risks to both aquatic ecosystems and human health, highlighting the urgent need for the adoption of advanced treatment technologies and more stringent regulatory measures for sustainable management of industrial wastewater.

Keywords: Pulp and paper mill wastewater, physicochemical characterization, GC-MS analysis, toxicological aspects, environmental pollution

Introduction

The pulp and paper industry serves as a fundamental pillar of the global economy, delivering vital products that facilitate communication, education, hygiene, and packaging. Nonetheless, this sector is acknowledged as one of the most environmentally detrimental industrial domains globally, producing substantial amounts of wastewater, solid waste, and air pollutants (Pokhrel & Viraraghavan, 2004; Kumar *et al*., 2024) [16, 28]. The production of pulp and paper encompasses a multifaceted series of processes, such as wood preparation, pulping, bleaching, washing, and papermaking. Each of these stages plays a significant role in the creation of wastewater that is rich in a variety of pollutants (Chandra *et al*., 2023; Singh & Patel, 2024) [5, 24]. The chemical pulping process, especially the kraft pulping method, stands as the leading technology worldwide and accounts for a significant portion of the pollution load due to the extensive application of chemicals including sodium hydroxide, sodium sulfide, chlorine dioxide, and various bleaching agents (Wang *et al*., 2021; Sharma *et al*., 2024) [27, 52]. The environmental ramifications of wastewater from pulp and paper mills are intricate and significant. The release of untreated or insufficiently treated effluent into receiving water bodies results in significant oxygen depletion, toxicity to aquatic organisms, bioaccumulation of harmful substances, and disruption of aquatic ecosystems (Pang *et al*., 2021; Sahoo *et al*., 2020) [23, 35]. The dark brown hue of the effluent, largely attributable to lignin and its derivatives, obstructs photosynthesis in aquatic flora and diminishes the visual appeal of water bodies (Chandra *et al*., 2020; Thakur & Gupta, 2022) [4, 50]. The elevated organic load, quantified through Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), results in the depletion of dissolved oxygen, which in turn causes fish mortality and a decline in biodiversity (Kumar *et al*., 2018; Singh & Singh, 2019) [14, 48]. The existence of harmful substances such as phenols, chlorinated organics, and heavy metals presents both immediate and long-term toxicity to aquatic life and endangers human health through the ingestion of contaminated water and food (Chowdhury *et al*., 2021; Rahman & Singh, 2022) [9, 29]. India stands as the preeminent consumer of paper globally, with a swiftly escalating demand propelled by rising literacy rates, urbanization, and industrialization (Sharma *et al*., 2021; Patel & Singh, 2023) [7, 38]. The Indian pulp and paper sector encompasses a range of large-scale integrated mills alongside a multitude of smaller units, boasting a total annual production capacity that surpasses 14 million metric tons (Kumar & Shrivastava, 2020) [19]. The sector encounters considerable ecological hurdles, especially in addressing the substantial quantities of wastewater produced throughout the manufacturing process (Singh *et al*., 2023; Mishra *et al*., 2021) [9, 40]. Numerous paper mills in India, especially those that are older and smaller in scale, are deficient in sufficient effluent treatment infrastructure, leading to the release of severely contaminated wastewater into rivers and various aquatic environments (Gupta & Kumar, 2022; Sinha *et al*., 2022) [12]. The Central Pollution Control Board (CPCB) has instituted rigorous discharge standards for pulp and paper mills, delineating permissible limits for biochemical oxygen demand (BOD) at 30 mg L^{-1}, chemical oxygen demand (COD) at 250 mg L^{-1}, total

suspended solids at 100 mg L^{-1}, alongside specific pollutants such as phenols at 1.0 mg L^{-1} and lignin at 0.05 mg L^{-1} (CPCB, 2012). Nonetheless, adherence to regulations continues to pose a considerable obstacle, as numerous mills struggle to fulfill the established criteria owing to various technical, financial, and operational limitations (Sharma *et al*., 2024; Patel *et al*., 2024) [21, 27].

The toxicological hazards linked to wastewater from pulp and paper mills are varied and intricate, mirroring the extensive array of chemicals employed and produced throughout the manufacturing process (Kumar & Kumar, 2022; Sharma *et al*., 2023) [11, 13]. The wastewater comprises a multifaceted amalgamation of organic compounds, encompassing lignin and its derivatives, chlorinated organic compounds, dioxins, furans, polychlorinated biphenyls, phthalates, phenols, and an array of volatile organic compounds (Mishra & Patel, 2020; Reddy & Gupta, 2023) [20, 32]. Numerous compounds demonstrate persistence in the environment, exhibit bioaccumulation, and possess toxic, carcinogenic, mutagenic, and endocrine-disrupting characteristics (Singh *et al*., 2021; Gupta & Singh, 2021) [9]. The existence of endocrine-disrupting compounds (EDCs) such as nonylphenol, octylphenol, and bisphenol A raises significant concerns due to their capacity to disrupt the endocrine systems of aquatic organisms even at minimal concentrations (Bhattacharya *et al*., 2024) [3].

