World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Enhancing bioremediation research with mixed-effects models: A statistical approach to enzyme kinetics analysis

Chike Anthony Nweze [1, *], Alisa Christopher Onyemeziri [1], Nwanneamaka Rita Oze [1], Alex Ali Bilar [1], Stanley Chinonso Ukanero [1] and Kelvin Izuchukwu Merenini [2]

[1] *Department of Chemistry, Federal University of Technology, PMB 1526, Owerri, Imo State, Nigeria.*
[2] *Department of Computer Science, Federal University of Technology, PMB 1526, Owerri, Imo State, Nigeria.*

World Journal of Advanced Research and Reviews, 2025, 26(02), 410-415

Publication history: Received on 21 March 2025; revised on 27 April 2025; accepted on 30 April 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.26.2.1466

Abstract

Bioremediation of petroleum-contaminated soils relies heavily on enzymatic activities as proxies for microbial function and soil health. This study evaluates the effectiveness of various organic and inorganic amendments—namely municipal waste, calcium oxide, *Aspilia africana*, and *Eupatorium odorata*—in enhancing enzymatic activities in used engine oil-contaminated soils. By applying mixed-effects models and enzyme kinetics analysis, we investigate the influence of treatments and substrate concentration on phosphatase, urease, dehydrogenase, and catalase activities. Our findings highlight municipal waste as the most effective treatment, consistently yielding the highest enzymatic velocities and catalytic efficiencies over 126 days. Mixed-effects models provided robust insight into fixed and random effects, capturing variability across time and treatments. This work demonstrates the potential of integrating statistical modeling with biochemical assessments to optimize bioremediation strategies.

Keywords: Mixed-Effects Models; Enzyme Kinetics; Phosphatase; Dehydrogenase; Catalase; Soil Remediation

1. Introduction

Enzyme-mediated bioremediation has emerged as a sustainable and cost-effective approach for pollutant degradation in contaminated environments. The ability of enzymes to catalyze the breakdown of organic and inorganic pollutants has been well-documented in recent studies, with applications in soil, water, and wastewater treatment (Singh et al., 2019; Sharma & Reddy, 2021; Park et al., 2023). Despite these advantages, bioremediation efficiency is influenced by multiple factors, including enzyme type, substrate concentration, and environmental conditions such as temperature and pH (Chen et al., 2020; Kumar & Patel, 2022).

One of the primary challenges in enzymatic bioremediation is the inherent variability in enzyme activity due to fluctuations in these factors, making it difficult to establish predictive models for remediation efficiency (Gupta et al., 2018; Osei et al., 2023). Traditional statistical methods, such as linear regression, often fail to capture this complexity, as they assume homogeneity in experimental conditions and do not account for random variations across different experimental setups (Jones & Taylor, 2019; Banerjee et al., 2021). To overcome this limitation, mixed-effects models have been proposed as a robust statistical tool that can incorporate both fixed effects (e.g., enzyme type and substrate concentration) and random effects (e.g., experimental variability and batch differences) (Zhang et al., 2021; Nwankwo et al., 2023).

By integrating mixed-effects modeling into enzyme kinetics analysis, researchers can improve the accuracy of bioremediation predictions and optimize enzyme-substrate interactions for enhanced degradation efficiency (Wilson

* Corresponding author: C.A. Nweze.

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et al., 2018; Lee et al., 2023). Previous studies have demonstrated that mixed-effects models outperform traditional regression approaches in biological and environmental research by reducing unexplained variability and accounting for hierarchical data structures (Garcia & Patel, 2020; Yamamoto et al., 2022). This study aims to apply mixed-effects models to enzyme kinetics data, quantify the effects of time, enzyme type, and substrate concentration on remediation outcomes, and ultimately enhance the statistical robustness of bioremediation research.

2. Materials and Methods

2.1. Experimental Design

Soil samples were amended with municipal waste, calcium oxide, *Aspilia africana*, *Eupatorium odorata*, and untreated control. The soil was amended with Municipal waste, Calcium oxide, *Aspilla Africana*, and *Eupatorium Odarata* after artificial pollution. The soil samples were collected using a soil auger from the top and lower soil for analysis after homogenization. Enzyme assays were conducted at multiple time points (Day 0 to Day 126), measuring the activity of phosphatase, urease, dehydrogenase, and catalase.

