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Biostimulated Phytoremediation of Stabilized Leachate using Eco-Enzyme and Selected Macrophytes: Performance and Removal Kinetics

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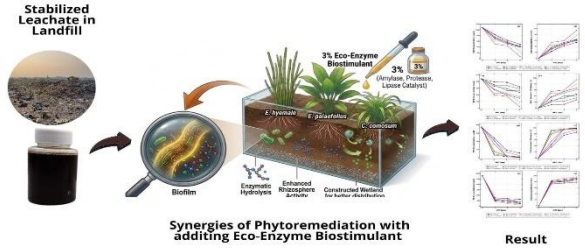
ABSTRACT

Stabilized leachate contains massive loads of recalcitrant organics and ammoniacal nitrogen, posing severe ecological risks and resisting conventional treatments. Integrating phytoremediation with biological catalysts, specifically eco-enzymes with macrophytes to treat toxic stabilized leachate, remains a significant research gap. This study evaluates the performance and removal kinetics of a biostimulated subsurface flow phytoremediation system. The experiment utilized C. comosum, E. palaefolius, and E. hyemale, biostimulated with a 3% (v/v) eco-enzyme dosage over a 9-day hydraulic retention time. First-order kinetic modeling revealed rapid temporal pollutant decay. Reactor R8 (E. hyemale with eco-enzyme) achieved superior removal efficiencies for Biochemical Oxygen Demand (94.35%) and Chemical Oxygen Demand (93.90%), with a rapid half-life of 2.44 days. Concurrently, reactor R6 (C. comosum with eco-enzyme) maximized Total Nitrogen removal at 97.10%. The core novelty demonstrates the auto-neutralization mechanism between the highly acidic eco-enzyme and alkaline leachate. This biochemical synergy accelerates enzymatic hydrolysis of complex

macromolecules into bioavailable carbon without inducing fatal osmotic shock to macrophyte roots. Ultimately, 3% eco-enzyme biostimulation overcomes the low biodegradability limitations of stabilized leachate, providing highly efficient bioengineering solutions for compliance standards.

Keyword: Biostimulation, eco-enzyme, phytoremediation, stabilized leachate

Graphical of Abstract



1. Introduction

The acceleration of global urbanization has led to increased municipal solid waste, producing highly toxic stabilized landfill leachate. This leachate is characterized by a low biodegradability ratio (BOD/COD) and a high load of recalcitrant organic matter that strongly resist conventional biological treatment [1], [2]. If discharged without adequate remediation, it poses severe risks to aquatic ecosystems and violates strict environmental regulatory frameworks [3]. Consequently, cost-effective bioengineering solutions, such as subsurface flow constructed wetlands (SSF-CWs), are essential for sustainable waste management [4], [5].

Phytoremediation leverages the ability of macrophytes to absorb and degrade contaminants through enzymatic activity in the rhizosphere zone. Furthermore, physical mechanisms like rhizofiltration intercept suspended solids within dense root structures [6], [7]. However, these natural processes often exhibit limited efficiency when treating stabilized

leachate due to its high toxicity and lack of bioavailable carbon [8]. The selection of resilient species like *E. palaefolius* and *E. hyemale* is critical for maintaining system stability, while *C. comosum* has shown remarkable adaptability to hydroponic treatment [9], [10], [11].

Phytoremediation leverages the ability of macrophytes to absorb and degrade contaminants through enzymatic activity in the rhizosphere zone. Furthermore, physical mechanisms like rhizofiltration intercept suspended solids within dense root structures [6], [7]. However, these natural processes often exhibit limited efficiency when treating stabilized leachate due to its high toxicity and lack of bioavailable carbon [8]. Additionally, the sustainable management of these robust macrophytes post-remediation is increasingly recognized as a vital component of holistic landfill operations to prevent the re-release of absorbed pollutants [12]. The selection of resilient species like *E. palaefolius* and *E. hyemale* is critical for maintaining system stability, while *C. comosum* has shown remarkable adaptability to hydroponic treatment [9].

Therefore, this research aims to bridge this gap by evaluating the performance of biostimulated phytoremediation for stabilized landfill leachate utilizing *C. comosum*, *E. palaefolius*, and *E. hyemale* coupled with targeted eco-enzyme supplementation. The experimental study is rigorously monitored over a designated 9-day hydraulic retention time (HRT) to empirically map the temporal pollutant breakdown. Ultimately, this research evaluates the first-order removal kinetics to calculate the rate constant (k) and half-life ($t_{1/2}$) alongside the statistical determination coefficient (R^2) to provide a sustainable model for complex wastewater management [13], [14]. The eight experimental reactors were systematically varied into distinct configurations: R1 (Control), R2 (*C. comosum*), R3 (*E. palaefolius*), R4 (*E. hyemale*), R5 (Unplanted + Eco-Enzyme), R6 (*C. comosum* + Eco-Enzyme), R7 (*E. palaefolius* + Eco-Enzyme), and R8 (*E. hyemale* + Eco-Enzyme).