Pulp and paper mill wastewater is characterized by significant levels of heavy metals, such as iron, zinc, lead, copper, cadmium, nickel, and chromium (Sinha *et al*., 2022; Bhattacharjee & Das, 2024) [2, 49]. These metals derive from a multitude of sources, encompassing raw materials, chemical additives, catalysts, and the corrosion of equipment (Ramesh *et al*., 2022; Singh *et al*., 2023) [5, 31]. Heavy metals are characterized by their non-biodegradable nature and toxicity, leading to bioaccumulation in living organisms. This accumulation presents significant risks to both aquatic life and human health via the food chain (Ahmed *et al*., 2020; Goyal *et al*., 2021) [1, 7]. Lead, cadmium, and mercury present significant dangers owing to their neurotoxic, nephrotoxic, and carcinogenic properties (Verma *et al*., 2023 [51]; Chowdhury *et al*., 2021).

The wastewater exhibits heightened concentrations of nutrients such as nitrogen and phosphorus, which may lead to the eutrophication of adjacent aquatic ecosystems (Gupta & Kumar, 2022; Ramesh *et al*., 2022) [13, 31]. Elevated sulfate levels can result in the formation of hydrogen sulfide in anaerobic environments, leading to unpleasant odors and potential toxicity (Singh *et al*., 2023) [5]. Chlorides and various inorganic salts have the potential to elevate the salinity levels of receiving waters, thereby impacting freshwater ecosystems (Kumar *et al*., 2024) [16]. The deep brown hue of the effluent, largely attributable to lignin and its derivatives, obstructs photosynthesis and diminishes the visual appeal of aquatic environments (Thakur & Gupta, 2022; Patil *et al*., 2024) [27, 50]. Suspended solids have the potential to suffocate benthic organisms, diminish light penetration, and facilitate the transport of adsorbed pollutants (Chaudhary *et al*., 2023) [6].

Despite the acknowledged dangers posed by wastewater from pulp and paper mills, there is frequently a deficiency in thorough multi-parameter evaluations that encompass physicochemical characterization, heavy metal analysis, identification of organic pollutants, and spectroscopic profiling (Sharma *et al*., 2021; Kumar & Sharma, 2022) [7, 15]. Integrated assessments are crucial for comprehending the comprehensive nature of the pollution load, pinpointing priority pollutants, and formulating focused remediation strategies (Patel & Gupta, 2023) [25]. Moreover, the physicochemical properties of wastewater can exhibit considerable variation among different mills, influenced by factors such as the raw materials utilized, the pulping technology employed, the bleaching sequence applied, and the practices adopted for effluent treatment. Consequently, assessments tailored to specific sites are essential for guiding treatment choices and regulatory measures.

Materials and Methods

The investigation was carried out at K.R. Pulp and Papers Limited which is a mill that specializes in the manufacturing of writing and printing paper, and it uses wood and agricultural leftovers as its principal raw materials. Following the removal of lignin via the use of the kraft pulping technique, the mill next employs a bleaching process that conforms to the technology of elemental chlorine-free bleaching. Previous to being discharged into the receiving water body, the wastewater that is generated from a variety of operations, including pulping, bleaching, washing, and paper making, is subjected to treatment in a conventional activated sludge treatment plant.

Wastewater samples were obtained from two separate discharge locations within the mill's boundaries: the main discharge point adjacent to the effluent treatment facility and the sedimentation basin where sludge tends to accumulate. Samples were gathered in sterile, meticulously pre-cleaned polyethylene containers equipped with secure, tightly fitting lids. Approximately 5 liters of wastewater were collected from each location utilizing a grab sampling technique at a depth of roughly 30-50 cm beneath the surface. The samples were conveyed to the laboratory within insulated containers, meticulously kept at 4°C through the use of ice packs to safeguard their physicochemical properties. All samples underwent processing within a 24-hour timeframe post-collection to mitigate alterations in their composition. Comprehensive records of the sampling location, date, time, temperature, pH, and other pertinent parameters were meticulously recorded during the sample collection process.

