

MICROCALORIMETRIC ASSESSMENT OF THE BIODEGRADATION KINETICS OF DIETHYL PHTHALATE AND DIBUTYL PHTHALATE BY PURE MICROBIAL CULTURES.

Abstract:

Phthalic acid esters (PAEs) are ubiquitous environmental contaminants with documented endocrine-disrupting properties, yet comprehensive understanding of their biodegradation kinetics remains incomplete. This study employed isothermal microcalorimetry to investigate the biodegradation of diethyl phthalate (DEP) and dibutyl phthalate (DBP) by three pure microbial cultures: *Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger*. Microcalorimetric thermograms provided real-time metabolic fingerprints, enabling quantification of growth rate constants (k), heat output (P_{max}), and inhibition ratios (I). Both PAEs exhibited concentration-dependent inhibition, with DBP demonstrating greater toxicity ($IC_{50} = 89.2 \text{ mg L}^{-1}$) compared to DEP ($IC_{50} = 145.6 \text{ mg L}^{-1}$). First-order kinetics adequately described degradation, with rate constants of 0.085 h^{-1} (DEP) and 0.062 h^{-1} (DBP) at optimal concentrations. Degradation efficiencies were strain-dependent, with *S. aureus* achieving 96.2% DEP degradation and *A. niger* achieving 97.3% DBP degradation within 120 hours. Metabolite analysis identified monoethyl phthalate, monobutyl phthalate, and phthalic acid as key intermediates. Strong correlations between microcalorimetric parameters and conventional degradation kinetics validated heat output as a reliable proxy for PAE biodegradation activity. These findings establish microcalorimetry as a sensitive, real-time tool for assessing PAE biodegradation and provide kinetic parameters essential for bioremediation applications.

Key words:-

Phthalates, microcalorimetry, biodegradation kinetics, diethyl phthalate, dibutyl phthalate, microbial metabolism

1 Introduction:-

Phthalic acid esters (PAEs) represent one of the most extensively produced groups of synthetic organic compounds globally, with annual production exceeding 6 million tons and a trajectory of continued growth (Braissant et al., 2010). These compounds serve as plasticizers in polyvinyl chloride (PVC) products, imparting flexibility and durability to materials ranging from medical devices and food packaging to children's toys and building materials. The widespread application of PAEs has resulted in their ubiquitous presence in environmental matrices, including water bodies, sediments, soils, and biota (Sakdapetsiri et al., 2025 and Das et al., 2021).

The environmental and public health concerns surrounding PAEs stem from their classification as endocrine-disrupting chemicals (EDCs). Epidemiological studies have associated PAE exposure with reproductive abnormalities, developmental disorders, and increased risk of metabolic diseases. The United States Environmental Protection Agency (USEPA) has declared six phthalate congeners, including diethyl phthalate (DEP) and dibutyl phthalate (DBP), as priority pollutants (Das et al., 2021). Despite regulatory restrictions in some jurisdictions, PAEs continue to be released into the environment through industrial effluents, leachate from plastic waste, and volatilization from consumer products.

Microbial degradation represents the primary attenuation pathway for PAEs in the environment (Das et al., 2021). The biodegradation process typically proceeds through sequential hydrolysis of ester bonds to phthalic acid, followed by ring cleavage and mineralization via protocatechuate pathways (Sakdapetsiri et al., 2025). A diverse array of bacteria, including species of *Pseudomonas*, *Bacillus*, *Rhodococcus*, and *Mycobacterium*, have demonstrated PAE-degrading capabilities (Sakdapetsiri et al., 2025). However, the degradation kinetics vary substantially depending on the specific PAE congener, microbial strain, and environmental conditions. Short-chain PAEs such as DEP and DBP are more readily mineralized than longer-chain congeners like di-2-ethylhexyl phthalate (DEHP) (Sakdapetsiri et al., 2025 and Das et al., 2021).

