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Research Article

GC-MS Investigation of Kraft Lignin Degradation by the Newly Isolated Bacterial Strain *Pusillimonas noertemannii* Derived from Coir Pith

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Abstract

This study investigates the microbial degradation of kraft lignin (KL) by *Pusillimonas noertemannii*, focusing on the transformation of lignin into valuable monomers. Coir pith, a lignocellulosic biomass by-product, serves as a sustainable resource for producing KL. The research explores the effects of KL concentrations (4000 ppm and 8000 ppm) on degradation products. Using gas chromatography-mass spectrometry (GC-MS) analysis, a diverse array of compounds was identified, including key products such as Cyclohexane, 1,3,5-triphenyl- and various phenolic derivatives. At 4000 ppm, significant monomers included 3-Tetradecene and 2,4-Di-tert-butylphenol, while at 8000 ppm, Styrene emerged as a prominent product, indicating enhanced metabolic pathways at higher lignin concentrations. The study reveals *Pusillimonas noertemannii's* ability to convert complex lignin into aromatic compounds and long-chain hydrocarbons. This highlights its potential for bioremediation and lignin valorization. The persistence of aromatic compounds suggests applications in polymer synthesis and plastic stabilization. Overall, the research provides insights into microbial lignin degradation and lays the groundwork for optimizing conditions to enhance microbial activity in lignin valorization.

Key words: kraft lignin, *Pusillimonas noertemannii, Biodegradation, G*as chromatography-mass spectrometry

Introduction

The industrial reliance on non-renewable fossil fuels for synthetic polymers and chemicals has raised significant environmental concerns, leading to a search for sustainable alternatives from renewable resources [1]. Lignocellulosic biomass, particularly coir pith—a by-product of coconut husk processing—presents a promising solution [2]. Traditionally considered waste, coir pith is rich in lignin, cellulose, and hemicellulose, making it a valuable resource for producing sustainable materials [3]. Valorizing coir pith addresses waste management challenges while contributing to the production of bioplastics and biofuels, thereby fostering a circular economy [4]. By extracting lignin from coir pith, researchers aim to mitigate environmental impacts and develop eco-friendly materials that align with sustainability goals. Coir pith's composition—approximately 30% lignin and 50% cellulose—enhances its applicability, particularly in agriculture [5]. Lignin provides structural integrity, while cellulose contributes to moisture retention and soil aeration [6]. Additionally, coir pith's high cation exchange capacity improves soil fertility and reduces fertilizer needs. To maximize coir pith's potential, pretreatment strategies are employed to depolymerize lignin, facilitating its degradation by specific microbes [7]. This process exposes cellulose and hemicellulose for microbial consumption and helps extract valuable intermediates, transforming waste into useful resources that bolster agricultural productivity and environmental conservation [8]. For example, hydrogen peroxide pretreatment effectively solubilizes lignin, producing coir pith black liquor, which can be recovered through acid precipitation, promoting industrial reuse [9]. Kraft lignin, a byproduct of the kraft pulping process, shares similar recovery processes and underscores the importance of pretreatment for optimizing lignin extraction from lignocellulosic biomass [10]. Microbial degradation of lignin, primarily by fungi and certain bacteria, is crucial for converting biomass into simpler compounds [11,12]. While fungi, especially white-rot fungi, produce oxidative enzymes like lignin peroxidases and laccases that efficiently degrade lignin, bacterial strains, such as Sphingomonas paucimobilis, also show potential, albeit with lower efficiency [13]. As the demand for sustainable materials increases, bioplastics derived from renewable biomass sources offer a promising alternative to petroleum-based plastics [14]. These bioplastics can be synthesized by microorganisms, including bacteria, fungi, and algae, through the production of biopolymers such as polyhydroxyalkanoates (PHAs), polylactic acid (PLA), and polyhydroxybutyrate (PHB) [15, 16]. These biopolymers are produced via metabolic processes under nutrient-limited conditions, with PHAs recognized for their versatility and biodegradability, making them suitable for various applications, from packaging to medical devices [17]. Researches focused on harnessing lignin from coir pith and utilizing bacterial strains like Bacillus megaterium and Pseudomonas aeruginosa to convert kraft lignin into industrially useful PHB [18]. Additionally, this research aims to explore the lignin-degrading capabilities of bacteria such as *Pusillimonas noertemannii*, investigating their enzymatic activities and the conversion of lignin into value-added products. By establishing a framework for valorizing coir pith lignin, the research seeks to provide insights into economically viable methods for producing bioplastics while addressing environmental concerns related to plastic waste. Understanding the mechanisms of lignin degradation is critical, given its resistance to microbial breakdown due to its complex structure. This investigation will also identify low molecular weight aromatic compounds resulting from kraft lignin degradation, further enhancing the understanding and utilization of coir pith waste in sustainable practices.