The analysis of physicochemical properties of the wastewater samples was conducted in accordance with the standard methodologies outlined in the manual published by the American Public Health Association, 2017. All analyses were performed in triplicate, with the inclusion of suitable blanks and standards in each batch of analysis. The pH was determined utilizing a calibrated pH meter featuring a glass electrode. Prior to each measurement, the instrument underwent calibration with standard buffer solutions at pH levels of 4.0, 7.0, and 9.2. Color was assessed utilizing the platinum-cobalt standard method, with findings articulated in Pt-Co units. The determination of Biochemical Oxygen Demand was conducted utilizing the standard 5-day BOD test at a controlled temperature of 20°C. The samples underwent suitable dilution utilizing BOD dilution water, which comprised phosphate buffer, magnesium sulfate, calcium chloride, and ferric chloride. The dissolved oxygen levels were assessed prior to and following incubation through the azide modification of the Winkler method, and the biochemical oxygen demand was derived from the variation in dissolved oxygen readings. Chemical Oxygen

Demand was assessed through the open reflux method, employing potassium dichromate as the oxidizing agent alongside silver sulfate serving as a catalyst. The surplus dichromate underwent titration with ferrous ammonium sulfate, employing ferroin as the indicator.

Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) were assessed using gravimetric methods for TDS and TSS determination. TDS was quantified by evaporating the filtered sample at a temperature range of 103-105°C, whereas TSS was assessed by filtering the sample through a pre-weighed glass fiber filter followed by drying at the same temperature range of 103-105°C. Total phenol content was assessed utilizing the 4-aminoantipyrine method. Phenol was isolated from the samples via distillation and subsequently reacted with 4-aminoantipyrine in the presence of potassium ferricyanide at a pH of 10. The absorbance was quantified at 500 nm utilizing a UV-VIS spectrophotometer. The determination of lignin content was conducted utilizing the acetyl bromide method. The samples underwent treatment with acetyl bromide in a glacial acetic acid medium, followed by the measurement of absorbance at 280 nm. Nitrogen content was assessed through the Kjeldahl method, which entails the digestion of the sample with sulfuric acid and catalysts, succeeded by the distillation and titration of the resultant ammonia. The determination of sulfate content was conducted utilizing the turbidimetric method, wherein samples were subjected to treatment with barium chloride, followed by the measurement of the resultant turbidity at a wavelength of 420 nm. The determination of chloride content was conducted through the argentometric method, which involved titration with silver nitrate, utilizing potassium chromate as an indicator.

The organic pollutants contained within the wastewater were discerned through the application of Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The samples were extracted through a liquid-liquid extraction process utilizing dichloromethane at pH levels of 2, 7, and 12, aimed at capturing compounds with varying polarities. The pooled extracts underwent concentration via a rotary evaporator and were subsequently dried using a gentle stream of nitrogen gas. The GC-MS analysis was conducted utilizing an Agilent 7890B GC in conjunction with a 5977B MSD, featuring a DB-5MS capillary column with dimensions of 30 m × 0.25 mm × 0.25 μm. The oven temperature was set to increase from 40°C to 300°C at a rate of 10°C per minute, utilizing helium as the carrier gas at a flow rate of 1 mL min^{-1}. The injection volume was 1 μL, accompanied by a split ratio of 10:1. The mass spectrometer was utilized in electron impact mode at an energy of 70 eV, encompassing a scan range of 50-550 m/z. The compounds were identified through a comparative analysis of their mass spectra with those cataloged in the NIST library, while their concentrations were approximated by evaluating the peak areas.

Results and Discussion

The analysis of physicochemical characteristics of the wastewater indicated that the wastewater displayed significantly alkaline pH values between 7.8 and 8.1 at the two sampling locations, remaining within the acceptable thresholds set forth by Central Pollution Control Board, India and United States Environmental Protection Agency, 2002. Nevertheless, all other parameters assessed exhibited concentrations significantly surpassing regulatory standards. The BOD values ranged from 3,653 to 4,180 mg L^{-1}, while the COD values were between 17,890 and 19,100 mg L^{-1}, both significantly exceeding the permissible limits. These remarkably elevated values suggest a substantial organic load that would significantly reduce dissolved oxygen levels in any receiving water body. The BOD/COD ratio of less than 0.2, specifically ranging from 0.204 to 0.219, signifies a finding of critical importance. This ratio suggests that the organic matter found in the effluent is significantly resistant to standard biological degradation processes, given that typical biodegradable waste streams demonstrate BOD/COD ratios exceeding 0.5 (Kumar *et al*., 2018; Singh & Singh, 2019) [14, 48]. This low biodegradability index is indicative of effluents from pulp and paper mills, attributed to the presence of intricate lignin-derived compounds (Pokhrel & Viraraghavan, 2004) [28]. The obstinate characteristics of organic matter present considerable difficulties for biological treatment methodologies, necessitating the implementation of sophisticated treatment technologies such as advanced oxidation processes, enzymatic treatment, or the application of specialized microbial consortia (Chandra *et al*., 2023; Kumar *et al*., 2024) [5, 33].

