Biodegradation Of Chlorpyrifos Using *Pseudomonas Aeruginosa* Isolated from Agricultural Wastewater in Kaduna State

Balarabe, M.A.[1*], Shehu, A.B.[2], Usman, H.A.[1], Adamu, H.[2], Abdullahi, Z.[1]

[1] Department of Biochemistry, Kaduna State University, Kaduna, Nigeria.

[2]Department of Food Technology/ Home Economics, (NAERLS), Ahmadu Bello University, Zaria, Kaduna, Nigeria.

*[1]**Corresponding Author***: mariya.abubakar@kasu.edu.ng* Tel: +2348109624250

DOI: https://doi.org/10.5281/zenodo.19851115

Abstract

This study evaluated the biodegradation potential of bacteria isolated from agricultural wastewater contaminated with chlorpyrifos pesticide. Wastewater samples were collected from drainage canals in Nasarawa, Kaduna State, Nigeria and analyzed for organophosphorus pesticide contamination using gas chromatography-mass spectrometry (GC-MS). Chlorpyrifos concentrations ranged from 105.6 ± 0.4 mg/L to 131.8 ± 0.2 mg/L. Bacterial isolates were identified using morphological, biochemical, and 16S rRNA gene sequencing techniques. Optimal degradation was observed at 30°C and pH 7. The isolate identified as *Pseudomonas aeruginosa* degraded 60.43% of chlorpyrifos at 30 mg/L after 28 days. The metabolite 3,5,6-trichloro-2-pyridinol (TCP) was detected, confirming chlorpyrifos degradation. These findings demonstrate the potential application of this *P. aeruginos*a for the bioremediation of pesticide-contaminated agricultural wastewater.

Keywords: Chlorpyrifos; bioremediation; agricultural wastewater, organophosphorus pesticide.

INTRODUCTION

Pesticides are chemical compounds, used extensively in modern agriculture to enhance crop yield and quality. However, their indiscriminate use has resulted in contamination of environmental matrices. These chemicals have increased agricultural production with less labor (Usharani and Muthujumar, 2013). Chlorpyrifos (0, 0-dieethyl 0-(3,5,6- trichloro pyridyl) phosphorothioate is a broad-spectrum organophosphorus insecticide widely applied to control chewing and sucking pests (Raj and Kumar, 2022). Extensive research has demonstrated how pesticides affect environment owing to their persistence and toxicity. Studies have shown that surface waters in agricultural regions are especially vulnerable to pesticide pollution due to runoff and drainage (Vryzas, 2018). Agricultural wastewater refers to excess water that drains from a field during surface irrigation at the low end of furrows, boundary strips, basins and flooded regions. Conventional remediation techniques for pesticide-contaminated water such as chemical oxidation, incineration and photodecomposition are often costly, inefficient and may generate toxic byproducts (Muhammad, 2010). This study aimed to isolate and identify chlorpyrifos-degrading bacteria from agricultural wastewater in Kaduna State and to evaluate their biodegradation efficiency under controlled laboratory conditions.

MATERIALS AND METHODS

Chemicals

Analytical-grade chlorpyrifos (99% purity) was used as the test compound. All solvents and reagents employed were of analytical grade and obtained from standard commercial suppliers.

Sampling Site

Wastewater samples were collected from two agricultural drainage canals located in Nasarawa, Kaduna State, Nigeria (10°46`N 7°38`E). A total of two samples (one from each site) were collected in sterile 15 mL containers. Samples were transported on ice to the laboratory and processed immediately.

Isolation

Mineral Salt Medium (MSM) was used for bacterial enrichment. A stock solution of chlorpyrifos (1000 mg/L) was prepared in analytical-grade acetone. One milliliter of the stock solution was added to 100 mL MSM to obtain a final concentration of 10 mg/L. Chlorpyrifos served as the sole carbon and energy source during enrichment. Serial dilutions (10^{-3} and 10^{-4}) were prepared to obtain countable colonies (30–300 CFU per plate). Preliminary observations indicated high microbial load in the wastewater samples; therefore, these dilution factors were appropriate for ensuring discrete colony formation and accurate enumeration. Plates were incubated at 30°C for 24 h. Distinct colonies were repeatedly sub-cultured to obtain pure isolates (Faria *et al.,* 2017).

Identification

Bacterial isolates were characterized using standard morphological and biochemical tests. Molecular identification was performed by PCR amplification and sequencing of the 16S rRNA gene (Mwaura *et al.,* 2018).