2. Research Methods

2.1. Initial Leachate and Eco-Enzyme Preparation

The stabilized leachate utilized in this research was directly sourced from the active Ngipik Landfill located in Gresik, East Java. The initial baseline characterization of this raw wastewater recorded Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) concentrations at 439 mg/L and 2695 mg/L, respectively [2]. Furthermore, the initial physical and nitrogenous parameters exhibited severe contamination levels, with Total Suspended Solids (TSS) at 500 mg/L and Total Nitrogen (TN) reaching 4955.92 mg/L [2]. Importantly, the calculated biodegradability ratio (BOD/COD) was exceptionally low at exactly 0.16, strictly categorizing the effluent as highly stabilized leachate that requires advanced biostimulation [2].

To overcome the severe recalcitrance of this stabilized effluent, a catalytic eco-enzyme solution was synthesized through a strictly controlled 3-month anaerobic fermentation process [7]. The raw biological substrate consisted of an optimal stoichiometric mixture of non-chlorinated tap water, fresh fruit peels, and brown sugar formulated at a precise 10:3:1 volume ratio [8]. These active organic constituents were thoroughly homogenized and securely enclosed within airtight fermentation vessels to ensure an optimal anoxic environment [9]. Following the maturation period, the crude liquid was systematically filtered using Whatman 40 filter paper to eliminate residual colloidal particles that could interfere with subsequent remediation phases.

The comprehensive post-fermentation characterization confirmed that the resulting liquid biostimulant successfully generated a highly acidic environment with a recorded pH of 4.96 [15]. In addition to massive organic loads, this specific catalytic liquid exhibited exceptionally

high hydrolytic activities, including amylase at 15.30 U/mL and protease at 12.15 U/mL. Furthermore, the lipase concentration was quantified at exactly 8.45 U/mL, providing the necessary biological agents to break down complex lipids, as detailed in **Table 3** [15]. Ultimately, this robust enzymatic profile serves as the primary biostimulation catalyst designed to radically accelerate the degradation of complex macromolecules within the treatment reactors.

2.2 Acclimatization & Range Finding Test (RFT)

The biological remediation framework carefully employed *C. comosum*, *E. palaefolius*, and *E. hyemale*, which were selected for their robust phytoextraction potential [9]. Prior to exposure, all fresh botanical specimens underwent a rigorous 7-day acclimatization process within a controlled greenhouse environment utilizing clean hydroponic water without any leachate mixture [13]. This preliminary stabilization phase was conducted in high-density polyethylene (HDPE) containers measuring 30 cm × 18 cm × 24 cm. This mandatory conditioning period was specifically designed to guarantee optimal physiological health and to establish a robust baseline root system for the aquatic macrophytes [14].

The RFT was conducted in a reactor with a volume capacity of 5 L and dimensions of 26 cm x 23 cm x 24 cm, holding exactly 1 L of liquid with 3 individual plants per reactor [16]. The 1 kg wet weight biomass per reactor was distributed among three individual plants to maintain a consistent contact surface area between the macrophyte roots and the stabilized leachate [9]. The RFT growing medium consisted of soil measuring 0.053–0.075 mm at the top and gravel measuring 10–20 mm at the bottom, each 7 cm high. The first RFT phase exposed the plants to escalating stabilized leachate-to-tap-water volume ratios of 0%:100%, 25%:75%, 50%:50%, 75%:25%, and 100%:0% (v/v). Subsequently, the second RFT phase utilized specific stabilized leachate : eco-enzyme volume ratios of 100%:0%, 99%:1%, 97%:3%, and 95%:5% (v/v)[17].

Both phases of the Range Finding Test (RFT) were conducted over a 7-day observation period to assess both acute and chronic phytotoxicity [9], [16]. The physiological responses were evaluated daily through visual morphological changes and the monitoring of structural integrity in the stems and roots to determine the lethal percentage. The first phase of the Range Finding Test (RFT) confirmed that all selected macrophyte species successfully tolerated the maximum undiluted stabilized leachate concentration of 100%:0% (v/v) without exhibiting acute phytotoxicity. The second RFT phase established that the maximum safe threshold for maintaining cellular integrity was a leachate-to-eco-enzyme ratio of 97%:3% (v/v), which was definitively selected as the optimal biostimulant dosage for the primary phytoremediation treatment.

