Journal of Advances in Microbiology

21(3): 36-45, 2021; Article no.JAMB.66263
ISSN: 2456-7116

Studies on Lignin Degradation Activity by *Pseudomonas aeruginosa* Isolated from Kware Lake

Muhammad Hizbullahi Usman[1*] and Aliyu Ibrahim Dabai[2]

[1]Department of Microbiology, Sokoto State University, Nigeria.
[2]Department of Microbiology, Usman Danfodiyo University, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2021/v21i330334
Editor(s):
(1) Dr. Foluso O. Osunsanmi, University of Zululand, South Africa.
Reviewers:
(1) Daniel Benharroch, Ben-Gurion University of the Negev, Israel.
(2) Pavithra S, Vels University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/66263

Original Research Article

Received 01 January 2021
Accepted 05 March 2021
Published 30 March 2021

ABSTRACT

Background: Wood is naturally degraded by wood degrading microorganisms and modified and partly degraded residual of lignin goes into soil. It is an amorphous and complex aromatic compound. In the plant cells, lignin can be converted via phenylalanine and tyrosine by transamination. Africa generates a huge amount of waste from agricultural and household activities. This huge amount of waste can be exploited as a sustainable raw material for many industrial processes other than just simply burning it as a solid fuel.
Aims: The study aimed at isolating and screening *Pseudomonas aeruginoas* for lignin degradation.
Methods: The bacterium was characterized and identified according to morphological and biochemical characteristics. The bacterial DNA was extracted using DNA isolation kit and used for molecular analysis. Four (4) dyes such as Methylene blue, Congo red, iodine and Malachite green were screened on bacterial isolate for its ability to decolorize the dyes. The effect of some growth parameters such incubation time, temperature, pH and agitation were studies on the isolate for lignin degradation as well as bacterial biomass production.
Results: The effect of some growth parameters such incubation time, temperature, pH and agitation were studies on the isolate for lignin degradation as well as bacterial biomass production. From the results, it was observed that the isolate showed higher zone of inhibition on Congo red (14.00 mm) and Methylene blue (10.00 mm), while no decolorization was observed on Malachite green. The growth parameters studied indicated the optimum condition required for both lignin degradation and

**Corresponding author: E-mail: muhammadhiz04@gmail.com;*

bacterial biomass production as follows; 48 hours (65%, 0.41g), 40^{o}C (77%, 0.31g), pH 7 (58%, 0.26g) and 100rpm (72%, 0.42). The FTIR revealed two peaks at 3375 cm^{-1} which attributed to O-H stretching while the second peak 1687.5 cm^{-1} corresponded to C=O stretching.
Conclusion: The results of GC-MS detected the presence of 2,5-Hexanediol, 2-Octynoic acid, n-Hexadecanoic acid and i-Propyl9,12-octadecenadienoate.

Keywords: Wood; lignin; dyes; Pseudomonas aeruginosa and degradation.

1. INRODUCTION

Agricultural and wood residues produced after harvesting and processing of plants are abundant biomass on earth, and these biomass resources have remarkable energy capacity [1]. Several tons (estimated to be around 10–50 billion produced annually worldwide of these wastes are produced annually from corn, wheat, soybean, timbers, etc., and are mainly composed of lignin, cellulose and hemicelluloses [2]. The lignocellulose biomass, recognized as the most abundant biopolymer on earth, is a predominant component of the cell wall of plants. It is an inexhaustible raw material for biofuel production. The plant cell wall is a heterogeneous complex of carbohydrate polymers (cellulose and hemicellulose) and an aromatic polymer (lignin) [3,4].

Lignin, among the three major wood components, represents most difficulty for microbial degradation because of its complex structure and because cross linking to hemicellulose makes wood such a compact structure. Nevertheless, wood is naturally degraded by wood degrading microorganisms and modified and partly degraded residual of lignin goes into soil. It is an amorphous and complex aromatic compound with a substantial molecular weight, which is mainly composed of three main lignin precursors (p-hydroxyphenyl, guaiacyl, and syringyl units) linked via C-C or C-O bonds formed by radical coupling reactions. In the plant cells, lignin can be converted via phenylalanine and tyrosine by transamination [5,6]. Lignin gives the plant a rigid structure that provides protection against the hydrolysis of cellulose and hemicellulose. In spite of the plethora of lignocellulose in nature, the expensive cost of hydrolyzing them into smaller monosaccharides has made the cost of using them unappealing. Therefore, this has led to the search for cheaper means of hydrolyzing lignocellulose biologically. Lignocellulose is used in many industrial processes for the production of chemicals, fuels, polymer precursors, paper and pulp, food and flavor compounds [7].