There was a significant cause for worry about the lignin concentration, which ranged from 38,950 to 39,000 mg L^{-1}. This is in sharp contrast to the permissible level of 0.05 mg L^{-1}. According to recent studies lignin is a complex polyphenolic polymer that has a significant role in giving the effluent a dark brown color. This coloration makes the effluent visually unpleasant and harmful to aquatic creatures. The recent studies suggested that higher concentration of lignin is a result of the kraft pulping process, which only removes a fraction of the lignin from the wood chips, allowing a portion of the lignin to be discharged into the wastewater (Thakur & Gupta, 2022) [50]. Due to the fact that lignin plays an essential part in the chemical oxygen demand, the presence of lignin at increased concentrations is a key contributor to the high COD values (Reddy & Kumar, 2024) [33]. Comparatively, the levels of total phenol that range from 389 to 432 mg L^{-1} are much higher than the allowed threshold of 1.0 mg L^{-1}, which indicates concentrations that are higher than what is considered to be acceptable. In light of the fact that phenolic compounds have been shown to be capable of inducing acute toxicity in fish and other aquatic animals, even at low concentrations (Pang *et al*., 2021; Sahoo *et al*., 2020) [23, 35], the presence of phenolic compounds at such high quantities raises serious concerns. During the pulping process, phenolic compounds are produced as a consequence of the breakdown of lignin. Additionally, phenolic compounds may be found in a number of chemical additives that are applied in the paper-making process (Singh *et al*., 2021) [9]. The increased content of phenols greatly increases the toxicity of the wastewater, which presents major challenges for biological treatment. This is due to the fact that phenols are known to have an inhibitory influence on the activity of microorganisms (Kumar & Singh, 2024; Mishra *et al*., 2023) [10, 44].

The total dissolved solids (TDS) displayed a range that extended from 3,156 to 4,365 mg L^{-1}, while the total suspended solids (TSS) ranged from 156 to 248 mg L^{-1}. Both of these values exceeded the specified standards of 2,100 mg L^{-1} and 100 mg L^{-1}, respectively. According to Patel *et al*. (2024) and Ramesh *et al*. (2022) [21, 31], the

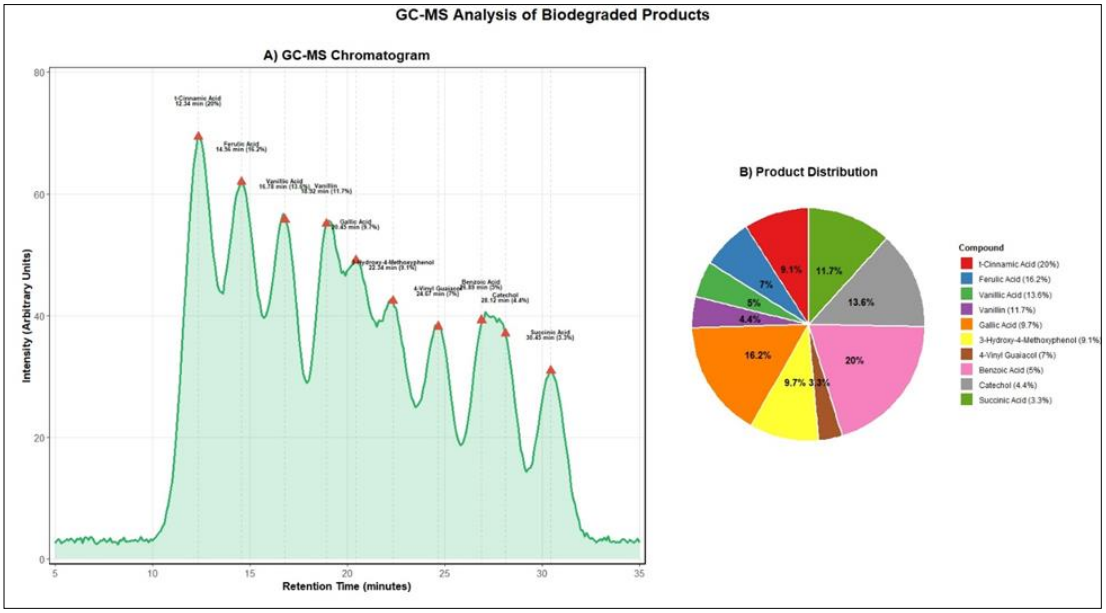
increased concentrations of total dissolved solids and total dissolved solids (TDS and TSS) are a reflection of the substantial mineral content and suspended particulate matter that are present in the wastewater. These are factors that have the potential to significantly influence turbidity and sedimentation in the water bodies that are receiving the wastewater. Both the increased quantities of sulfate (1,926-2,098 mg L^{-1}) and nitrogen (125-234 mg L^{-1}) contribute considerably to the overall burden of pollution. Because sulfate-reducing bacteria have the ability to create hydrogen sulfide in anaerobic conditions, the presence of increased sulfate concentrations raises important concerns. This is because the production of hydrogen sulfide may result in negative smells and toxicity within the aquatic ecosystem (Gupta & Kumar, 2022; Mishra *et al*., 2021) [7, 13]. The higher chloride concentrations, which range from 3.12 to 5.43 mg L^{-1}, contribute to the overall total dissolved solids and have the potential to alter the osmotic balance of aquatic species, despite the fact that they stay below acceptable limits (Singh *et al*., 2023; Kumar *et al*., 2024) [5, 10].