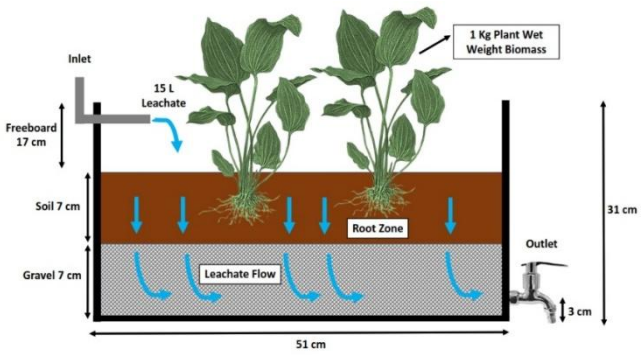
2.3 Reactor Set-Up and Experimental Variations

The experimental utilized eight laboratory-scale phytoremediation reactors from HDPE tanks with dimensions of 51 cm x 32 cm x 30 cm. To optimize hydraulic conductivity and prevent clogging, the substrate consisted of two layers: a bottom layer of gravel (0.85–1.18 mm) and an upper layer of soil (0.053–0.075 mm) (Sari et al., 2018). The selection of soil particles (0.053–0.075 mm) and gravel (10–20 mm) was strategically implemented to ensure uniform leachate flow distribution and mitigate potential clogging within the critical root zone. This specific fertile soil was meticulously selected for its 5.2% rich organic matter content and optimal 6.8 pH to continuously support phytostabilization [18]. The effective pore water volume of each static batch unit was precisely calibrated to hold exactly 15 L of liquid under semi-outdoor conditions. The 15 L pore water volume was precisely calculated based on the media porosity to guarantee that the 9-day HRT was accurately maintained under batch conditions. Deploying the reactors in a semi-outdoor environment with temperatures ranging

from 28.5 to 30.4 °C served to simulate real-world field conditions while preserving the plants' metabolic stability

The reactors were equipped with an inlet pipe placed in the upper center of the side wall to introduce the inflow, while an outlet tap was installed in the lower center of the opposite side wall to facilitate drainage and sampling. The outlet tap was placed 3 cm above the reactor base. The outlet tap was constructed using ½ - inch PVC, while the inlet used ¾-inch 90° PVC elbow. The substrate media size was conditioned by screening in accordance with ISO 565/3310-1 & ASTM E11. The comprehensive experimental framework, encompassing leachate characterization, plant acclimatization, range finding tests, and the main SSF-CW operation, is systematically illustrated in Fig. 1.

Eight reactors were constructed using a dual-layer substrate consisting of gravel and fertile soil with a total media height of 14 cm (7 cm per layer), an arrangement previously shown to optimize pollutant filtration and plant stability [19]. Reactors R5-R8 received a percentage of leachate : Eco-enzme in 97%:3% (v/v), determined as optimal via a Range Finding Test to maximize microbial stimulation without inducing acidic tissue damage [9]. The eight experimental reactors study were systematically varied into distinct configurations R1 (Control), R2 (*C. comosum*), R3 (*E. palaefolius*), R4 (*E. hyemale*), R5 (Unplanted + Eco-Enzyme), R6 (*C. comosum* + Eco-Enzyme), R7 (*E. palaefolius* + Eco-Enzyme), and R8 (*E. hyemale* + Eco-Enzyme). All reactors were monitored over a designated 9-day HRT to continuously assess the temporal kinetics of pollutant removal.



158

159 **Fig. 1.** Phytoremediation Reactor Design of Stabilized Leachate Treatment.

160 ## 2.4. Analytical Procedures and Removal Kinetics

161 The operational treatment reactors were rigorously evaluated over a continuous 9-day
162 hydraulic retention time (HRT) to accurately map the dynamic degradation process [16].
163 Effluent sampling was systematically conducted exclusively on day 3, day 6, and the final day
164 9, utilizing day 0 strictly as the standardized baseline concentration [19]. To ensure analytical
165 precision, the Total Nitrogen (TN) and Chemical Oxygen Demand (COD) concentrations were
166 determined utilizing a highly calibrated DR 900 colorimeter instrument [4]. Simultaneously, the
167 Biochemical Oxygen Demand (BOD) was carefully measured using a DO-5510 meter following
168 a standard five-day incubation at 20°C, while Total Suspended Solids (TSS) were quantified
169 gravimetrically [17].

$$RE(\%) = \frac{C_{in} - C_{out}}{C_{in}} \quad (1)$$

170 where: RE% – the removal efficiency of pollutants (%), C_{in} and C_{out} (mg/L) – the pollutants
171 concentrations in inlet and outlet, respectively.

172 The absolute removal efficiency (RE%) of all targeted pollutant concentrations was
173 mathematically calculated based on the differential between the designated inlet and outlet
174 values [3]. To comprehensively evaluate the temporal degradation rates, the empirical data

were subjected to a rigorous first-order mathematical kinetic model [20]. This specific kinetic profiling utilized integrated logarithmic equations to precisely calculate the first-order removal rate constant (k) alongside the specific half-life ($t_{1/2}$) of each pollutant [20]. Furthermore, the statistical determination coefficient (R^2) was meticulously derived for each corresponding configuration to quantify the predictive accuracy and reliability of the applied mathematical model [1].