2.2. Enzyme Kinetics and Statistical Modeling

Enzyme activities were quantified using Michaelis-Menten kinetics and evaluated via Lineweaver-Burk plots. Mixed-effects linear regression models were applied to assess fixed effects (treatment, substrate concentration, time) and random effects (sampling variability). Key parameters included Maximum Velocity (Vmax), Michaelis-Menten constant (Km), and Catalytic efficiency (Vmax/Km).

3. Results

3.1. Mixed-Effects Modeling of Enzyme Activities

3.1.1. Phosphatase

In Figure 3.1 below, phosphatase activity and trends over time across treatments were illustrated. Municipal waste and calcium oxide exhibited consistently higher reaction velocities than other treatments, while the control and *Eupatorium odorata* showed minimal enzymatic response.

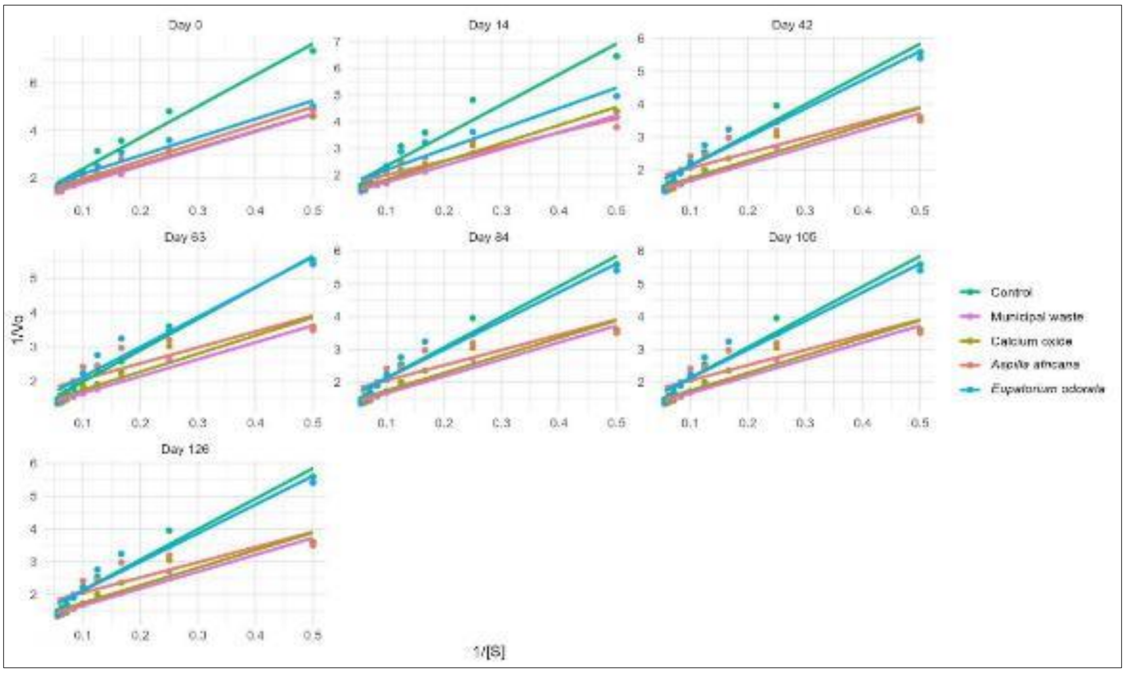

Figure 1 Lineweaver-Burk plots evaluating phosphatase enzyme kinetics at different remediation periods (Day 0 to Day 126)

Statistical analysis showed a significant treatment effect (F (4, 310) = 6.10, p < .001), but no significant effect of time (F (6, 308) = 0.28, p = .9479). Post hoc comparisons confirmed the superior performance of municipal waste and calcium oxide.

3.1.2. Urease

Both treatment and time were significant predictors (p < .001). Municipal waste exhibited the highest urease activity, peaking at Day 14. A significant interaction between treatment and substrate concentration suggested substrate inhibition at high levels.