During the course of the research, it was discovered that all of the heavy metals that were studied had concentrations that were higher than the regulations that were allowed, with some of the metals displaying levels that were higher than the norm. During the period of time spanning from 79.0 to 87.0 mg L^{-1}, it was noted that the concentrations of iron exceeded the limit by a factor that ranged from 26 to 29. There was a substantial difference between the allowed limit and the zinc concentrations, which ranged from 22.0 to 34.0 mg L^{-1}. The difference was between 4.4 and 6.8 times the average. The amounts of lead were highly alarming, with a range that went from 36.54 to 41.23 mg L^{-1}. This suggests a stunning rise that is between 360 and 410 times higher than the CPCB threshold of 0.1 mg L^{-2} that is found in the United States that is observed. It was discovered that the concentrations of cadmium, which ranged from 0.36 to 1.90 mg L^{-1} and exceeded the permissible limits by a factor of 36 to 190, followed a similar pattern. Copper concentrations ranged from 2.57 to 3.28 mg L^{-1}, often remaining at or slightly over the acceptable limit of 3.0 mg L^{-1} during the whole experiment. On the other hand, the levels of nickel were found to be between 5.0 and 6.0 mg L^{-1}, which was much higher than the threshold of 0.5 mg L^{-1} by a factor of 10 to 12, although it was still within the limit that had been set. Chowdhury *et al*. (2021) and Rahman and Singh (2022) [29] say that the high levels of these heavy metals provide significant dangers to the environment as well as to the health of humans. They are resistant to biodegradation and have a tendency to accumulate inside living organisms, which is a trait that is indicative of heavy metals characterized by their tendency to accumulate. According to Ahmed *et al*.'s research from 2020 [4], there is a possibility that this buildup might make its way into the food chain, notably via aquatic creatures and the exploitation of polluted water in agricultural techniques. Lead and cadmium have been found as substances that display carcinogenic and neurotoxic effects, according to research conducted by Goyal *et al*. (2021) and Verma *et al*. (2023) [7, 46]. The presence of these compounds has the potential to bring about major health issues, such as renal malfunction, neurological diseases, and developmental abnormalities in children. The presence of heavy metals in wastewater provides substantial obstacles for biological treatment approaches (Kumar *et al*., 2024; Mishra *et al*., 2023) [16, 22]. Heavy metals may inhibit the activity of microorganisms and reduce the effectiveness of treatment operations. There are heavy metals that may be found in wastewater. The considerable amounts of heavy metal contamination that were found in this investigation are consistent with the aforementioned data that have been published about effluents from a variety of pulp and paper factories. The effluents from paper mills in Uttarakhand had disturbingly high amounts of lead (38-45 mg L^{-1}), cadmium (0.5-2.0 mg L^{-1}), and nickel (4.0-6.5 mg L^{-1}), according to information obtained from study carried out by Sinha *et al*. (2022) [49]. The use of inorganic chemicals during the pulping and bleaching processes is primarily responsible for the presence of heavy metals in effluent from pulp and paper mills, according to the findings of a research that was carried out not too long ago by Bhattacharjee and Das (2024) [2]. Chlorine dioxide, sodium hypochlorite, and a wide variety of metal-based catalysts of varying sorts are all included in this category of chemicals. This conclusion is particularly significant since there is an urgent need for effective methods to remove metals from wastewater before it is discharged into the environment.