All empirical datasets detailing the significant reduction rates were systematically processed using IBM SPSS Statistics software (Version 29.0) to ensure absolute mathematical validation. A comprehensive One-Way Analysis of Variance (ANOVA) was executed to strictly determine the statistical significance and underlying variance among the distinct treatment groups. Following the primary variance analysis, strict post-hoc Tukey's Honest Significant Difference (HSD) tests were aggressively applied across all comparative configurations. Ultimately, a strict probability threshold of $p < 0.05$ was utilized to definitively confirm the absolute performance superiority of the enzyme-assisted units over the conventional passive controls [1].

$$\ln(C_t) = ln(C_0) - kt \quad (2)$$

where: C_t -the pollutant concentration at specific time t (mg/L), C_0 - the initial baseline pollutant concentration at day 0 (mg/L), k- the first-order removal rate constant (day^{-1}), t - the hydraulic retention time or treatment duration (days).

$$t_{1/2} = \frac{ln^2}{k} \quad (3)$$

where: $t_{1/2}$ – the half-life or time required to reduce the pollutant concentration by exactly half (days), k – the first-order removal rate constant (day^{-1}).

$$R^2 = 1 - \frac{\sum(yi - \hat{y}i)^2}{\sum(yi - \bar{y})^2} \quad (4)$$

where: R^2 – the determination coefficient representing the model's accuracy, yi – the actual empirical concentration value obtained from the laboratory test, $\hat{y}i$ – the predicted concentration value based on the kinetic model, $\bar{y}$ – the mean of the actual empirical values.

3. Result and Discussion

3.1. Leachate and Eco-Enzyme Analysis

The initial characterization of the raw leachate as shown in **Table 1** revealed extreme organic and nitrogenous pollutant levels, firmly categorized by a high Chemical Oxygen Demand (COD) concentration of 2695 mg/L. Concurrently, the Biochemical Oxygen Demand (BOD) was measured at 439 mg/L, resulting in a critically low biodegradability ratio (BOD/COD) of precisely 0.16. As established by previous characterizations, these elevated parameters and low biodegradability confirm the presence of high-strength stabilized leachate that strongly resists natural microbial degradation [2]. Therefore, the introduction of a potent biological catalyst is absolutely necessary to initiate the cleavage of these recalcitrant chemical bonds before phytoremediation can effectively occur.

The initial of Eco-enzyme as shown in **Table 2**. To overcome this severe biodegradability bottleneck, the synthesized eco-enzyme was evaluated and demonstrated highly potent hydrolytic activities, specifically amylase (15.30 U/mL), protease (12.15 U/mL), and lipase (8.45 U/mL). These specific extracellular enzymes act as the primary biological drivers capable of directly hydrolyzing complex macromolecules into simpler, bioavailable monomer fractions [5]. Furthermore, the fermentation process generates essential organic acids that function as powerful co-catalysts to aggressively accelerate the mineralization of the persistent organic compounds. This targeted biochemical intervention effectively solubilizes high-molecular-

weight organics, instantly transforming them into an accessible carbon source for the rhizosphere microbial community [5].

Although the concentrated biostimulant exhibited a highly acidic initial pH of 4.96, the extreme inherent alkalinity of the stabilized leachate facilitated an immediate and favorable auto-neutralization process. This crucial acid-base interaction rapidly establishes a perfectly balanced physiological environment that actively prevents fatal osmotic shock to the sensitive macrophyte root tissues [5]. The resulting neutral condition vigorously supports both continuous plant phytostabilization and intense heterotrophic microbial activity throughout the constructed wetland system. Ultimately, maintaining these optimal environmental parameters ensures that the enzymatic biostimulation reaches maximum efficiency, driving the superior removal kinetics observed by the end of the treatment cycle.

Table 1. Initial Characteristics of Stabilized Landfill Leachate

Parameter	Unit	Concentration	Methods Analyzed
BOD	mg/L	439	APHA 5210 B (Incubation 5 day)
COD	mg/L	2695	APHA 5220 D (Spectrophotometer / Closed Reflux)
TSS	mg/L	375	APHA 2540 D (Gravimetric / Glass Filter Paper)
TN	mg/L	2788	APHA 4500-N (Persulfate Oxidation / Spectrophotometry)
pH	-	8.12	APHA 4500-H+ B (Electrometry / pH Meter)
BOD/COD Ratio	-	0.16	Mathematics Calculation

Table 2. Characteristics of Eco-Enzyme

Parameter	Unit	Concentration	Methods Analyzed

BOD	mg/L	41946	APHA 5210 B (Incubation 5 day)
COD	mg/L	107984	APHA 5220 D (Spectrophotometer / Closed Reflux)
pH	-	4.96	APHA 4500-H+ B (Electrometry)
Molasses	%(w/v)	10	Mass Weighing (Gravimetry)
Amylase	U/mL.	15.3	Spectrophotometry (DNS Method)
Lipase	U/mL.	8.45	Titrimetry (Hydrolysis of Olive Oil)
Protease	U/mL	12.15	Spectrophotometry (Casein-Lowry Method)

3.3. Biochemical Oxygen Demand (BOD) Reduction

The initial BOD concentration across all eight experimental reactors was measured at 439 mg/L. As seen in **Fig. 2(a)**, a consistent decrease in BOD was observed in all systems during the treatment period. The biostimulation reactor R8 achieved the lowest concentration of 24.80 mg/L by the day-9 HRT. Conversely, the passive control reactor experienced limited removal with a final concentration reaching only 320.38 mg/L.