Due to the recalcitrant nature of lignin toward degradation, the conversion of lignocellulose to biofuels and other renewable energy resources involves several pretreatment processes, such as biological, chemical, mechanical and thermal processes. The biological pretreatment process involves using microorganisms for the conversion and degradation of lignocelluloses streams into sugars to produce biofuel [3]. Naturally occurring bacterial species from different niches have developed cellular mechanisms to acquire energy from plant biomass through the production and release of carbohydrate-active enzymes [8]. These enzymes degrade plant cell, which, as a result, synthesize monosaccharides that can be used biofuels and other value-added products [1].

Numerous microorganisms such as bacteria, fungi, actinomycetes, and cyanobacteria have been reported which are capable to degrade lignocellulosic waste and other wood containing fibers. There is a growing concern among researchers to isolate bacteria and fungi directly from lignocellulosic waste contaminated sites due to acclimatized microbial genome pool with degrading enzyme-producing capabilities [9]. Culture-dependent screening detects lignin-degrading prokaryotes by their growth on lignin model substrates and lignin derived molecules. Inoculation of lignin-rich media with environmental samples enables the enrichment of ligninolytic strains, hence facilitating their isolation and characterization [10]. Soils, waste water from the paper industry, and decaying lignocellulosic materials have frequently been chosen as original ecosystems for ligninolytic strains [11,12]. While some other interesting environments remain to be explored, such as waterlogged archaeological wood that suffers from microbial degradation by uncharacterized erosion, cavitation, and tunneling bacteria [13].

Africa generates a huge amount of waste from agricultural and household activities. This waste can be exploited as a sustainable raw material for many industrial processes other than just

simply burning it as a solid fuel. Therefore, the purpose of this study was to investigate whether *Pseudomonas aeruginosa* isolated from Kware Lake could be used for degradation of lignin.

2. MATERIALS AND METHODS

2.1 Sample Collection

Soil sediment sample was collected from Kware Lake in the morning. Ten grams (10 grams) of the soil sediments was collected each from five different locations at the deep bottom of Lake using polyethene bags. The samples were immediately transported to Laboratory for further analysis.

2.2 Isolation of Lignin Degrading Bacteria

The isolation of lignin degrading bacteria was done using minimal salt medium as described by Hassan and El-hannafy [14]. After serial dilution, 0.5% soil suspension was inoculated in mineral salt media (MSM). One gram of wood powdered was added to 50 mL and incubated at 30°C with shaking for Ten days. After incubation, the isolates were sub-culture into freshly prepared nutrient agar. Bacterial colonies was selected according to their shape, growth and sub cultured again using Luria Bertani medium and incubated at 30°C for 48 hours to achieved maximum growth rate and subsequently used for selection of bacterial isolates capable to utilize lignin as a sole carbon source.

2.3 Morphological and Biochemical Identification of Bacteria Isolate

Macroscopic observation on the nature of color, shape, size, elevation and surfaces of the isolates were observed. The characterizations of isolates were done using standard procedure described by Cheesbrough [14]; Oyeleke and Manga [15]. The bacterial isolates were characterized based on the biochemical reactions. The tests employed in this study were motility, gas production, starch hydrolysis Methyl Red, Vogues Proskauer, catalase, and urease. Morphological characterization for bacterial and fungal isolate was carried out through gram staining.

2.4 Molecular Identification of Bacteria

The genomic DNA of bacteria was extracted by the DNA Isolation Kit according to the protocol described by Aiya [16]. PCR reaction was carried out in 200 µl reaction containing template DNA, primers, dNTPs and Taq polymerase. The reaction was cycled 35 times as 94^{o}C for 30 seconds, 58^{o}C for 30 seconds and 72^{o}C for 1 minute 30 seconds, followed by final extension at 72^{o}C for 10 minutes. The PCR products were analyzed on 1% agarose gel in 1x TBE buffer, run at 100V for 45 hours. Gels were stained with ethidium bromide and photographed. The sequence was aligned with corresponding sequences of 16SrDNA from the database using BLAST from the website http://www.ncbi.nlm.nih.gov/blast (Altschul *et al.,* 1997). Multiple alignments were generated by the CLUSTAL W program and phylogenetic tree was constructed by neighbor-joining algorithm using MEGA 6 Software [17].

2.5 Effect of Some Growth Parameters on Crude Lignin Degradation and Bacterial Biomass Production

The effect of growth parameters were determined as in one factor one time method as described by Murugesan and Vembu [18] with slight modification. The growth parameters include incubation time (24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours), temperature (25^{o}C, 30^{o}C, 35^{o}C, 40^{o}C and 45^{o}C), pH (6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 and 10) and agitation rate (50rpm, 100rpm, 150rpm and 200rpm). The lignin degradation rate was expressed in percentage and bacterial biomass was estimated. All the experiments were carried out in triplicates and the results were expressed as mean ± standard deviation

2.6 Degradation of Lignin by *Pseudomonas aeruginosa* under Optimum Growth Conditions

A twenty four (48) hours old culture of *Pseudomonas aeruginosa* was inoculated into 50 ml of mineral salt medium (MSA) incorporated with 5% lignin extract from plant. The growth conditions such as incubation time, temperature, pH and agitation whose show highest activity were chosen and used in this study. After the period of incubation, the preparation was centrifuge at 5,000 rpm and filtered using Whatman filter 12. The supernatant was used for FTIR and GC-MS analysis.