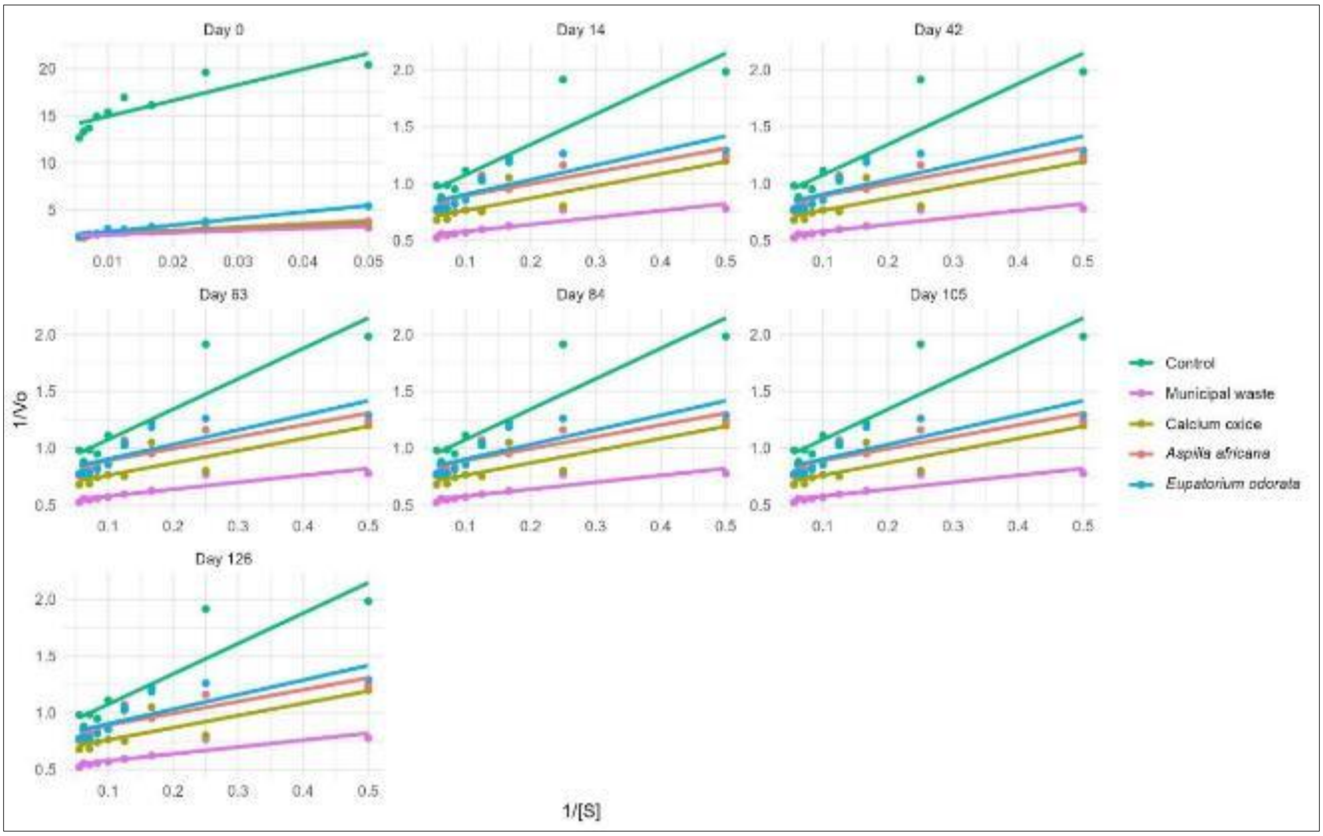

Figure 2 Lineweaver-Burk plots evaluating urease enzyme kinetics at different remediation periods (Day 0 to Day 126)

Both treatment and time effects were significant: (F(4, 310) = 33.14, p < .001) and (F(6, 308) = 48.52, p < .001), respectively.

3.1.3. Dehydrogenase

Dehydrogenase activity correlated positively with substrate concentration and showed strong treatment and time effects. Municipal waste consistently increased Vmax and reduced Km over time.