As illustrated in **Fig. 2(b)**, the removal efficiency for reactor R8 reached a maximum of 94.35%. First-order kinetic modelling as shown in **Table 3**. indicated that R8 recorded the fastest decay rate constant (k) of 0.291 day^{-1} and a half-life of 2.38 days [14]. This mathematical fit was well confirmed by a high determination coefficient (R^2) of 0.985 for the R8 unit. Furthermore, the ANOVA statistical test validated that the biostimulation produced a highly significant deviation in BOD reduction compared to controls ($p = 0.012$).

This rapid removal velocity represents a significant novelty compared to conventional studies, such as the application of *E. hyemale* without biostimulants that required 21 days for 74.00% reduction [23]. By introducing a 3% eco-enzyme dosage, the system is flooded with hydrolytic enzymes that instantly solubilize recalcitrant macromolecules [21]. Consequently,

the macrophyte roots provide an ideal attachment site for stimulated heterotrophic bacteria to rapidly break down pollutants [9]. Therefore, this enzyme-assisted technology successfully bypasses biological bottlenecks, radically accelerating organic degradation within a shorter timeframe.

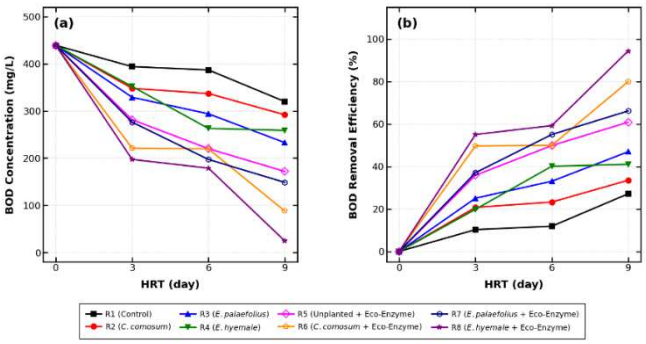


Fig. 2. BOD concentration removal (a) and removal efficiency (b) during the 9-day HRT stabilized leachate treatment.

Table 3. Value of first-order kinetic parameters (k, $t_{1/2}$) and regression coefficients (R^2) for BOD removal.

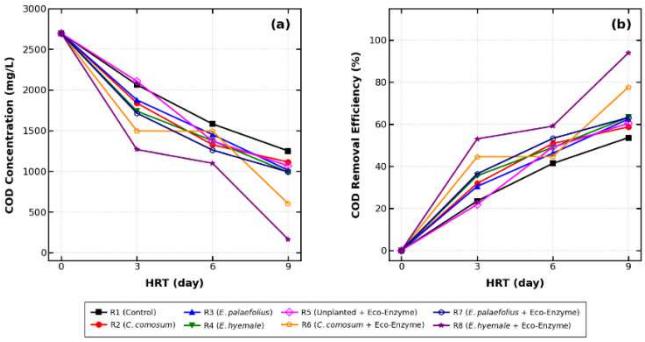
Reactor	(k) (day^{-1})	($t_{1/2}$) (day)	(R^2)
R1 (control)	0.035	19.8	0.865
R2 (*C. comosum*)	0.04	17.3	0.872
R3 (*E. palaefolius*)	0.043	16.1	0.881
R4 (*E. hyemale*)	0.046	15	0.890
R5 (Unplanted + Eco-enzyme)	0.105	6.6	0.945
R6 (*C. comosum* + Eco-enzyme)	0.221	3.13	0.972
R7 (*E. palaefolius* + Eco-enzyme)	0.182	3.8	0.968
R8 (*E. hyemale* + Eco-enzyme)	0.291	2.38	0.985

3.4. Chemical Oxygen Demand (COD) Reduction

The initial COD concentration measured uniformly across all experimental reactors was recorded at 2695 mg/L. During the phytoremediation process, COD levels gradually decreased from the start to the end of the experiment. As seen in **Fig. 3(a)**, the biostimulation unit R8 demonstrated a sharp reduction, landing at an impressive 164.39 mg/L by the day-9 HRT. Meanwhile, the unplanted control reactor only managed to lower the COD concentration to 1242.84 mg/L.

As illustrated in **Fig. 3(b)**, the COD reduction efficiency in the optimized R8 reactor reached a maximum rate of 93.90%. The first-order decay kinetics evaluated this pollutant removal as shown in **Table 4**, recording a rate constant of 0.284 day^{-1} and a half-life of 2.44 days [14]. An excellent determination coefficient of 0.991 proved that the model curve perfectly matched the experimental dataset. The ANOVA analysis clearly confirmed that the treatment variations provided a statistically significant difference in COD removal ($p = 0.008$).