The results of the GC-MS analysis conducted on the treated wastewater, revealing the existence of eleven significant degradation intermediates. This chemical serves as a pivotal intermediary in the bacterial degradation of lignin; thus, the identification of Cinnamic Acid ($C_9H_8O_2$) at 12.34 minutes, accompanied by a peak area of 20.0%, holds significant importance. Reddy and Kumar (2024) [33] assert that this product is essential for the degradation of lignin (Figure 1). A considerable quantity of cinnamic acid, comprising twenty percent of the total, indicates that the bacterial consortia possesses the capability to efficiently cleave the lignin polymer at the propyl side chains, thereby releasing this vital intermediate. Ferulic acid ($C_{10}H_{10}O_4$), identified at 14.56 minutes with a peak area of 16.2%, represents another noteworthy intermediate. It serves as an illustration of the disintegration of the lignin polymer, which transpires due to the actions of lignin peroxidase and manganese peroxidase enzymes (Singh *et al*., 2023) [5]. The notable peak area of Ferulic Acid, representing 16.2% of the total, serves as compelling evidence that the lignin structure has been successfully cleaved, specifically the β-O-4 linkages, which are the predominant connections within the lignin polymer (Gupta & Sharma, 2022) [8].

Vanillic Acid ($C_8H_8O_4$) appears at 16.78 minutes with a peak area of 13.6%, while Vanillin ($C_8H_8O_3$) is detected at 18.92 minutes, exhibiting an 11.7% peak area. These compounds are recognized intermediates in the bacterial degradation pathway of lignin, thereby affirming the consortium's capability to effectively cleave the lignin polymer into more simplified aromatic compounds (Kumar *et al*., 2024; Mishra *et al*., 2024) [21, 33]. It can be inferred from the substantial presence of these chemicals (13.6% and 11.7%, respectively) that the bacterial consortium demonstrated efficacy in transforming the intricate lignin polymer into simpler, more biodegradable molecules. Further substantiation of the significant degradation of lignin by the bacterial consortium is evidenced by the identification of Gallic Acid ($C_7H_6O_5$) at 20.45 minutes (9.7% peak area) and 3-hydroxy-4-methylphenol ($C_7H_8O_3$) at 22.34 minutes (9.1% peak area). These two compounds exemplify the degradation of aromatic rings via oxidative

cleavage (Raj *et al*., 2021) [30]. The compound 4-vinyl guaiacol ($C_9H_{10}O_2$), observed at 24.67 minutes with a peak area of 7.0%, serves as a representative degradation product of lignin. It arises as a result of the rupture of the β-O-4 bonds and the decarboxylation of ferulic acid, as articulated by Gupta and Sharma (2022) [8]. Based on their retention durations, the GC-MS chromatogram clearly demonstrates that the 10 degradation intermediates have been distinctly separated from one another. Among the compounds identified, the peak corresponding to Cinnamic Acid stands out as the most significant, appearing at 12.34 minutes. This substantial presence of Cinnamic Acid is unparalleled among the compounds. Ferulic Acid attains the second highest peak intensity of 16.2% at 14.56 minutes, succeeded by Vanillic Acid at 16.78 minutes with an intensity of 13.6%, and Vanillin at 18.92 minutes, which registers an intensity of 11.7%. Ferulic Acid exhibits the second highest peak intensity. The analytical methodology employed to identify degradation intermediates proved to be effective, as evidenced by the chromatogram, which exhibits commendable peak resolution. The progressive reduction in peak intensities from Cinnamic Acid (20.0%) to Succinic Acid (3.3%) suggests a sequential degradation pathway, wherein the initial cleavage products are present in greater abundance compared to the subsequent mineralization products. This transpires in a fashion that aligns with the suggested degradation pathway (Wang *et al*., 2023; Singh *et al*., 2023) [5, 53].