2.7 Fourier Transform Infrared Analysis

For the structural characterization, Fourier Transform Infrared Spectroscopy (FTIR) was performed. The pigments obtained were concentrated using a vacuum concentrator for 24

hours and the powder was mixed with small amount of KBr and mixed thoroughly. The preparation was then pressed in a sample holder and analyzed by computerized Fourier Transform Infrared Spectroscopy system, which generated the absorbance spectra showing the unique chemical bonds and the molecular structure of the sample material [19].

2.8 Gas Chromatography- Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis was performed using GC-MSQP2010 Plus (Shimadzu, Japan) and Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following; Column Elite-1 fused silica capillary column (30m x 0.25mm 1D x µl df, composed of 100% Trisil). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2 µL was employed (Split ratio of 20:0) injector temperature 250˚C; ionsource temperature 200˚C. the oven temperature was programmed from 60.0 (for 0.00 minute) with an increase of 160˚C (Isothermal for 2.00 minutes) ending with a 2.00 minutes isothermal at 280˚C. Mass spectra were taken at 70eV; a scan interval of 0.5s and fragments from 45 to 700Da. Total GC running time was 19 minutes. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas, Software adopted to handle mass spectra and chromatogram was a turbomass. The detection employed the NIST Ver.2.0 year 2009 library [20].

2.9 Statistical Analysis

Data obtained were analyzing using SPSS (Version 20) statistical package. Descriptive statistic through simple graphs, tables and charts were used for presentation of data.

3. RESULTS AND DISCUSSION

A bacterium with potential to degrade the lignin was isolated from Kware Lake. The isolate was screened based on the colonial, morphological and biochemical characterization and identified as *Pseudomonas aeruginosa* according to Bergey's Manual of Determinative Bacteriology. Soil served as a potential source for isolation of lignin degradation bacteria as plant biomass decomposed and releasing intermediates which served as a carbon source for growth and metabolisms. Jiang-Hao et al. [13] reported that soils, waste water from the paper industry, and decaying lignocellulosic materials have frequently been chosen as original ecosystems for ligninolytic strain of bacteria.

Table 1. Morphological biochemical characterization of bacteria isolate

Characteristics	Isolate
Colonial characteristics	Negative Bacillus
Gram reaction	Green
Pigmentation	Irregular
Shape	Flat
Elevation	Smooth
Surface	
Biochemical characterization	+
Catalase	-
Coagulase	-
Methyl Red	-
Voges-Proskauer	-
Indole	-
Urease	+
Citrate	-
Glucose	-
Lactose	-
Sucrose	+
Gas	-
H_2S Production	

Table 2 presented the result of screening of *Pseudomonas aeruginosa* and its ability to decolorized dyes thereby producing clearing zone of inhibition. Four dyes including Congo red, methylene blue, iodine and malachite green were used in this study. It was observed that the isolate effectively decoulorized Congo red, producing 14.00 mm zone of inhibition than the other dyes used. Similarly, 10.00 mm inhibition zone was observed on Methylene blue while no zone of inhibition was observed on Malachite green. Decolorization of dyes with aromatic chromophores has been used as an indicator of aromatic-degrading activity, because of its closed structure to that of lignin fragments which is reasonably simple to use and also time saving [13,21]. Patel et al. [22] screened laccase-producing bacteria on the different dye containing agar plate. The isolates PHP7 and PKD5 were showing clear zone in toluidine blue, methylene blue and congo red, while the isolates PHP6, PSG3, PSG9, PPS1 and PPS10 were showing clear zone in only two, toulidine blue and methylene blue. The isolates PHP8 and

PPS4 were showing clear zone in only toluidine blue and PKD9 shows clear zone in only toluidine blue. None isolates showed clear zone in malachite green.

Table 2. Screening of *Pseudomonas aeruginosa* for Laccase Production

Dyes	Zones of Inhibition (mm)
Methylene blue	10.00
Congo red	14.00
Iodine	4.00
Malachite green	0.00

The sequence results of isolate showed 100 percent identity to *Pseudomonas* sp. The phylogenetic tree constructed by MEGA 6 showed that the isolate use in this study was closely related to the *Pseudomonas aeruginosa* strain NR_114471.