Biodegradation Experiment

Biodegradation experiment was conducted in MSM supplemented with chlorpyrifos at 20, 30 and 40 mg/L. Cultures were incubated at 30 ℃ and pH 7 for 28 days with continuous shaking at 150 rpm. Control flasks containing chlorpyrifos without bacterial inoculation were maintained in parallel. Samples were withdrawn at 0, 7, 14, 21, and 28 days for analysis. The percentage degradation of chlorpyrifos was calculated using the equation:

$$X\,(\%) = \frac{(Cck - \mathrm{Cx})}{Cck}\ X\ 100$$

Where:

- Cck = concentration in control
- Cx = concentration in inoculated sample

Proceedings of the 7th Biennial International Conference of Organization for Women in Science for the Developing World (OWSD) Nigeria National Chapter — Hosted by OWSD FUTA Chapter, Federal University of Technology Akure, Nigeria, 7–10 July 2025, ISBN: 978-978-61927-9-6

Analytical Methods

Chlorpyrifos and its metabolites were extracted from culture media and analyzed using GC-MS. Identification of compounds was based on retention times and comparison of mass spectra with entries in the NIST library. TCP was identified as the major degradation product (Xu *et al.*, 2017).

Statistical Analysis

All experiments were conducted in triplicates. Data were expressed as mean ±standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was carried out using IBM SPSS software version 23.

RESULTS

GC-MS analysis revealed the presence of several organophosphorus pesticides in the wastewater samples.

Table 1: Concentration of Pesticides in Wastewater Samples

Sample	Ethroprophos (ppm)	Chlorpyrifos (ppm)	Disulfoton (ppm)	Ronnel (ppm)	Phosphorodithioc acid (ppm)
NA1	35.1 ± 0.100^{a}	105.6 ± 0.4^{a}	0.40 ± 0.020^{a}	104.9 ± 0.20^{a}	25.15 ± 0.06^{a}
NB1	32.9 ± 0.200^{b}	131.8 ± 0.2^{b}	0.02 ± 0.001^{b}	0.42 ± 0.03^{b}	40.21 ± 0.09^{b}

Key:NA1=NasarawaA1, NB1=NasarawaB1
Values are expressed as Mean ± SD (n = 3). Different superscripts within the same column indicate significant difference ($p < 0.05$).

Physicochemical Parameters of the Wastewater Samples

Table 2 & 3 revealed concentration of electrical conductivity, alkalinity and sodium adsorption ratio concentrations. Metal analysis showed concentration for nitrate, chlorine, lead, chromium, calcium and sodium on all two sites.

Table 2: Physicochemical Properties of Wastewater Samples

Sample	EC (ppm)	pH	Alkalinity (mg/L)	SAR (meq/L)
NA1	121 ± 0.2^{a}	6.78 ± 0.02^{a}	82.3 ± 0.04^{a}	0.63 ± 0.02^{a}
NB1	142 ± 0.3^{b}	6.87 ± 0.12^{a}	112.3 ± 0.1^{b}	0.23 ± 0.11^{b}

Key: NA1 = Nasarawa A1, NB1 = Nasarawa B1
Values are expressed as Mean ± SD (n = 3). Different superscripts within the same column indicate significant difference ($p < 0.05$).

Table 3: Concentration of Metals in Wastewater Samples

Sample	NO_3 (mg/L)	Cl (mg/L)	Pb (mg/L)	Cr (mg/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)
NA1	6.29 ± 0.21^a	33.9 ± 0.04^a	0.91 ± 0.2^a	0.08 ± 0.01^a	24.8 ± 0.4^a	5.6 ± 0.2^a	10.5 ± 0.1^a
NB1	10.6 ± 0.15^b	28.22 ± 0.3^b	0.44 ± 0.19^b	0.01 ± 0.0^b	30.6 ± 0.5^b	4.7 ± 0.2^b	11.0 ± 0.0^a

Key: NA1 = Nasarawa A1, NB1 = Nasarawa B1
Values are expressed as Mean ± SD (n = 3). Different superscripts within the same column indicate significant difference ($p < 0.05$).

Isolation and characterization of pesticides degrading microorganisms

The bacterial isolates exhibiting superior pesticide degradation capabilities were subjected to identification through 16S rRNA sequence analyses. As a result, the degrading bacteria MS10 were identified as *Pseudomonas aeruginosa*, deposited in the NCBI GenBank with assigned accession numbers; MK334344. The phylogenetic tree analyses were constructed based on the neighbor-joining tree method.