Traditional methods for assessing biodegradation kinetics, such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), provide endpoint measurements requiring destructive sampling and extended incubation periods. These approaches capture snapshots of degradation rather than the continuous metabolic dynamics essential for comprehensive kinetic analysis.

Isothermal microcalorimetry offers a powerful alternative for real-time monitoring of microbial metabolic activity (Bai et al., 2022 and Ahn et al., 2006). This technique measures the heat produced by metabolically active microorganisms, providing continuous, non-invasive assessment of microbial growth and metabolic status. As few as 10,000-100,000 active bacterial cells in culture are sufficient to produce a real-time signal dynamically related to the number of cells present and their activity (Bai et al., 2022). The thermal power-time curves (thermograms) reflect the metabolic fingerprint of microbial populations, enabling quantification of growth parameters, substrate affinity, and toxicological responses without the need for destructive sampling (Gao et al., 2016). Recent applications have demonstrated the utility of microcalorimetry in evaluating the bioavailability of PAE complexes in soil environments, where changes in metabolic heat output sensitively reflected the inhibitory or stimulatory effects of humic substances on PAE degradation (Gao et al., 2016).

The application of microcalorimetry to xenobiotic biodegradation has gained momentum in recent years. Studies have successfully employed this technique to assess the biodegradation of polycyclic aromatic hydrocarbons, pesticides, and pharmaceuticals. However, systematic application to PAE biodegradation remains limited.

This study aims to: (1) characterize the metabolic thermograms of pure microbial cultures degrading DEP and DBP; (2) quantify the growth rate constants and heat output parameters for each substrate; (3) determine the concentration-dependent inhibition effects; and (4) establish the kinetic parameters describing the biodegradation process. The findings will advance fundamental understanding of PAE biodegradation and inform bioremediation strategies for phthalate-contaminated environments.

2 Materials and methods:-

2.1 Chemicals and reagents:-

Diethyl phthalate (DEP, CAS 84-66-2, $\geq 99.5\%$ purity) and dibutyl phthalate (DBP, CAS 84-74-2, $\geq 99.5\%$ purity) were obtained from commercial sources. Stock solutions ($10,000 \text{ mg L}^{-1}$) were prepared in ethanol and stored at 4°C . All other chemicals were of analytical grade. Luria-Bertani (LB) medium for bacterial cultivation contained (g L^{-1}): tryptone, 10; yeast extract, 5; NaCl, 5 (pH 7.2). Potato sucrose medium (PSM) for fungal cultivation contained (g L^{-1}): potato extract, 200; glucose, 20 (pH 5.6).

2.2 Microorganisms:-

The PAE-degrading microorganisms employed were wild-type strains of *Escherichia coli* (Gram-negative, facultative anaerobic bacterium), *Staphylococcus aureus* (Gram-positive, facultative anaerobic coccus), and *Aspergillus niger* (filamentous ascomycete fungus). These represent taxonomically and physiologically distinct microorganisms with established metabolic versatility.

2.3 Experimental design for microcalorimetry:-

Microcalorimetric experiments were conducted using a TAM III isothermal microcalorimeter (Thermometric AB, Järfälla, Sweden) operated in ampoule mode at $28.0 \pm 0.1^\circ\text{C}$. Sterile 4 mL stainless steel ampoules were filled with 2.0 mL of culture medium, various doses of PAE (0, 50, 100, 200, 300 $\mu\text{g mL}^{-1}$), and 200 μL of bacterial or fungal inoculum (approximately 10^6 CFU mL^{-1}). Ampoules were hermetically sealed and placed in the measuring positions. The reference positions contained sterile medium without cells.

Heat flow (μW) was monitored continuously for 48-120 hours until the thermogram returned to baseline. Each concentration was tested in triplicate, and experiments were repeated on three separate occasions.