The effect of some growth parameters such as incubation time, temperature, pH, agitation played significant role in lignin degradation and bacterial biomass production. The rate of lignin degradation by *Pseudomonas aeruginosa* was affected by incubation time, temperature, pH, agitation. The effects of incubation time on lignin degradation revealed that at 48 hours, the degradation of lignin by *Pseudomonas aeruginosa* was 65% and produced highest biomass of 0.41 gram. The variation of degradation rate and biomass production by the isolate in relation with incubation time might be attributed to nature of growth of organisms, as some bacteria have shorter generation time than others. It was also observed that the rate of lignin degradation increases as the days increases and gradually declined as the organisms reached the maximum degradation and biomass productions. This implies that as the numbers of days increased, the number of bacteria also increased which would increase the growth and lignin degradation.

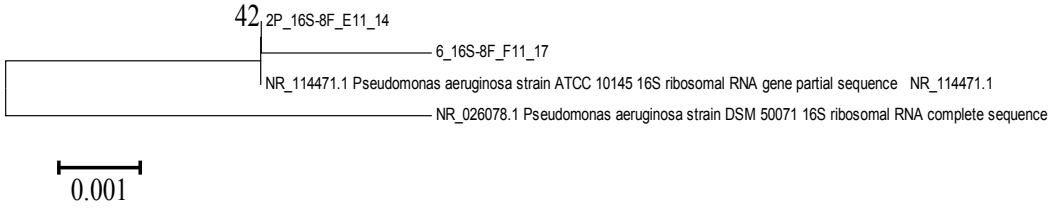

Fig. 1. The phylogenetic tree by neighbor joining of *Pseudomonas* sp.

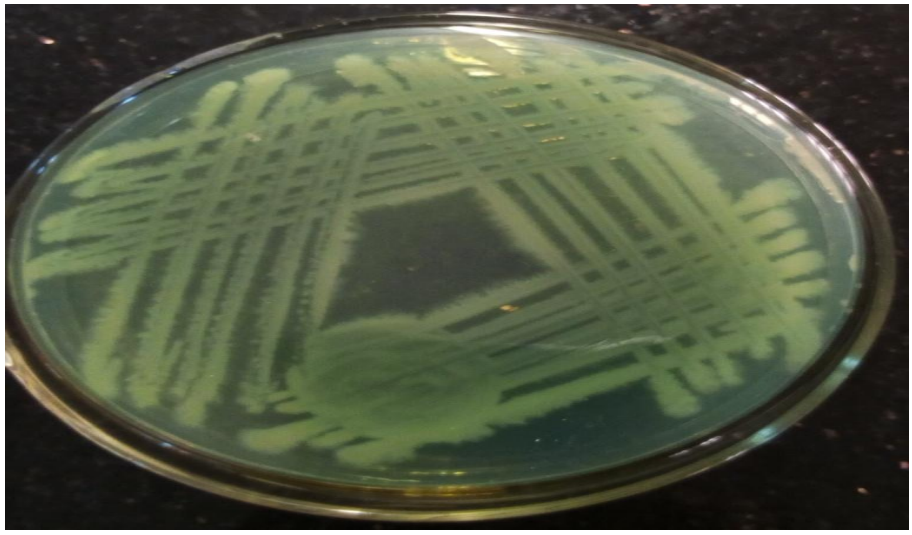

Fig. 2. Growth of *Pseudomonas aeruginosa* on nutrient agar plate

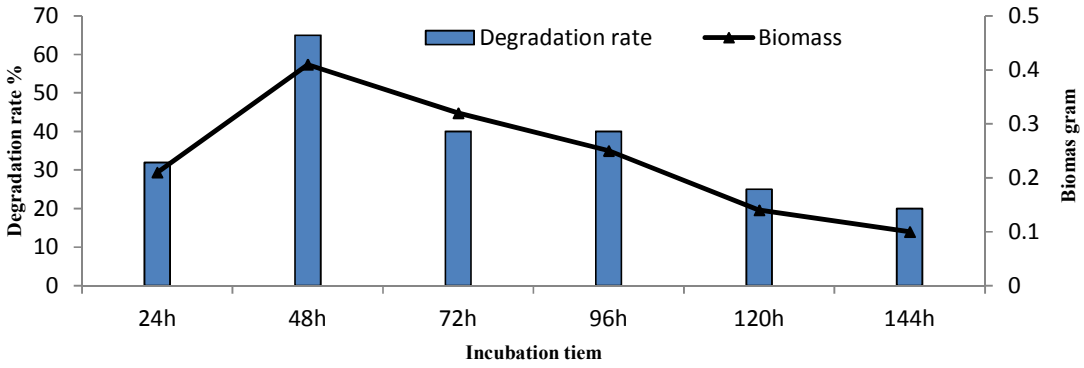

Fig. 3. Effect of incubation time on lignin degradation and biomass production by *Pseudomonas aeruginosa*