Materials and Methods

Kraft lignin production from coir pith and isolation of lignolytic bacteria

For analysis, a sample of coir pith (CP) was taken from a landfill in Tumkur, Karnataka, India, and kept at 4°C. Ten grams of CP were ground, treated with five milliliters of 1% sulfuric acid, heated to 80°C for half an hour, cooled, and then treated with one hundred milliliters of 4% sodium hydroxide, which was then boiled for half an hour in order to extract KL [19]. After being autoclaved, the filtrate was kept in an airtight container. Five grams of CP were added to Minimal Salt Media-Lignin (MSM-L) and incubated for seven days at 35°C in the dark on a rotary shaker set to 120 rpm in order to isolate lignolytic bacteria. Using serial dilutions and plating on MSM-L agar plates, pure cultures were produced [20].

Degradation of KL with lignolytic bacteria

The characterized bacteria i.e *Pusillimonas noertemannii* (ID PP528199.1.) was taken in nutrient broth. To investigate lignin degradation using inoculated bacteria in the presence of concentrated Kraft lignin, the procedure began by preparing Minimal Salt Medium (MSM) [21]. The medium was formulated with essential nutrients, including potassium phosphate, magnesium sulfate, ammonium nitrate, and appropriate carbon sources. After preparing the MSM, bacterial cultures were grown in a separate flask until they reached the logarithmic phase of growth. Following this, aliquots of the actively growing bacterial culture were inoculated into flasks containing MSM supplemented with 4000 ppm and 8000 ppm of Kraft lignin, ensuring that each flask was mixed thoroughly to achieve uniform distribution of the lignin [22]. The flasks were then incubated in the dark at 35°C with shaking at 120 rpm for a duration of 7 days to promote lignin degradation. Throughout the incubation, samples were periodically taken to monitor bacterial growth and assess the degradation of lignin. Without the inoculation of bacteria, The MSL media was used as control in GCMS analysis

GC-MS analysis

The acidified supernatants from control and treated samples were extracted with three volumes of ethyl acetate [22]. The organic layer was collected, dewatered over anhydrous sodium sulfate, and filtered through Whatman no. 54 filter paper before being dried under nitrogen gas. The residues were analyzed as trimethylsilyl (TMS) derivatives using Lundquist and Kirk's method (1971). Specifically, 100 µL of dioxane and 10 µL of pyridine were added to the residues, followed by silylation with 50 µL of BSTFA and TMCS. The mixture was heated at 60°C for 15 minutes with periodic shaking. A 1 µL aliquot was injected into a gas chromatography-mass spectrometry (GC-MS) system, utilizing a PE-5MS capillary column and helium as the carrier gas at 1 mL/min. The column temperature program began at 50°C for 5 minutes, ramped to 300°C at 10°C/min, and held for 5 minutes. Mass spectra were recorded in full-scan mode (30–550 m/z) at 70 eV. Standard monomeric phenolic compounds were also derivatized and analyzed. Identification of low molecular weight lignin-related compounds was performed by comparing mass spectra with the NIST library and matching retention times with authentic compounds.