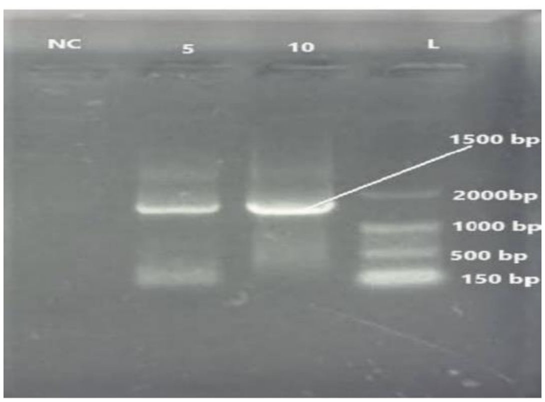

Figure 1: Agarose gel electrophoresis of 16S rRNA gene of isolate MS10**.**

Keys: NC: Negative control, 10: *Pseudomonas species,* L: Ladder (1500bp)

Biodegradation Study in Liquid Media

A pure culture of microbial isolate was prepared and used to biodegrade 20, 30 & 40 mg/L of chlorpyrifos separately. The results of chlorpyrifos degradation by the isolated bacterial strain were illustrated in Table 4.

Proceedings of the 7th Biennial International Conference of Organization for Women in Science for the Developing World (OWSD) Nigeria National Chapter — Hosted by OWSD FUTA Chapter, Federal University of Technology Akure, Nigeria, 7–10 July 2025, ISBN: 978-978-61927-9-6

Table 4: Spectrophotometric Degradation Assessment of Chlorpyrifos in Mineral Salt Liquid Media Inoculated with *Pseudomonas species* at 30℃ for Different Concentrations

	Absorbance value			
Incubation time (Days)	**(At 20 mg/L)**	**(At 30 mg/L)**	**(At 40 mg/L)**	**Control**
0	0.12 ± 0.009b	0.01 ± 0.001d	0.44 ± 0.010b	0.39 ± 0.030a
7	0.21 ± 0.040a	0.38 ± 0.070b	0.55 ± 0.090a	0.39 ± 0.033a
14	0.23 ± 0.033a	0.52 ± 0.110a	0.60 ± 0.050a	0.35 ± 0.070a
21	0.15 ± 0.002b	0.35 ± 0.060b	0.33 ± 0.010b	0.35 ± 0.000a
28	0.01 ± 0.000c	0.21 ± 0.002c	0.15 ± 0.020c	0.34 ± 0.044a
Percentage (%) Degradation	13.7	60.43	30.6	

Values are presented as Mean ± SD. And $p \leq 0.05$ was considered statistically significant. While Tukey comparison test was used for post hoc analysis.

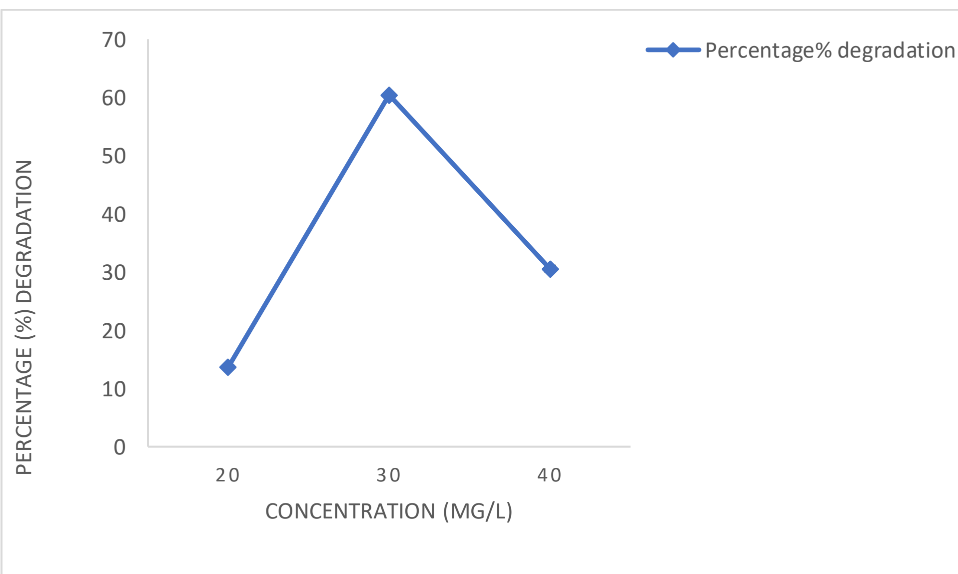

Figure 2: Percentage (%) degradation of *Pseudomonas aeruginosa* at different concentrations