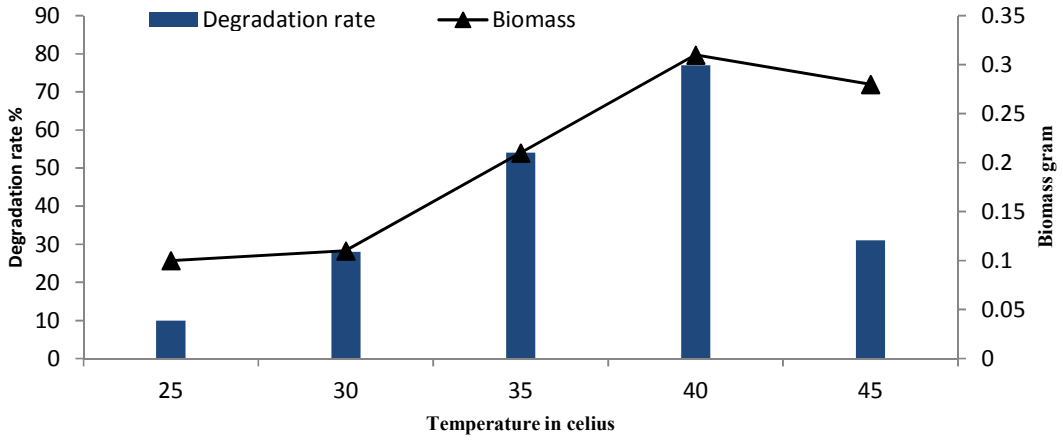

Fig. 4. Effect of incubation temperature on lignin degradation and biomass production by *Pseudomonas aeruginosa*

The rate of lignin degradation and biomass production by the bacterial isolated is also affected by changing in temperature. It was observed that *Pseudomonas aeruginosa* reached highest peak of 82% lignin degaradation at 35^{o}C. The difference of temperature on lignin degradation and biomass production might be attributed to enzymes activities during growth, as highest activities of enzymes occur at optimum temperature. It was reported that the optimum tempertature of laccase production by bacteria such as B. subtilis occurred at mesophilic character thereby showing maximum enzyme production at 40°C [23].

The effect of pH on lignin degradation and biomass production is presented on Figure 3. It was observed that the rate of degradation and biomass production was slow at pH 6.0 and 6.5. As pH increase to 7.0, 7.5 and rising toward alkalinity there was gradual increase of lignin degradation and biomass production. This is similar with the finding of Murugesan and Vembu [18] who reported that *Bacillus* sp. produced high laccase activity at pH 7.5 and gradually decrease from 7.5 to 10.0. However, they noted that the ability of the isolate to produce enzymes at alkaline pH makes it suitable for bioremediation and various industrial applications.

The effect of agitation on the growth and lignin degradation by *Pseudomonas aeruginosa* was determined and the results are shown in Figure 6. It was observed that the growth of the isolated strain increased with increase in speed of agitation as higher degradation was achieved at 100 rpm and gradually declined as agitation rate increase. The increase in agitation rate up to 200 rpm did not increase the degradation, but gave the maximum bacterial growth. This is probably

at higher agitation rate the enzyme structure might be changed [18]. However, at lower agitation rate there was both reduction of both degradation and growth of *Pseudomonas aeruginosa,* this indicated that mixing of growth media and supply of oxygen was insufficient. The amount of dissolved oxygen in the fermentation medium is greatly influenced by the speed of agitation. Excessive agitation results in a higher mechanical force, which may produce the cell destruction and uptake of nutrients, thereby lowering the enzyme production [24]. Therefore, optimizing the agitation of fermentation media is a very important factor to provide sufficient oxygen, mixing and uptake of nutrients by the microbe [18]. This is similar with the finding of Mahmoud et al. [25] who reported that the maximum laccase production by the strains *Streptomyces lydicus* was achieved at 150 rpm under shaking condition.