Results

KL (4000ppm) derived monomers obtained by the degradation of *Pusillimonas noertemannii*

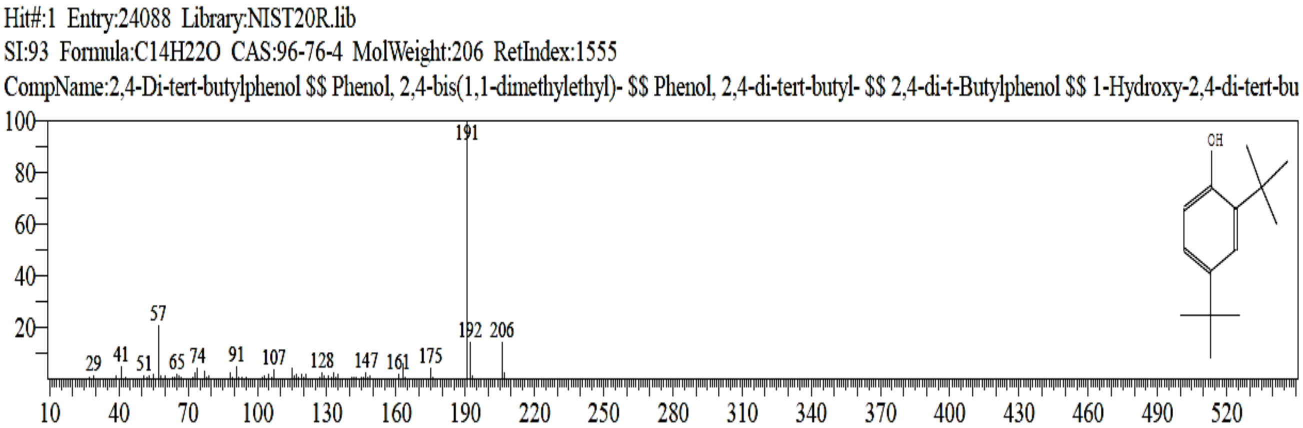
In this study, we investigated the degradation of 4000 ppm concentrated kraft lignin by the bacterium *Pusillimonas noertemannii*. The analysis of the degradation products revealed a diverse range of monomers, with varying retention times and peak areas indicative of their relative abundances **(Table 1)**. The primary degradation products included 3-Tetradecene and 9-Octadecene, with retention times of 6.618 and 8.220 minutes, respectively, accounting for

2.16% and 2.79% of the total area. Other notable compounds included Cycloheptasiloxane, tetradecamethyl- and 2,4-Di-tert-butylphenol, with peak areas of 148,784 and 134,807, representing 2.91% and 2.64% of the total area. The most significant product was Cyclohexane, 1,3,5-triphenyl, which showed an impressive peak area of 2,785,116, comprising 54.46% of the total area, indicating it was a major product of lignin degradation. Further analysis identified Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1), which contributed 13.70% of the total area, highlighting its significance in the degradation pathway. The results demonstrated that *Pusillimonas noertemannii* effectively converted kraft lignin into various monomers, suggesting its potential for bioremediation and lignin valorization.

Concentration (ppm)	**Monomer**	**Retention Time (minutes)**	**Peak Area (%)**
4000	3-Tetradecene	6.618	2.16
	9-Octadecene	8.220	2.79
		13.858	6.56
	Cyclohexane, 1,3,5-triphenyl-	15.326	54.46
	Phenol, 2,4-di-tert-butyl	9.018	2.64
	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	11.563	2.51
	Eicosane	10.164	1.74
		9.531	3.46
	1-Heptadecene	10.678	2.61
8000	Ethylbenzene	2.989	0.54
	Styrene	3.339	4.50
		13.858	16.64
	Cyclohexane, 1,3,5-triphenyl	14.322	25.32
	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, trans	10.166	1.71
		10.578	6.79
	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite	20.493	5.19
	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	Various	Significant Peaks