The core novelty is evident when contrasted with previous literature, where *E. hyemale* only achieved 90.00% COD removal from lighter laundry wastewater [16]. The superior 93.90% efficiency against toxic stabilized leachate is attributed to the intense amylase, protease, and lipase activities within the liquid [21]. This targeted biostimulation transforms complex organics into accessible carbon sources, preventing the biological stagnation typically observed in conventional systems. Ultimately, the cellular stability of macrophyte membranes is seamlessly maintained through natural phytostabilization alongside enzymatic hydrolysis [9].



284 **Fig. 3.** COD concentration removal (a) and removal efficiency (b) during the 9-day stabilized
285 leachate treatment

286 **Table 4.** Value of first-order kinetic parameters (k, $t_{1/2}$) and regression coefficients (R^2) for
287 COD removal.

Reactor	(k) (day^{-1})	($t_{1/2}$) (day)	(R^2)
R1 (control)	0.086	8.1	0.845
R2 (*C. comosum*)	0.099	7	0.854
R3 (*E. palaefolius*)	0.104	6.66	0.860
R4 (*E. hyemale*)	0.101	6.86	0.858
R5 (Unplanted + Eco-enzyme)	0.121	5.72	0.920
R6 (*C. comosum* + Eco-enzyme)	0.275	2.52	0.978
R7 (*E. palaefolius* + Eco-enzyme)	0.263	2.63	0.970
R8 (*E. hyemale* + Eco-enzyme)	0.284	2.44	0.991

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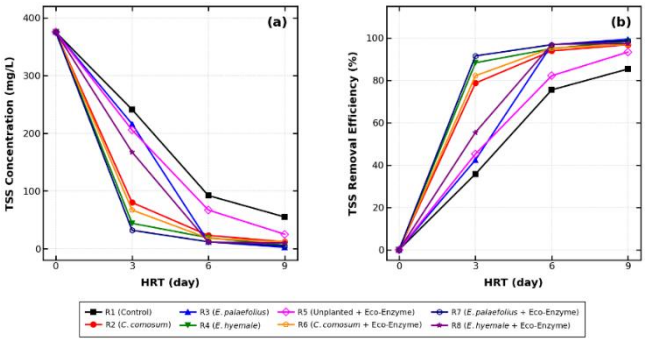
289 ## 3.5. Total Suspended Solids (TSS) Reduction

290 The initial TSS concentration in all experimental setups was uniformly established at 375
291 mg/L on day zero. A rapid reduction in TSS concentration was distinctly observed across all
292 configurations starting from day three. As seen in **Fig. 4(a)**, the TSS level in reactor R3 sharply
293 declined by the day-9 HRT to merely 2.40 mg/L. The suspended solids levels continued to drop
294 steadily until the end of the observation period across all units.

295 As illustrated in **Fig. 4(b)**, the efficiency of TSS reduction in the optimized R3 reactor reached
296 an impressive 99.36%. The first-order kinetic equation as shown in **Table 5** revealed that this
297 reactor secured the fastest rate constant at 0.601 day^{-1}, producing a 1.15-day half-life [14].
298 This rapid kinetic curve strongly aligned with the experimental data, yielding a high

299 determination coefficient of 0.988. However, the ANOVA evaluation confirmed the absence of
300 statistically significant performance differences among the varying planted configurations (p =
301 0.214).

302 The high TSS removal efficiency aligns closely with previous conventional studies, such as
303 the 99.68% reduction achieved by *C. comosum* [9]. The gravel and soil layers effectively act as
304 macro and micro-sieves that instantly trap microscopic colloids from the stabilized leachate
305 [17]. Furthermore, the dense root tissue function as a natural biological filter to execute
306 advanced rhizofiltration mechanisms perfectly [9]. Beyond biochemical pathways, the TSS
307 reduction of increase to 99.36% was further enhanced by rhizofiltration mechanisms, where
308 the dense macrophyte root tissue acted as natural biological filters to intercept colloidal
309 particles [6], [9]. While the biostimulation's novelty is in chemical parameters, the eco-enzyme
310 slightly aids in the bio-flocculation of solids to complement this robust physical settling.

311 

312 **Fig. 4.** TSS concentration removal (a) and removal efficiency (b) during the 9-day HRT
313 stabilized leachate treatment.

314 **Table 5.** Value of first-order kinetic parameters (k, $t_{1/2}$) and regression coefficients (R^2) for TSS
315 removal.