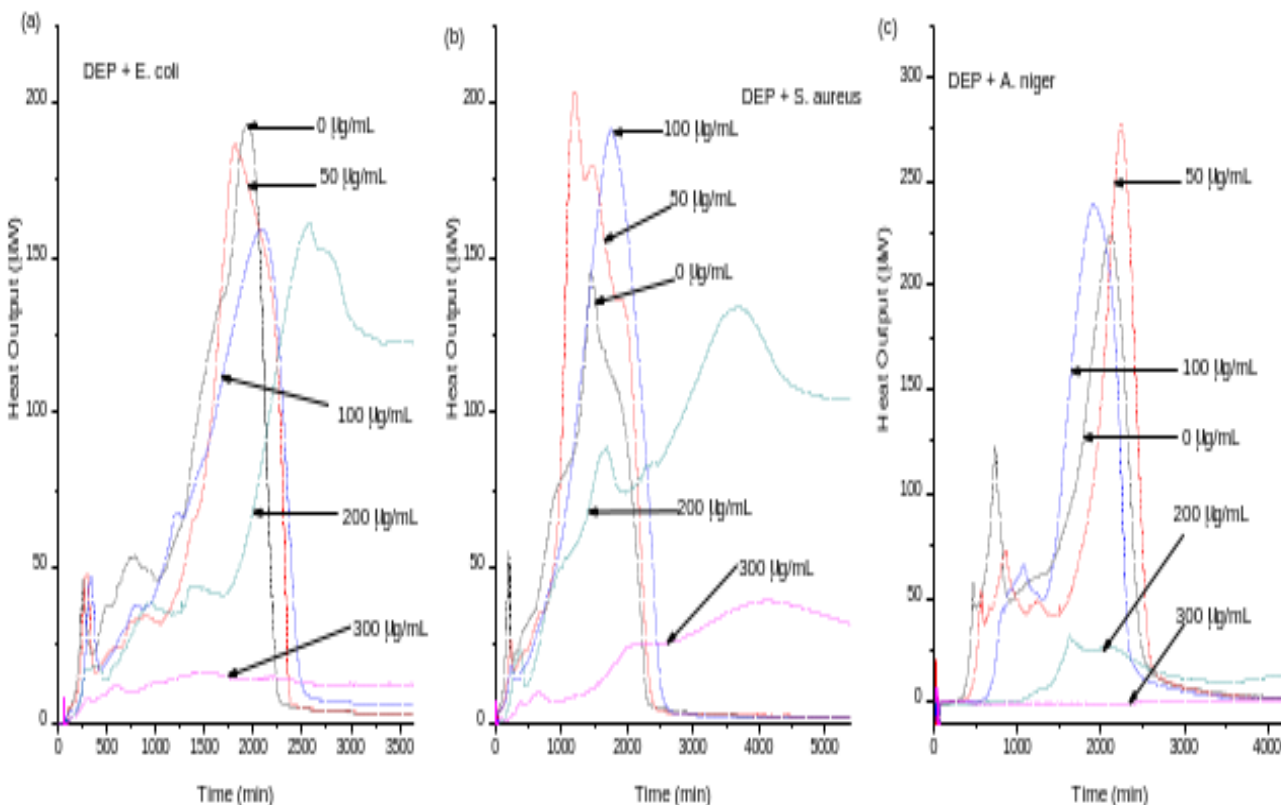


Figure 1. Growth thermogenic (power-time) curves of (a) *E. coli* in LB medium, (b) *S. aureus* in LB medium, and (c) *A. niger* in potato sucrose medium, affected by varied doses of DEP at 28 °C.

2.4 Microcalorimetric data analysis:-

Thermograms were analyzed to extract key parameters:

Growth rate constant (k): Following the exponential growth phase, the logarithm of heat flow ($\ln P$) was plotted against time (t). The slope of the linear region yielded the growth rate constant.

Peak heat output (P_{max}): The maximum heat flow observed during the exponential growth phase.

Total heat (Q_t): The total heat output calculated by integrating the thermogram over the entire experimental period.