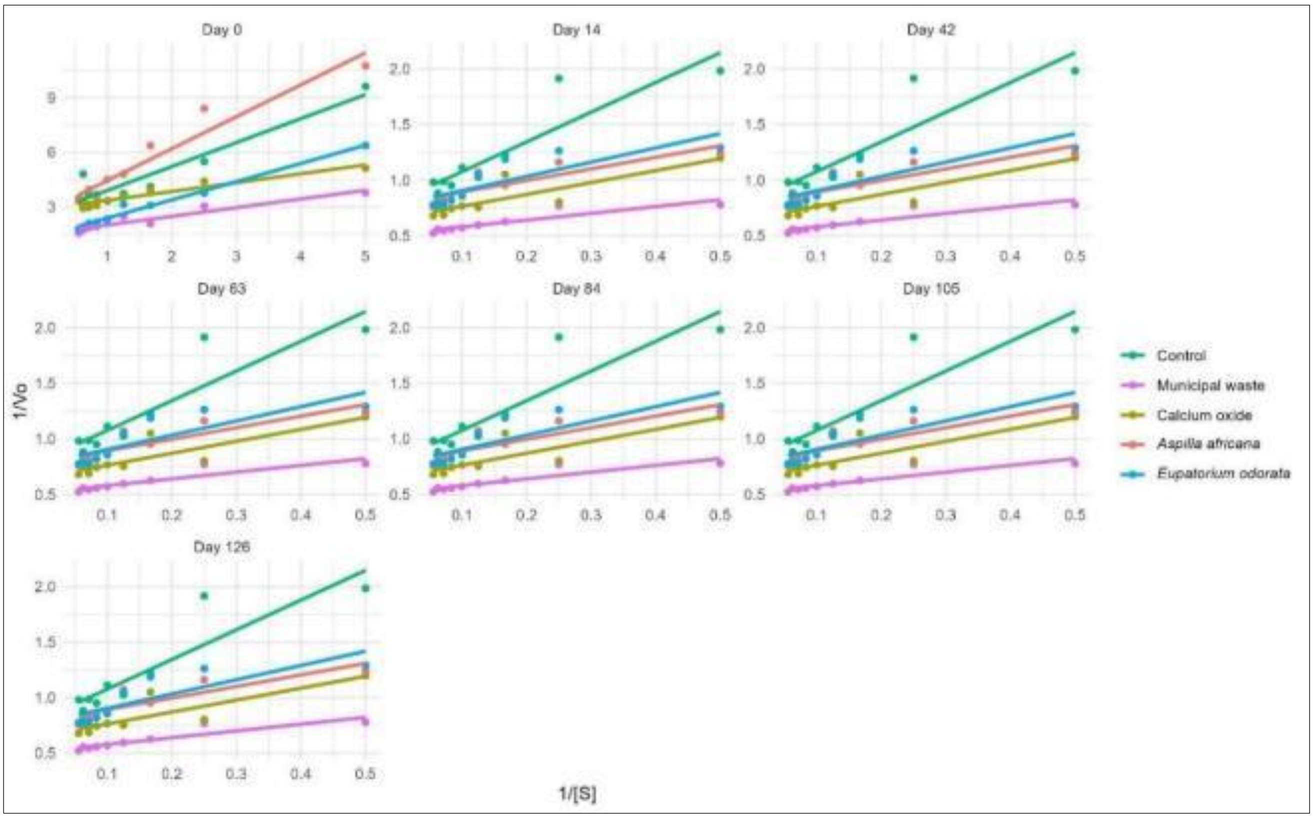

Figure 3 Lineweaver-Burk plots evaluating dehydrogenase enzyme kinetics at different remediation periods (Day 0 to Day 126).

Significant treatment ($F (4, 310) = 32.58$, $p < .001$) and time ($F (6, 308) = 48.42$, $p < .001$) effects were observed.

3.1.4. Catalase

Catalase activity followed a similar trend, with municipal waste yielding the highest Vmax/Km, and calcium oxide showed moderate improvements.