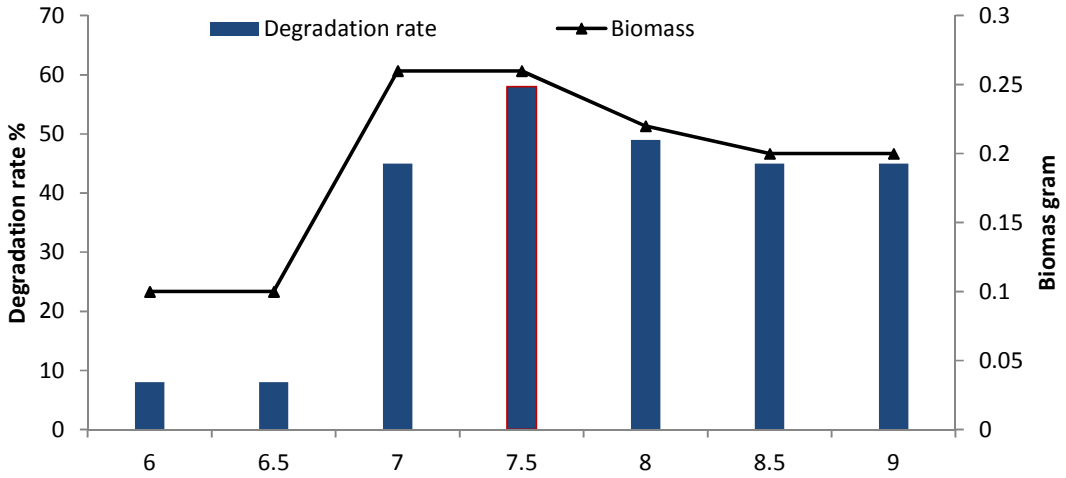

Fig. 5. Effect of pH on lignin degradation and biomass production by *Pseudomonas aeruginosa*

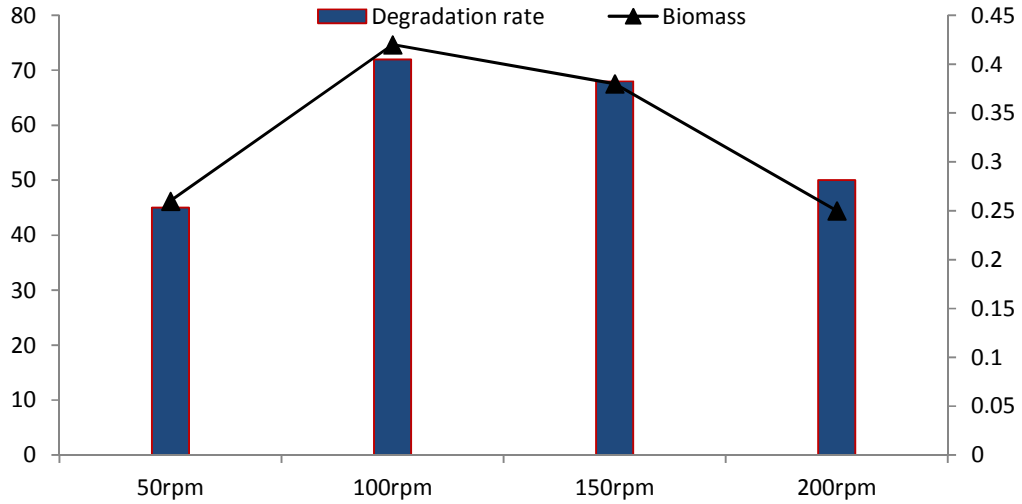

Fig. 6. Effect of agitation on lignin degradation and biomass production *Pseudomonas aeruginosa*

The result of Fourier transformed infrared spectroscopy of lignin degradation revealed two absorption peaks. The peak at 3375 cm^{-1} is attributed to O-H stretching while the second peak 1687.5 cm^{-1} corresponded to C=O stretching. Some compounds detected by GC-MS includes the following 2,5-Hexanediol, 2-Octynoic acid, n-Hexadecanoic acid, i-Propyl9,12-octadecenadienoate and (R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol. The Hexanediol and have been widely used in the production of bread, dairy products, and alcoholic beverages. Hexanediol is a versatile building block for the synthesis of various chiral phosphine ligands. Also hexanediol is used as intermediate in the preparation of optically active tetrahydrofurans used in biodegradable polymers, perfumes and medicines [26]. Hemalatha et al. [26] reported to used yeast *Pichia farinose* MTCC *246 for the production of Hexanediol using batch production.

It was reported that n-hexadecanoic acid (C16H32O2) possess Anti-inflammatory, antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-Alpha reductase inhibitor, potent mosquito larvicide [27]. 2-octynoic acid (2-OA) is widely used in the environment including perfumes, lipstick and many common food avorings [28]. It has been reported that 2-OA may be associated with fatty acid pathway [29]. Darong *et al.* [30] reported that 2-OA inhibits HCV RNA replication, viral protein expression, and infectious virus production in human hepatocytes.

Table 3. GC-MS peak for lignin degradation by *Pseudomonas aeruginosa*

Peak #	R.Time	Height%	Molecular Formular	Name
1	3.311	1.67	$C_6H_{14}O_2$	2,5-Hexanediol
2	4.069	2.14	$C_8H_{12}O_2$	2-Octynoic acid
3	13.734	24.10	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
4	15.207	66.40	$C_{21}H_{38}O_2$	i-Propyl9,12-octadecenadienoate
5	15.844	2.85	$C_{17}H_{34}O$	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol

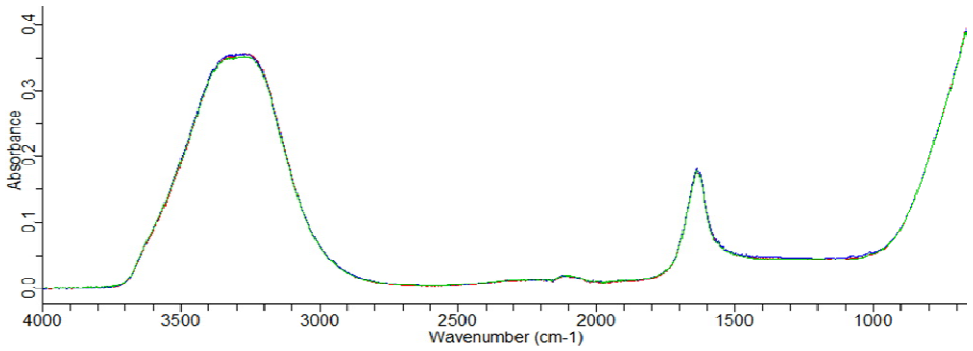

Fig. 7. FTIR Spezctra of lignin degradation by *Pseudomonas aeruginosa* Isolated from Kware Lake

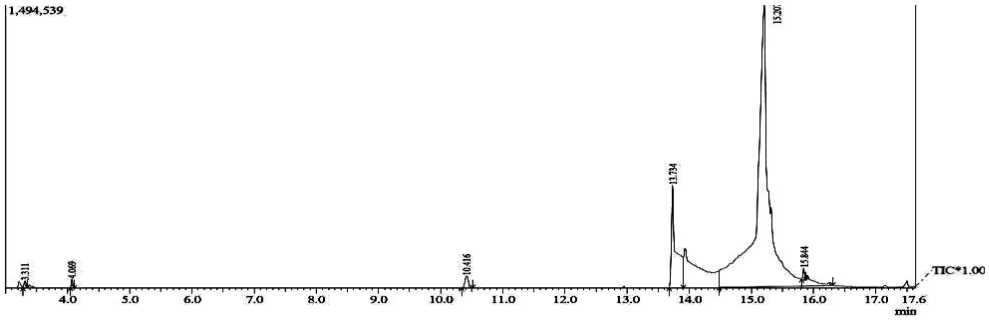

Fig. 8. GC-MS chromatogram of crude lignin degradation by *Pseudomonas aeruginosa*

4. CONCLUSION

This study demonstrated the ability of *Pseudomonas aeruginosa* for lignin degradation. From the results it clearly proving that growth condition affect lignin degradation and biomass production by the bacterium. The results of GC-MS detected the presence of 2, 5-Hexanediol, 2-Octynoic acid, n-Hexadecanoic acid and i-Propyl 9, 12-octadecenadienoate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chen Y, Wang W, Zhou D, Jing T, Li K, Zhao Y, Tang W, Qi D, Zhang M, Zang X. Biodegradation of lignocellulosic agricultural residues by a newly isolated *Fictibacillus sp.* YS-26 improving carbon metabolic properties and functional diversity of the rhizosphere microbial community. Bioresour. Technol. 2020;310:123381.
[CrossRef] [PubMed]
2. Sharma HK, Xu C, Qin W. Biological pretreatment of lignocellulosic biomass for biofuels and bioproducts: An overview. Waste Biomass Valoriz. 2019;10:235–251.
[CrossRef]
3. Mathews SL, Pawlak J, Grunden AM. Bacterial biodegradation and bioconversion of industrial lignocellulosic streams. Appl. Microbiol. Biotechnol. 2015;99:2939–2954.
[CrossRef]
4. Enagbonma BJ, Babalola OO. Potentials of termite mound soil bacteria in ecosystem engineering for sustainable agriculture. Ann. Microbiol. 2019;69:211–219.
[CrossRef]
5. Kang X, Kirui A, Dickwella Widanage MC, Mentink-Vigier F, Cosgrove DJ, Wang T. Lignin-polysaccharide interactions in plant secondary cell walls revealed by solid-state NMR. Nat Commun. 2019;10(1):347.
DOI: https://doi.org/10.1038/s41467-018-08252-0
6. Yi Xiong, Yaru Zhao, Kuikui Ni, Yue Shi and Qingfang Xu. Characterization of ligninolytic bacteria and analysis of alkali-lignin biodegradation products. Polish Journal of Microbiology. 2020;69(3):339–347
7. Zwetsloot MJ, Ucros JM, Wickings K, Wilhelm RC, Sparks J, Buckley DH, Bauerle TL. Prevalent root-derived phenolics drive shifts in microbial community composition and prime decomposition in forest soil. Soil. Biol. Biochem. 2020;145:1–13.
[CrossRef]
8. Harun R. Danquah MK, Forde GM. Microalgal biomass as a fermentation feedstock for bioethanol production. J. Chem. Technol. Biotechnol. 2010;85:199–203.
[CrossRef]
9. Kumar A, Chandra R. Lignolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. Heliyon. 2020;6:e03170.
10. Perestelo F, Rodriguez A, Pérez R, Carnicero A, de la Fuente G, Falcon MA. Short communication: Isolation of a bacterium capable of limited degradation of industrial and labelled, natural and synthetic lignins. World J Microbiol Biotechnol. 1996;12:111–112.
11. Huang XF, Santhanam N, Badri DV, Hunter WJ, Manter DK, Decker SR, Vivanco JM, Reardon KF. Isolation and characterization of lignin-degrading bacteria from rainforest soils. Biotechnol Bioeng. 2013;110:1616–1626.
12. Shi Y, Chai L, Tang C, Yang Z, Zheng Y, Chen Y, Jing Q. Biochemical investigation of kraft lignin degradation by *Pandoraea sp.* B-6 isolated from bamboo slips. Bioprocess Biosyst Eng. 2013;36(12):1957–1965.
13. Jiang-Hao T, Anne-Marie P, Théodore B, Eric Gelhaye, Pascal P. Occurrence of lignin degradation genotypes and phenotypes among prokaryotes. Appl Microbiol Biotechnol. 2014;98:9527–9544.
14. Cheesbrough M. District laboratory practice for tropical countries. 2nd edition. Cambridge University Press. Low price edition. 2006;62-70.
15. Oyeleke B, Manga SB. Essential of Laboratory in Microbiology. 1st edition. Tolbase Publisher, Niger State, Nigeria. 2008;119-142.
16. Aiya C. Klebsiella and enterobacter isolated from mangrove wetland soils in thailand and their application in biological decolorization of textile reactive dyes. Int.