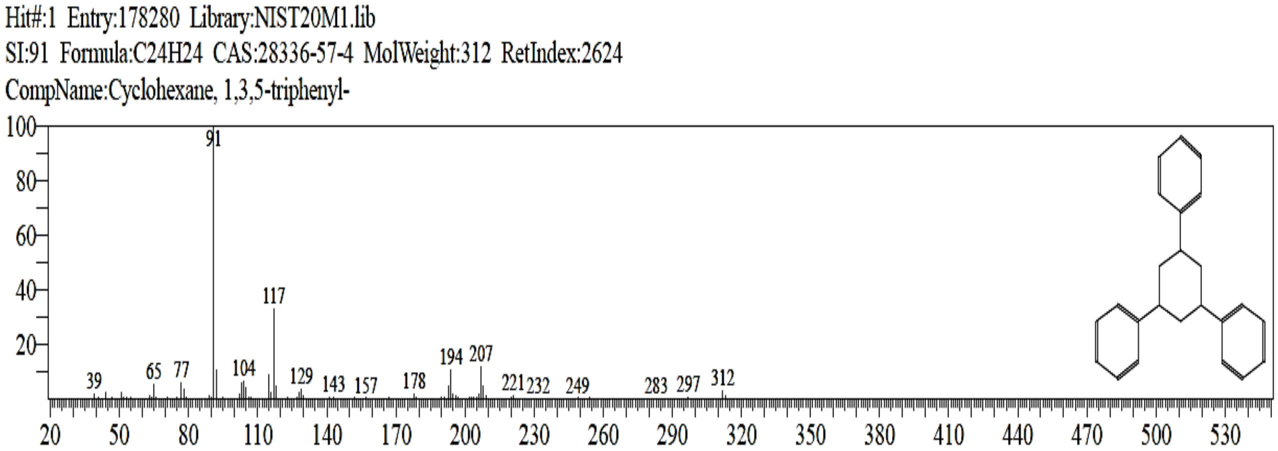
Table.1 Biodegradation by lignolytic bacteria different concentrations of kraft lignin

Fig: a



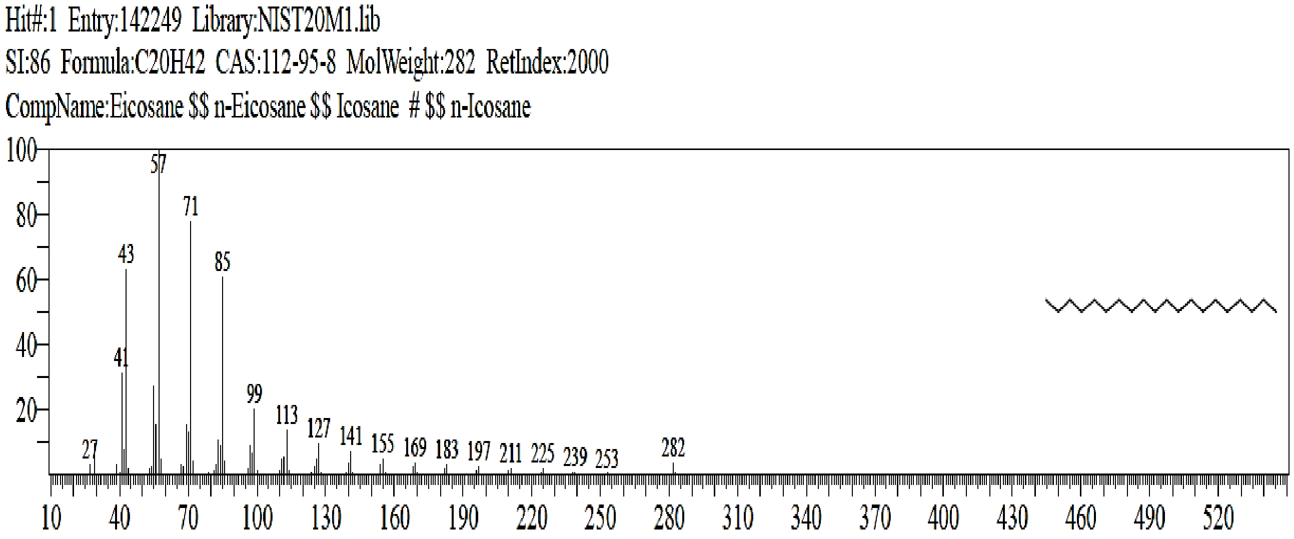
Hit#:1 Entry:24088 Library:NIST20R.lib
SI:93 Formula:C14H22O CAS:96-76-4 MolWeight:206 RetIndex:1555
CompName:2,4-Di-tert-butylphenol $$ Phenol, 2,4-bis(1,1-dimethylethyl)- $$ Phenol, 2,4-di-tert-butyl- $$ 2,4-di-t-Butylphenol $$ 1-Hydroxy-2,4-di-tert-bu