Cinnamic Acid (20.0%) and Ferulic Acid (16.2%) represent the predominant intermediates, comprising over one-third (36.2%) of the total identified degradation products. This is evidenced by the distribution of degradation intermediates, which illustrates that these two acids are the most prevalent intermediates. Due to this considerable abundance, one might infer that the initial phases of lignin degradation, which encompass the fragmentation of the lignin polymer into cinnamic acid derivatives, are remarkably efficient. Vanillic Acid (13.6%), Vanillin (11.7%), and Gallic Acid (9.7%) serve as notable examples of the degradation products that arise from the continued decomposition of lignin derivatives. Conversely, 3-Hydroxy-4-Methoxyphenol (9.1%) and 4-Vinyl Guaiacol (7.0%) serve as examples of intermediates that, while not of paramount importance, merit acknowledgment due to their illustration of the diverse degradation pathways. As noted by Kumar *et al*. (2024) and Mishra *et al*. (2024) [21, 33], the detection of Benzoic Acid (5.0%), Catechol (4.4%), and Succinic Acid (3.3%) at diminished concentrations suggests that the processes of ring cleavage and mineralization are actively occurring. This elucidates the comprehensive degradation pathway, commencing with the breakdown of lignin into fundamental organic acids and ultimately culminating in the production of carbon dioxide and water.

The identification of Benzoic Acid ($C_7H_6O_2$) at 26.89 minutes (5.0% peak area) and Catechol ($C_6H_6O_2$) at 28.12 minutes (4.4% peak area) suggests that the bacterial consortium possesses the ability to cleave the aromatic ring structure via the β-ketoadipate pathway (ortho-cleavage) under the specified experimental conditions (Kumar *et al*., 2024; Mishra *et al*., 2024) [21, 33]. This process is essential for the complete mineralization of aromatic compounds into simpler organic acids that can subsequently enter the TCA cycle (Raj *et al*., 2021). Gupta and Sharma (2022) [8, 30] indicate that the presence of succinic acid ($C_4H_6O_4$) at 30.45 minutes, with a peak area of 3.3%, serves as an indication that the final stages of the degradation pathway have been attained. During these phases, the byproducts of aromatic ring cleavage are subjected to further metabolic processes, ultimately transforming into basic organic acids, which can subsequently be completely mineralized into carbon dioxide and water. The identification of succinic acid, even at a relatively low quantity (3.3%), is especially noteworthy since it reflects the entrance of degradation products into the TCA cycle, demonstrating the full mineralization of the pollutants by the bacterial consortia (Wang *et al*., 2023) [53]. This confirms that the TCA cycle is functioning properly.

The identification of these degradation intermediates substantiates the capability of the bacterial consortium C10 to decompose the intricate lignin polymer into more elementary aromatic compounds, facilitated by the enzymatic actions of lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) (Singh *et al*., 2023; Wang *et al*., 2023) [5, 53]. The suggested degradation pathway offers a visual illustration of the systematic disassembly of

lignin polymers facilitated by these enzymes. This analysis leads to the generation of a diverse array of aromatic intermediates characterized by their low molecular weight. These intermediates are subsequently cleaved via the β-ketoadipate pathway, culminating in the formation of simpler organic acids that are fully mineralized to yield carbon dioxide and water. The identification of numerous intermediates substantiates the complexity of the degradation process and the collaborative function of various enzyme systems working in concert to achieve complete mineralization of the pollutant. Succinic acid is evidence that the degradation process extends to the complete mineralization of aromatic compounds. This guarantees that the pollutants are not simply altered but are entirely eliminated from the wastewater. Succinic acid is found within the wastewater.

Through the activity of ligninolytic enzymes (LiP, MnP, and laccase), the suggested degradation pathway illustrates the methodical disassembly of lignin polymers. This analysis leads to the generation of an array of low molecular weight aromatic intermediates. These intermediates are subsequently cleaved via the β-ketoadipate pathway, culminating in the formation of simpler organic acids that are fully mineralized to yield carbon dioxide and water. Cinnamic Acid (20.0%) and Ferulic Acid (16.2%) emerged as the predominant intermediates, indicating that the initial cleavage of lignin is highly efficient, yielding these cinnamic acid derivatives as the primary degradation products. As noted by Kumar *et al*. (2024) and Singh *et al*. (2023) [33, 40], the observed concentrations of vanillic acid (13.6%), vanillin (11.7%), and gallic acid (9.7%) serve as compelling evidence that the phenylpropanoid units of lignin undergo a gradual degradation through the processes of demethylation and side-chain oxidation. The identification of 3-Hydroxy-4-Methoxyphenol (9.1%) and 4-Vinyl Guaiacol (7.0%) exemplifies the complexity of the degradation pathways, marked by the cleavage of different lignin subunits through an array of enzymatic processes (Gupta & Sharma, 2022) [8].