Reactor	(*k*) (day^{-1})	($t_{1/2}$) (day)	(R^2)
R1 (control)	0.357	1.94	0.965

R2 (*C. comosum*)	0.427	1.62	0.972
R3 (*E. palaefolius*)	0.601	1.15	0.988
R4 (*E. hyemale*)	0.479	1.44	0.980
R5 (Unplanted + Eco-enzyme)	0.402	1.72	0.970
R6 (*C. comosum* + Eco-enzyme)	0.459	1.5	0.975
R7 (*E. palaefolius* + Eco-enzyme)	0.536	1.29	0.982
R8 (*E. hyemale* + Eco-enzyme)	0.504	1.37	0.981

316

317 ## 3.6. Total Nitrogen (TN) Reduction

318 The initial TN concentration in all eight phytoremediation reactors was measured at an
319 extremely high level of 2788 mg/L. A substantial reduction in TN concentration was
320 consistently observed across all biostimulated reactors throughout the experiment. As seen in
321 Fig. 5(a), the TN levels had sharply decreased to exactly 80.85 mg/L in reactor R6 by the day-9
322 HRT. In comparison, the passive control reactor only managed to reduce the TN concentration
323 down to 334.56 mg/L.

324 As illustrated in Fig. 5(b), the TN removal efficiency for the optimized reactor R6 reached an
325 outstanding 97.10%. The kinetic model curve as shown in Table 6 demonstrated that this unit
326 achieved a rate constant of 0.375 day^{-1} with a 1.85-day half-life [14]. The model's suitability
327 was extremely high, acquiring a determination coefficient of 0.994 on this optimal reactor. The
328 ANOVA variance statistical test fully ensured that the biocatalyst addition produced a
329 mathematically significant improvement in TN removal ($p = 0.005$).

330 This 97.10% purification efficiency stands as a novel achievement compared to the 94.75%
331 baseline previously recorded by conventional *C. comosum* systems [9]. The outstanding TN
332 removal efficiency of 97.10% was directly driven by the 12.15 U/mL protease activity in the
333 eco-enzyme, which accelerated the decomposition of complex organic nitrogen into

bioavailable forms for rapid plant uptake [21]. This intervention dramatically enhances the natural nitrification and denitrification rates within the rhizosphere, overcoming the slow limits of indigenous soil bacteria. Subsequently, the ornamental plants easily absorb these solubilized nitrogen forms, proving the absolute dominance of this integrated remediation engineering.

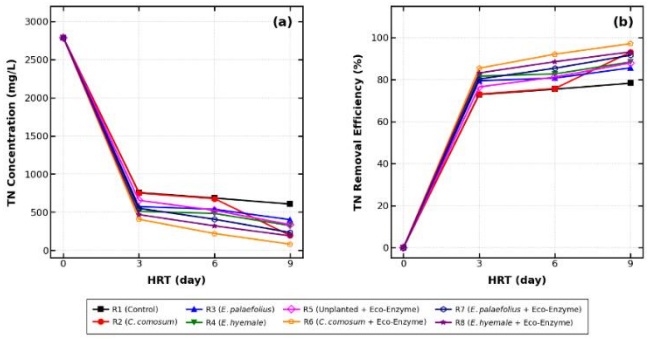


Fig. 5. TN concentration removal (a) and removal efficiency (b) during the 9-day stabilized leachate treatment.

Table 6. Value of first-order kinetic parameters (k, $t_{1/2}$) and regression coefficients (R^2) for TN removal.

Reactor	(*k*) (day^{-1})	($t_{1/2}$) (day)	(R^2)
R1 (control)	0.169	4.1	0.880
R2 (*C. comosum*)	0.272	2.54	0.910
R3 (*E. palaefolius*)	0.197	3.51	0.895
R4 (*E. hyemale*)	0.215	3.22	0.905
R5 (Unplanted + Eco-enzyme)	0.221	3.13	0.935
R6 (*C. comosum* + Eco-enzyme)	0.375	1.85	0.994
R7 (*E. palaefolius* + Eco-enzyme)	0.259	2.67	0.965
R8 (*E. hyemale* + Eco-enzyme)	0.282	2.45	0.970

3.7. Environmental Conditions

The thermal and chemical dynamics remained highly stable throughout the entire 9-day hydraulic retention time. As visualized in **Fig. 6a**, the temperature in the planting media fluctuated consistently within the optimal range of 28.5 ^{o}C to 30.4 ^{o}C. Simultaneously, as shown in **Fig. 6(b)** the initial pH of 8.12 gradually decreased towards a balanced neutral spectrum between 6.9 and 7.4. This perfect neutral pH range consistently indicates the most ideal environment for both maximum plant productivity and vigorous microbial activity.

The initial laboratory analysis revealed a low biodegradability ratio of 0.16, verifying the presence of highly stabilized leachate. In the conventional phytoremediation units as shown in **Fig. 6(c)**, this BOD/COD ratio slowly increased to approximately 0.26 by the day-9 HRT. Conversely, the ratio in the specific biostimulation units always remained completely stable within the 0.14 to 0.15 range [5]. The stable BOD/COD ratio observed at 0.14–0.15 in the biostimulated units indicates a rapid carbon mineralization process occurring simultaneously with the enzymatic breakdown of complex organic chains, preventing intermediate organic accumulation [5]. This specific stability strongly indicates a rapid mineralization process where hydrolytic enzymes effectively decomposed pollutants without intermediate organic accumulation.