Fig: b



Hit#:1 Entry:178280 Library:NIST20M1.lib
SI:91 Formula:C24H24 CAS:28336-57-4 MolWeight:312 RetIndex:2624
CompName:Cyclohexane, 1,3,5-triphenyl-

Fig: c



Hit#:1 Entry:142249 Library:NIST20M1.lib
SI:86 Formula:C20H42 CAS:112-95-8 MolWeight:282 RetIndex:2000
CompName:Eicosane $$ n-Eicosane $$ Icosane # $$ n-Icosane

Figure 1. a,b,c Monomers commonly present in different concentrations of KL after degraded by lignolytic bacteria

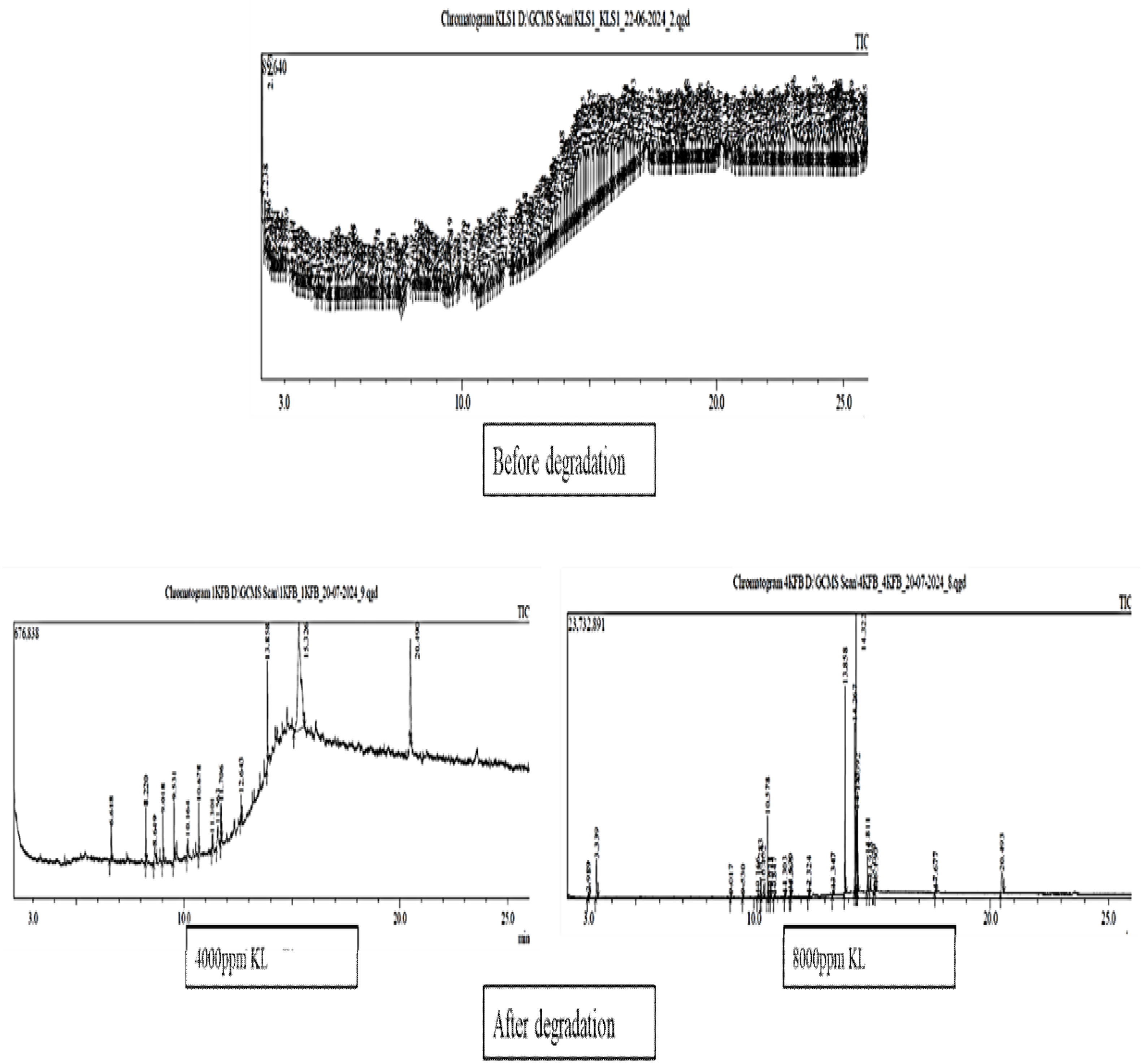


Figure 2. GCMS report. a- Before degradation, b-after degradation with 4000ppm Kl, c- after degradation with 8000ppm KL

KL (8000ppm) derived monomers obtained by the degradation of *Pusillimonas noertemannii*

The degradation of 8000 ppm concentrated kraft lignin by the bacterium *Pusillimonas* *noertemannii* resulted in the identification of several monomeric compounds, showcasing the bacterium's capability to transform lignin into simpler, valuable substances. Notably, Styrene emerged as the predominant product, exhibiting a significant peak area of 5,370,802, which accounted for 4.50% of the total area **(Figure 2)**. This suggests that *Pusillimonas noertemannii* effectively converted lignin into styrenic compounds. The analysis also revealed various other compounds, including Cyclohexane, 1,3,5-triphenyl- and multiple forms of (2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, with the latter demonstrating a peak area of 30,204,256, representing 25.32% of the total area. These findings indicate a substantial production of specific phenolic compounds during the degradation process. Additional notable products included n-Hexadecanoic acid and Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite, further illustrating the diversity of monomers generated. The presence of several Eicosane forms suggests that *Pusillimonas noertemannii* can degrade long-chain hydrocarbons effectively.

Monomers commonly present in both the concentrations

The analysis identified several compounds that could be associated with kraft lignin monomers **(Figure 1)**. 2,4-Di-tert-butylphenol was noted as a phenolic derivative, related to the structural motifs of lignin monomers, particularly those derived from guaiacyl and syringyl units [24]. Additionally, Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite was recognized as another phenolic structure that could indicate the presence of lignin-derived compounds. Furthermore, Cyclohexane, 1,3,5-triphenyl- was observed, suggesting the presence of aromatic rings characteristic of lignin, even though it is not a direct lignin monomer. The compound Styrene was also identified as an aromatic hydrocarbon that could relate to the monomeric structure of lignin [25]. Lastly, while Eicosane and other alkenes, such as 1-Heptadecene, were recognized, they were generally not directly associated with lignin monomers; instead, they were considered potential products of lignin degradation or related organic matter [26].

Comparison of degradation pathway with two different concentration of KL (4000ppm and 8000ppm)