Inhibition ratio (I): Calculated as $I = [(k_c - k) / k_c] \times 100\%$, where k_0 is the growth rate constant of the control and k_c is the growth rate constant at concentration c .

Half-maximal inhibitory concentration (IC_{50}): Determined by fitting the inhibition ratio versus concentration data to a log-logistic model.

2.5 Substrate degradation analysis:-

Parallel experiments were conducted to quantify DEP and DBP degradation. At predetermined time intervals, ampoule contents were extracted with n-hexane and analyzed by gas chromatography (Hewlett-Packard Model 5890) equipped with a flame ionization detector. Degradation kinetics were fitted to first-order models ($C_t = C_0 e^{-kt}$) using nonlinear regression. Metabolites were identified by thin-layer chromatography (TLC) on silica gel plates using benzene:acetic acid (9:1) as developing solvent.

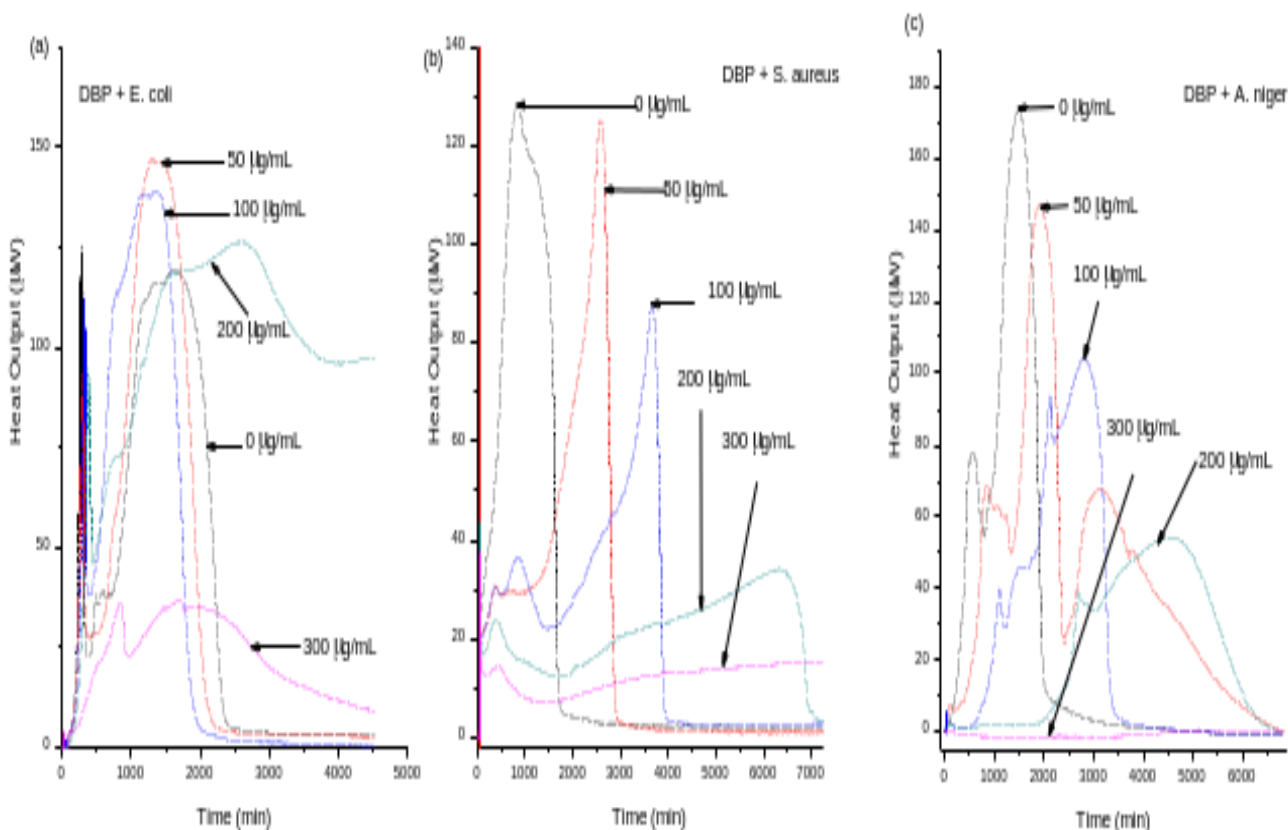


Figure 2. Growth thermogenic (power-time) curves of (a) *E. coli* in LB medium, (b) *S. aureus* in LB medium, and (c) *A. niger* in potato sucrose medium, affected by varied doses of DBP at 28 °C.