J. Environ. Res. Public Health 2020;17:7531.
17. Pace NR. A Molecular View of Microbial Diversity and the Biosphere. Science.1997;276:734-740.
18. Murugesan R, Vembu B. Production of extracellular laccase from the newly isolated *Bacillus sp.* PK4. African Journal of Biotechnology. 2016;15(34):1813-1826.
19. Ahmad AS, Ahmad WYW, Zakaria ZK, Yosof NZ. Applications of bacterial pigments as colorant: the malaysian perspective. New York: Springer Briefs in Molecular Science. 2012;3(7):s57-74.
20. Umar ZU, Mahaneem M. Analysis of phytochemical compound in water and methanol extracts of Malaysian propilis. International Journal of Pharma. and Bioscience. 2016;6(2):374-380.
21. Banat IM, Nigam P, Singh D, Marchant R. Microbial decolorization of textile-dye containing effluents: A review. Bioresour Technol. 1996;58:217–227.
22. Patel N, Shahane S, Shivam, Majumdar R, Mishra U. Mode of action, properties, production, and application of laccase: A review. Recent Pat. Biotechnol. 2019;13:19–32.
[CrossRef] [PubMed]
23. Tabinda S, Mustafa K, Mansoor A, Syeda MS, Rafeeq AK, Amir H. Medium optimization for the production of amylase by Bacillus subtilis RM16 in Shake-flask fermentation. Pakistan Journal of Pharmaceutical Sciences. 2016;29(2):439-444.
24. Purwanto LA, Ibrahim D, Sudrajat H. Effect of agitation speed on morphological changes in Aspergillus niger hyphae during production of tannase. World J. Chem. 2009;4:34-38.
25. Mahmoud MG, Rifaat HM, El-Sayed OH, El-Beih FM, Selim MS. Effect of inducers and process parameters on laccase production by locally isolated marine *Streptomyces lydicus* from Red Sea, Egypt. Int. J. Chem. Tech. Res. 2013;5:15-23.
26. Hemalatha R, Nivedha P, Gopinat M, Shivakumar K, Dhanasekar R. Production of 2, 5-Hexanediol Using Pichia farinose: A Response Surface Methodology. Int. J. Chem Tech Res. 2013;5(3):1178-1185.
27. Mustapha NA, Runner RTM. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Medicines. 2016;3(3):2-9.
28. Amano K, Leung PC, Rieger R, Quan C, Wang X. Chemical Xenobiotics and Mitochondrial Autoantigens in Primary Biliary Cirrhosis: Identification of Antibodies against a Common Environmental, Cosmetic, and Food Additive, 2-Octynoic Acid. Journal Immunology. 2005;174:5874–5883.
29. Kim J, Matsuyama S, Suzuki T. 4, 8-Dimethyldecanal the aggregation pheromone of *Tribolium castaneum* is biosynthesized through fatty acid pathway. J Chem Ecol. 2005;31:1381–1400.
30. Darong Y, Binbin X, Xiaohong W, Xiaoyan Y, Nianli L, Yimin G, Chen L, Haizhen Z. 2-Octynoic Acid inhibits hepatitis C virus infection through activation of amp-activated protein kinase. PLoS ONE. 2013;8(5):e64932.

© 2021 Usman and Dabai; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/66263