Proceedings of the 7th Biennial International Conference of Organization for Women in Science for the Developing World (OWSD) Nigeria National Chapter — Hosted by OWSD FUTA Chapter, Federal University of Technology Akure, Nigeria, 7–10 July 2025, ISBN: 978-978-61927-9-6

DISCUSSION

The water quality analysis of the agricultural wastewater samples collected from Nasarawa revealed contamination with several organophosphorus pesticides. Quantitative analysis indicated the presence of ethroprophos, ronnel, disulfoton, and phosphorodithioc acid in the samples, while chlorpyrifos was detected at concentrations ranging from 105.6 ± 0.4 to 131.8 ± 0.2 mg/L, as illustrated in Table 1. The elevated levels of these pesticides may be attributed to their extensive application in agricultural activities. Runoff and drainage from farmlands into irrigation channels can therefore introduce significant quantities of pesticides into surrounding aquatic ecosystems, thereby increasing the risk of environmental contamination (Phong *et al.*, 2010).

Physicochemical analysis of the wastewater samples showed that parameters such as pH, electrical conductivity (EC), sodium adsorption ratio (SAR), alkalinity, and metal concentrations were within ranges typically observed in agricultural drainage waters. The measured pH values for the samples (NA1 = 6.78 and NB1 = 6.87) were within the recommended range for irrigation water (6.5–8.4), indicating near-neutral conditions suitable for microbial activity ((FAO, 1985).

Using enrichment techniques, bacterial isolates capable of degrading chlorpyrifos were successfully obtained from the contaminated wastewater. The most efficient isolate was identified as *Pseudomonas aeruginosa* through morphological, biochemical, and molecular characterization using 16S rRNA gene sequencing. Members of the genus *Pseudomonas* are well known for their metabolic versatility and their ability to degrade a wide range of environmental pollutants, including pesticides and aromatic hydrocarbons. Chlorpyrifos degradation by the *Pseudomonas* strain varied with concentration. The highest degradation efficiency was recorded at 30 mg/L (60.43%), which was significantly greater ($p < 0.05$) than the degradation observed at 20 mg/L (13.7%) and 40 mg/L (30.6%). The enhanced degradation observed at this concentration may be attributed to optimal substrate availability, which stimulates enzymatic activity without causing toxic stress to the bacterial cells. In contrast, the reduced degradation observed at 40 mg/L suggests possible substrate inhibition or toxic effects of chlorpyrifos at elevated concentrations, which may suppress microbial metabolic activity. This indicates that 30 mg/L represents the optimum concentration for chlorpyrifos biodegradation by the isolate under the experimental conditions. Previous studies have reported that certain *Pseudomonas* strains harbor plasmids containing genes responsible for chlorpyrifos degradation (Xu *et al.,* 2008). This finding is consistent with the work of Bhagobathy and Malik (2008), who reported several *Pseudomonas* strains isolated from wastewater-irrigated agricultural soils capable of utilizing chlorpyrifos as a sole carbon source.

One of the key steps in chlorpyrifos biodegradation involves the formation and subsequent metabolism of 3,5,6-trichloro-2-pyridinol (TCP) and related intermediate compounds. These metabolites are further transformed through microbial enzymatic pathways, leading to partial mineralization of the pesticide. Microorganisms belonging to the genus *Pseudomonas* are particularly adaptable and possess metabolic pathways capable of degrading various xenobiotic compounds including hydrocarbons, petroleum products, and pesticides (Sarkar *et al.*, 2009).

GC–MS analysis of the degradation products further confirmed the biodegradation of chlorpyrifos by *Pseudomonas aeruginosa*. Several peaks were observed at different retention times, with a prominent peak at 9.150 min, corresponding to the metabolite 3,5,6-trichloro-2-pyridinol (TCP). The compound was identified based on comparison of its mass spectra with entries in the NIST library database. The detection of TCP provides strong evidence that chlorpyrifos removal occurred through microbial metabolic degradation rather than simple adsorption onto bacterial biomass. The formation of TCP is a well-established intermediate in the chlorpyrifos biodegradation pathway and indicates enzymatic hydrolysis of the parent compound.

Similarly, Maya *et al*. (2011) reported that several bacterial strains exhibited varying abilities to degrade chlorpyrifos and its metabolite TCP, with *Pseudomonas* species showing the highest degradation potential due to their wide environmental adaptability, followed by *Agrobacterium* and *Bacillus* species.

Although the 60.43% degradation observed in this study demonstrates promising potential for the bioremediation of chlorpyrifos-contaminated wastewater, complete mineralization was not achieved within the 28-day incubation period. Further optimization of environmental conditions, including the use of microbial consortia or nutrient supplementation, may enhance degradation efficiency and accelerate pesticide breakdown.