The degradation pathways of 4000 ppm and 8000 ppm kraft lignin by *Pusillimonas noertemannii* revealed distinct differences in the types and quantities of products formed, reflecting the influence of lignin concentration on microbial activity and metabolic outcomes. At 4000 ppm, the pathway indicated a progression from kraft lignin to intermediate products such as 3-Tetradecene and 9-Octadecene, leading to monomers like Cycloheptasiloxane and 2,4-Di-tert-butylphenol. This pathway culminated in the major products Cyclohexane, 1,3,5-triphenyl-, and Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite, demonstrating the bacterium's ability to convert complex lignin structures into a diverse array of compounds. In contrast, at 8000 ppm, the degradation pathway shifted towards the production of Styrene and Cyclohexane, 1,3,5-triphenyl- as key intermediate products. The resulting monomers, including (2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide and n-Hexadecanoic acid, indicated a more extensive breakdown of lignin-derived materials. The major products from this concentration included Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite, which suggests that higher lignin concentrations facilitated the production of more complex and valuable phenolic compounds. Overall, the increase in lignin concentration from 4000 ppm to 8000 ppm appeared to enhance the bacterium's metabolic pathways, leading to the generation of a greater diversity of monomers and a higher yield of specific products. This highlights the potential for *Pusillimonas noertemannii* in bioremediation and lignin valorization, with varying concentrations offering unique profiles of degradative outcomes.

Discussion

The GC-MS analysis of Kraft lignin degraded by *Pusillimonas noertemannii* across various concentrations revealed critical insights into the degradation products, particularly focusing on the prevalence of Cyclohexane, 1,3,5-triphenyl-, the persistence of phenolic compounds, and the generation of long-chain hydrocarbons. Cyclohexane, 1,3,5-triphenyl- emerged as the predominant compound in both the 4000 ppm and 8000 ppm analyses, showcasing peak areas that highlighted its significant accumulation. This abundance underscores a robust microbial pathway leading to aromatic structures, which are often stable and complex [27, 28]. The presence of this compound indicates that *Pusillimonas noertemannii* effectively metabolizes Kraft lignin, breaking it down into valuable aromatic intermediates [29,30]. The structural complexity of Cyclohexane, 1,3,5-triphenyl- suggests its potential applications in various industries, particularly in the synthesis of polymers and fine chemicals [31]. Furthermore, the analysis identified phenolic compounds, such as 2,4-Di-tert-butylphenol and Phenol, 2,4-bis(1,1-dimethylethyl)-, which confirmed the retention of aromatic structures post-degradation. The presence of these phenolic compounds indicates that even after microbial treatment, aromatic rings remain intact, which can be advantageous for specific applications [32,33]. For instance, phenolic compounds are known for their antioxidant properties and are commonly utilized as stabilizers in plastics and rubber, offering significant commercial value [33]. In addition to aromatic structures, the degradation process also resulted in the formation of long-chain hydrocarbons, including Eicosane and Tetradecene. These compounds reflect the microbial ability to produce aliphatic structures alongside aromatic ones [36]. Long-chain hydrocarbons have valuable applications as intermediates in the production of surfactants, lubricants, and fuels [36,37]. The dual production of aromatic and aliphatic compounds during the degradation of Kraft lignin suggests that *Pusillimonas noertemannii* possesses a versatile metabolic pathway, allowing for the conversion of complex lignin structures into a variety of useful monomers [39]. The analysis demonstrated that varying concentrations of kraft lignin (4000 ppm and 8000 ppm) influenced the types and quantities of degradation products generated, showcasing the bacterium's adaptability and metabolic versatility [38]. At both concentrations, key compounds such as Cyclohexane, 1,3,5-triphenyl- and phenolic derivatives like 2,4-Di-tert-butylphenol were prominently featured, indicating the retention of complex aromatic structures post-degradation. The identification of styrenic compounds and long-chain hydrocarbons further underscored the potential for *Pusillimonas noertemannii* to convert lignin into commercially valuable products for use in various industrial applications, including the synthesis of polymers, biofuels, and stabilizers for plastics [40]. The findings highlight not only the bacterium's efficacy in lignin valorization but also its potential role in bioremediation strategies targeting lignin-rich waste streams. Overall, this research contributes to the understanding of microbial lignin degradation pathways and emphasizes the significance of *Pusillimonas noertemannii* in developing sustainable approaches for lignin waste management and the production of high-value bio-based materials. Future investigations could explore optimizing conditions for enhanced microbial activity and further elucidate the metabolic pathways involved in lignin degradation.

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Conflict of Interest: The authors declare no conflict of interest.

Declaration of Non-Use of AI:

The authors confirm that no artificial intelligence tools were used in this study.

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