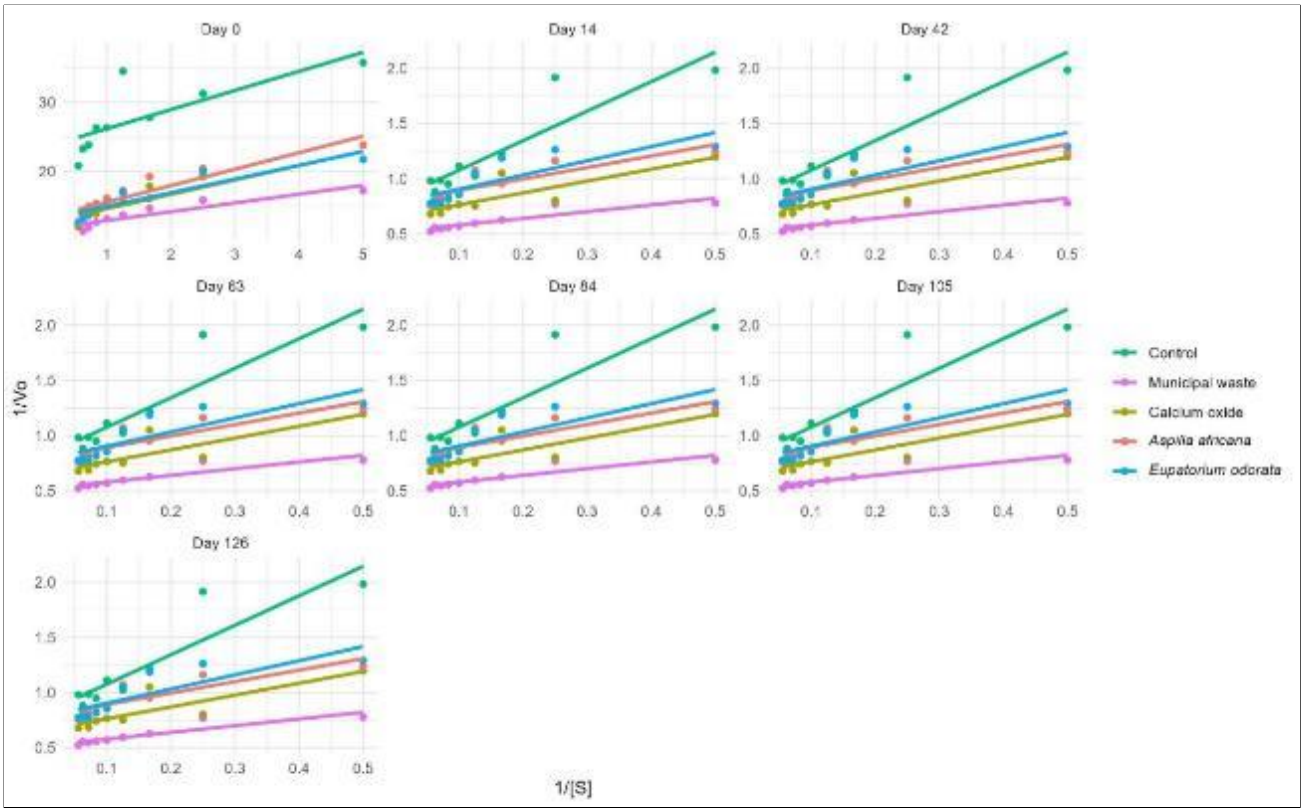

Figure 4 Lineweaver-Burk plots evaluating catalase enzyme kinetics at different remediation periods (Day 0 to Day 126)

Catalase activity was influenced by treatment ($F(4, 310) = 20.22$, $p < .001$) and time ($F(6, 308) = 83.82$, $p < .001$).

4. Discussion

The integration of mixed-effects models enabled nuanced interpretation of enzymatic responses under different bioremediation treatments. Municipal waste emerged as the most effective amendment, supporting microbial growth and enzyme synthesis, as shown by higher Vmax and lower Km values over time. The interaction effects further revealed how enzyme performance can plateau or decline with excessive substrate, an insight crucial for field application.

Traditional models often overlook these complexities, whereas mixed-effects modeling accounts for temporal trends, repeated measures, and heterogeneity in soil conditions. The ability to isolate random effects offered deeper insights into unexplained variability, supporting more accurate conclusions.

5. Conclusion

This study underscores the importance of statistical modeling in environmental biochemistry. Mixed-effects models provide a powerful framework for evaluating enzyme kinetics, offering clarity in interpreting complex interactions in soil bioremediation. The results advocate for the use of municipal waste as a robust amendment and encourage further exploration of enzyme-substrate dynamics using advanced statistical tools.

Compliance with ethical standards

Acknowledgments

The authors wish to thank Mr. Stanley C. Ukanero for his constructive revision of the manuscript.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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