A limitation of this study is the relatively small number of wastewater samples analyzed and the laboratory-scale experimental conditions employed. Future studies involving larger sampling areas and field-scale evaluations are recommended to better assess the practical applicability of *Pseudomonas aeruginosa* for environmental bioremediation.

CONCLUSION

This study confirmed the presence of a chlorpyrifos-degrading bacterium, *Pseudomonas aeruginosa*, isolated from contaminated agricultural wastewater in Nasarawa, Kaduna State. The isolate was evaluated for its biodegradation potential at chlorpyrifos concentrations of 20, 30, and 40 mg/L. The results showed that the bacterium achieved the highest biodegradation efficiency at 30 mg/L, with 60.43% degradation after 28 days of incubation under laboratory conditions. Gas chromatography–mass spectrometry (GC–MS) analysis confirmed the formation of the metabolite 3,5,6-trichloro-2-pyridinol (TCP), indicating the metabolic breakdown of chlorpyrifos. These findings demonstrate that *Pseudomonas aeruginosa* possesses promising potential for application in the bioremediation of chlorpyrifos-contaminated environments, particularly in agricultural wastewater systems.

REFERENCES

Bhagobathy, R. K., & Malik, A. (2008). Utilization of chlorpyrifos as a sole source of carbon by bacteria isolated from wastewater-irrigated agricultural soils in an industrial area of Western Uttar Pradesh, India. *Research Journal of Microbiology*, 3(5), 293–307.

FAO (1985). Water quality for agriculture. *FAO Irrigation and Drainage Paper 29 (Rev. 1)*. Food and Agriculture Organization of the United Nations, Rome.

Faria, Y., Aditi, Shafkat, S., Rahman, D., & Hossain, M. A. (2017). Study on the microbiological status of mineral drinking water. *The Open Microbiology Journal*, 11, 31–34.

Maya, K. S. R., Upadhyay, S. N., & Dubey, S. K. (2011). Kinetic analysis reveals bacterial efficacy for biodegradation of chlorpyrifos and its hydrolyzing metabolite TCP. *Process Biochemistry*, 46, 2130–2136.

Muhammad, S. G. (2010). Kinetic studies of catalytic photodegradation of chlorpyrifos insecticide in various natural waters. *Arabian Journal of Chemistry*, 3, 127–133.

Mwaura, A. N., Mbatia, B., Muge, E. K., & Okanya, P. W. (2018). Screening and characterization of hydrocarbonoclastic bacteria isolated from oil-contaminated soils from auto garages. *International Journal of Microbiology and Biotechnology*, 3, 11–24.

Phong, L. T., Van Dam, A. A., Udo, H. M. J., Van Mensvoort, M. E. F., Tri, L. Q., Steenstra, F. A., & Van der Zijpp, A. J. (2010). An agro-ecological evaluation of aquaculture integration into farming systems of the Mekong Delta. *Agriculture, Ecosystems & Environment*, 138(3–4), 232–241. https://doi.org/10.1016/j.agee.2010.05.004

Raj, A., Kumar, A., & Khare, P. K. (2024). The looming threats of profenofos organophosphate and microbes in action for their sustainable degradation. *Environmental Science and Pollution Research*, 31(10), 14367–14387.

Sarkar, S., Seenivasan, S., & Premkumar, R. (2009). Biodegradation of propiconazole by *Pseudomonas putida* isolated from tea rhizosphere. *Plant, Soil and Environment*, 55, 196–201.

Usharani, K., & Muthujumar, M. (2013). Optimization of aqueous methylparathion biodegradation by *Fusarium* sp. in batch scale process using response surface methodology. *International Journal of Environmental Science and Technology*, 10, 591–606.

Vryzas, Z. (2018). Pesticide fate in soil–sediment–water environment in relation to contamination prevention actions. *Current Opinion in Environmental Science & Health*, 4, 5–9. https://doi.org/10.1016/j.coesh.2018.03.001

Xu, G., Li, Z. W., Wang, Y., Zhang, S., et al. (2008). Biodegradation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol by a newly isolated *Paracoccus* sp. strain TRP. *International Biodeterioration & Biodegradation*, 62, 51–56.

Xu, X., Xu, Z., Shi, S., & Lin, M. (2017). Lignocellulose degradation patterns, structural changes and enzyme secretion by *Inonotus obliquus* on straw biomass under submerged fermentation. *Bioresource Technology*, 241, 415–423.