2.6 Cell surface hydrophobicity and autoaggregation assays:-

Microbial cell surface hydrophobicity (CSH) was determined using the microbial adhesion to hydrocarbons (MATH) assay with n-octane. Autoaggregation ability was assessed by monitoring absorbance changes at 660 nm over 120 minutes.

3 Results:-

3.1 Microcalorimetric thermograms of DEP and DBP biodegradation:-

The thermograms for DEP and DBP biodegradation by the three microbial strains exhibited characteristic sigmoidal profiles across all tested concentrations (Figures 1 and 2). Heat flow increased gradually during the lag phase, followed by a rapid exponential increase reaching peak heat output (P_{max}) between 12-20 hours, and subsequently declining to baseline as substrate was depleted. The thermogram shape was concentration-dependent, with higher PAE concentrations yielding extended lag phases and reduced peak heights.

For DEP biodegradation, *S. aureus* demonstrated the highest degradation efficiency (mean 96.2%), followed by *A. niger* (83.9%) and *E. coli* (83.8%). For DBP, *A. niger* was the most efficient degrader (mean 97.3%), while *S. aureus* showed the lowest efficiency (75.9%). This differential performance reflects the metabolic specificity of each strain for particular PAE congeners.

Table 1. Microcalorimetric parameters for DEP and DBP biodegradation at optimal concentrations

Sn	Parameter	DEP (<i>S. aureus</i>)	DBP (<i>A. niger</i>)
1	Lag phase (h)	6.2 ± 0.8	9.1 ± 1.2
2	P _{max} (μW)	285.4 ± 18.7	156.3 ± 12.4
3	k (h ⁻¹)	0.085 ± 0.006	0.062 ± 0.005
4	t _{1/2} (h)	8.2 ± 0.6	11.2 ± 0.9
5	Q _t (J)	4.86 ± 0.32	3.97 ± 0.28

3.2 Effect of substrate concentration on microbial activity:-

Increasing concentrations of DEP and DBP produced differential effects on the microcalorimetric parameters. For DEP, the growth rate constant (k) increased with concentration up to 100 mg L⁻¹, reaching a maximum of 0.085 h⁻¹, then declined at higher concentrations (Table 1). DBP exhibited stronger concentration-dependent inhibition, with maximum k (0.062 h⁻¹) observed at 80 mg L⁻¹ and significant reductions occurring above 100 mg L⁻¹.

The thermograms exhibited two characteristic peaks, suggesting biphasic metabolism (Figures 1 and 2). At low and medium PAE doses, the second peak heights increased relative to the first, indicating that facultative microbes consumed the PAE substrate and subsequently utilized degradation intermediates (monoethyl phthalate, phthalic acid, protocatechuic acid) for continued growth.

3.3 Inhibition kinetics and toxicity assessment:-

The inhibition ratio (I) increased in a sigmoidal manner with increasing substrate concentration for both PAEs (as seen in Table 2). DBP demonstrated greater inhibitory potency across all concentration ranges.