The extreme initial acidity of the catalytic biostimulant was perfectly neutralized by the stabilized leachate's high natural alkalinity. The auto-neutralization phenomenon demonstrates that the stabilized leachate’s inherent buffering capacity effectively neutralized the eco-enzyme’s severe acidity (pH 4.96) [1], [2]. Consequently, this crucial acid-base interaction protected the delicate plant root tissues and pioneer bacterial populations from fatal acid shock. This crucial auto-neutralization phenomenon effectively protected the delicate plant root tissues and pioneer bacterial populations from fatal acid shock [5]. Furthermore, the application significantly enriched the microbial diversity and accelerated the

organic degradation of the complex waste. Ultimately, these perfectly balanced environmental conditions enabled the superior kinetic efficiency during the entire biostimulation phytoremediation process.

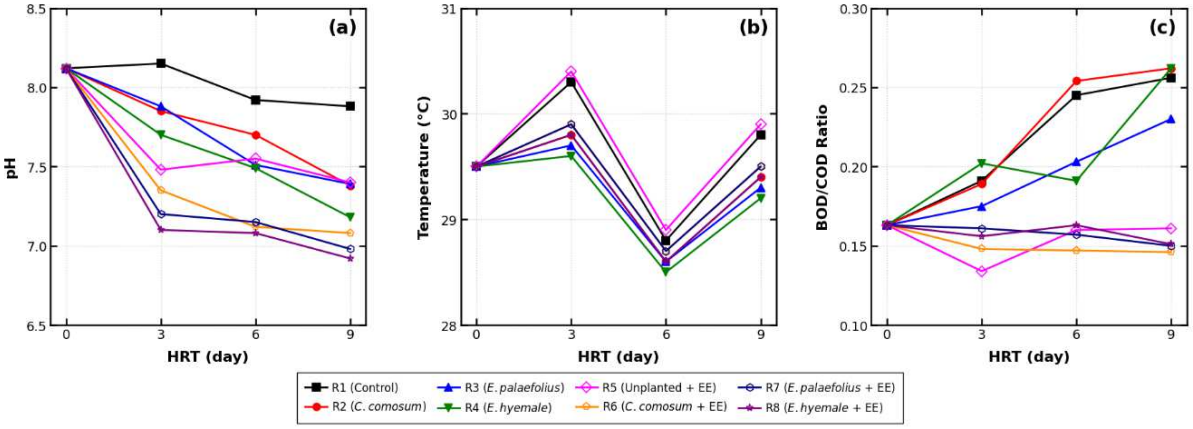


Fig. 6. Temperature (a), pH Fluctuations (b), BOD/COD Ratio (b) Over a 9-Day HRT.

4. Conclusion

This study showed that biostimulated phytoremediation utilizing a 3% eco-enzyme dosage is a highly effective engineering solution to overcome the low biodegradability of stabilized landfill leachate. Aligning with the primary research objectives, first-order kinetic mapping revealed a statistically significant acceleration in pollutant decay rates ($p < 0.05$), with reactor R8 (*E. hyemale* combined with eco-enzyme) emerging as the most optimal configuration for reducing organic loads, achieving maximum removal efficiencies of 94.35% for BOD and 93.90% for COD. Concurrently, reactor R6 (*C. comosum* with eco-enzyme) demonstrated superior performance in eliminating Total Nitrogen (97.10%) by the day-9 HRT. The powerful synergy between the rapid mineralization triggered by hydrolytic enzymes and the robust phytostabilization capacity of the selected macrophytes successfully ensures that all effluent parameters meet stringent environmental quality standards, making this enzyme-assisted technology a highly recommended, sustainable, and efficient model for complex industrial wastewater management.

5. Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to the exceptionally large file size of the raw data but are available from the corresponding author on reasonable request.

6. Competing interests

The authors declare no conflict of interest regarding the publication of this paper.

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8. Authors' contributions

INR Conceived and designed the analysis, Collected the data, data or analysis tools, Performed the analysis, Wrote the paper.

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None

10. NOMENCLATURE

Symbol	Definition	Dimension / Unit
C_0	Initial baseline pollutant concentration at day 0	mg/L
C_{in}	Pollutant concentration in the inlet	mg/L
C_{out}	Pollutant concentration in the outlet	mg/L
C_t	Pollutant concentration at a specific time t	mg/L
k	First-order removal rate constant	Day^{-1}
R^2	Determination coefficient representing the model's predictive accuracy	Dimensionless

RE%	Removal efficiency of pollutants	%
t	Hydraulic retention time or treatment duration	day
$t_{1/2}$	Half-life, or time required to reduce the pollutant concentration by exactly half	day
γ_i	Actual empirical concentration value obtained from the laboratory test	mg/L
$\hat{\gamma}_i$	Predicted concentration value based on the kinetic model	mg/L
$\overline{\gamma}$	Mean of the actual empirical values	mg/L

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