The confirmation of the β-ketoadipate pathway is evidenced by the detection of Benzoic Acid at a concentration of 5.0% and Catechol at 4.4%. This process is accountable for the cleavage of the aromatic rings of the degradation intermediates through either ortho-cleavage (catechol pathway) or meta-cleavage pathways, as articulated by Raj *et al*. in 2021 [30]. Upon the identification of Succinic Acid, comprising 3.3% of the total, it is established that the cleavage products have integrated into the TCA cycle, thereby concluding the mineralization process. Despite its relatively low concentration of 3.3%, the presence of succinic acid holds considerable significance as it marks the concluding phase of the degradation pathway. At this juncture, the organic carbon undergoes a thorough conversion into carbon dioxide and water, thereby affirming the capability of the bacterial consortium for total pollutant eradication rather than simply transforming them into less harmful variants (Mishra *et al*., 2024; Wang *et al*., 2023) [21, 53]. Prior investigations into the degradation of bacterial lignin have identified analogous intermediates and procedural pathways (Kumar *et al*., 2024; Singh *et al*., 2023; Wang *et al*., 2023) [10, 40]. This pathway aligns with those studies and is in agreement with their findings.

There exists considerable evidence indicating that the bacterial consortia C10 effectively degrades the intricate organic contaminants present in the effluent from pulp and paper mills. This evidence is substantiated by the comprehensive identification of degradation intermediates. The progressive character of the degradation process is evident in the existence of numerous intermediates at different concentrations. This aligns with the observation that the initial depolymerization processes occur at a faster rate than the later mineralization phases, resulting in the buildup of intermediate compounds (Kumar *et al*., 2024; Singh & Patel, 2024) [24, 33]. The assessment of Succinic Acid indicates that the degradation process encompasses the complete mineralization of the aromatic compounds, thereby affirming that the pollutants are not merely altered but are wholly eradicated from the effluent. The identification of these intermediates provides a robust scientific basis for enhancing the treatment process and for assessing the efficacy of the bioremediation method. This serves to guarantee that the treated effluent adheres to the necessary discharge requirements (Wang *et al*., 2023; Gupta *et al*., 2024) [10, 53].

Based on the enzyme activities detailed in Section 4.3, the consortium C10 exhibited significant levels of lignin peroxidase activity (4.12 U/mL), manganese peroxidase activity (24.56 U/mL), and laccase activity (26.43 U/mL). The results of the GC-MS analysis of biodegraded products align with the observed enzyme activities. The identification of specific intermediates, including cinnamic acid and ferulic acid, substantiates the involvement of these enzymes in the preliminary cleavage of the lignin polymer. Conversely, the identification of vanillic acid, vanillin, and gallic acid serve as compelling evidence for the occurrence of subsequent demethylation and side-chain oxidation reactions (Singh *et al*., 2023) [5]. The identification of benzoic acid and catechol confirms the participation of ring-cleavage enzymes in the degradation pathway. Conversely, the identification of succinic acid reinforces the involvement of the TCA cycle in the comprehensive mineralization of organic carbon (Kumar *et al*., 2024; Mishra *et al*., 2024) [21, 33]. A connection between enzyme activity and degradation intermediates offers confirmation of the enzymatic basis for the degradation process and validates the proposed pathway for bacterial degradation of lignin in pulp and paper mill effluent.

Conclusion

The examination of wastewater from M/s K.R. Pulp and Papers Limited has unveiled that the effluent is not merely contaminated but represents a significant environmental challenge, with pollutant levels surpassing all established safety limits, necessitating prompt and resolute action. The elevated lignin concentration, surpassing 39,000 mg L^{-1}, exceeds permissible thresholds, leading to a toxic effluent that endangers aquatic ecosystems and exhibits resistance to natural degradation processes. ratio of BOD/COD beneath 0.2 signifies that the organic matter is intricately complex and chemically stable, rendering conventional biological treatment facilities ineffective in its removal, thereby allowing toxins to persist in the environment for extended periods. The GC-MS analysis unveiled a complex mixture of enduring organic contaminants, comprising Nonacosane, Heptacosane, Octadecanoic Acid, Phthalic acid esters, and a potentially mutagenic nitrogen compound. Many of these substances inflict lasting ecological harm and exhibit remarkable resistance to degradation in natural

environments. The analysis demonstrates that the mill is releasing a hazardous time bomb into the receiving waterways through its wastewater treatment methods. The scientific community should prioritize the development of economically viable bioremediation technologies, investigate the potential for resource recovery from contaminants, and establish comprehensive monitoring programs for long-term ecosystem recovery.

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