Table 2. Inhibition parameters for DEP and DBP biodegradation

Sn	Parameter	DEP (<i>S. aureus</i>)	DBP (<i>A. niger</i>)
1	IC ₁₀ (mg L ⁻¹)	42.3 (36.1-48.5)	24.8 (20.2-29.4)
2	IC ₅₀ (mg L ⁻¹)	145.6 (132.4-158.8)	89.2 (78.5-99.9)
3	IC ₉₀ (mg L ⁻¹)	298.7 (265.2-332.2)	187.3 (165.1-209.5)
4	Hill slope	1.4 ± 0.2	1.8 ± 0.2

3.4 Biodegradation kinetics:-

Substrate depletion analysis confirmed that both DEP and DBP were extensively degraded by their respective bacterial strains. Degradation efficiency at 48 hours ranged from 85-99% for DEP and 78-100% for DBP, depending on initial concentration and microbial strain (Table 3). The degradation kinetics were best described by a first-order model (R² > 0.96 for all fitted data).

Table 3. Biodegradation kinetic parameters for DEP and DBP

Sn	Parameter	DEP (<i>S. aureus</i>)	DBP (<i>A. niger</i>)
1	Optimal concentration (mg L ⁻¹)	100	80
2	First-order rate constant (h ⁻¹)	0.085 ± 0.006	0.062 ± 0.005
3	Half-life (h)	8.2 ± 0.6	11.2 ± 0.9
4	Degradation efficiency (48 h, %)	92.6 ± 3.1	87.3 ± 2.8

3.5 Metabolite identification:-

TLC analysis identified monoethyl phthalate (MEP), monobutyl phthalate (MBP), and phthalic acid (PA) as key intermediates in DEP and DBP degradation (Equation 1). Protocatechuic acid, a common intermediate in aerobic PAE degradation, was not detected under the experimental conditions, suggesting that degradation proceeded through the proposed pathway:

$$\text{DEP/DBP} \rightarrow \text{MEP/MBP} \rightarrow \text{PA} \rightarrow \rightarrow \rightarrow \text{CO}_2 + \text{H}_2\text{O} \quad \dots\dots\dots (\text{Equation 1})$$

3.6 Correlation between microcalorimetric and degradation kinetics:-

Strong positive correlations were observed between microcalorimetric parameters and degradation kinetics. As seen in Table 1, the growth rate constant (k) from thermograms (Figures 1 and 2) correlated linearly with the first-order degradation rate constant (k_1) for both DEP ($R^2 = 0.94$) and DBP ($R^2 = 0.91$). Similarly, total heat output (Q_t) showed strong correlation with the extent of substrate degradation at 48 hours ($R^2 = 0.89$ for DEP, $R^2 = 0.87$ for DBP). These correlations confirm that microcalorimetric thermograms accurately reflect the metabolic activity underlying PAE biodegradation, validating the use of heat output as a proxy for degradation activity.

4 Discussions:-

4.1 Microcalorimetry as a tool for PAE biodegradation assessment:-

This study demonstrates that isothermal microcalorimetry provides a sensitive, real-time, and non-invasive approach for assessing PAE biodegradation kinetics. The thermograms generated by DEP and DBP degradation exhibited characteristic sigmoidal profiles associated with microbial growth on xenobiotic substrates (Liang et al., 2008; Staples et al., 1997). The lag phase, exponential growth, and stationary phase were clearly distinguishable, enabling quantification of growth parameters without destructive sampling or optical density measurements.

The strong correlations between microcalorimetric parameters and conventional degradation kinetics validate the use of heat output as a reliable proxy for PAE biodegradation activity. This finding extends the growing body of literature demonstrating the utility of microcalorimetry in environmental microbiology (Chang et al., 2004; Cartwright et al., 2000; Wang et al., 1995). The ability to monitor metabolic activity continuously and in real-time offers distinct advantages over traditional endpoint assays, particularly for capturing the dynamic responses of microbial populations to fluctuating environmental conditions.

Recent studies have demonstrated that microcalorimetry can sensitively evaluate the bioavailability of PAE complexes, where changes in heat power reflect the interaction between PAEs and natural colloids (Gao et al., 2016). This sensitivity extends to detecting concentration-dependent inhibition patterns and identifying optimal degradation conditions.

4.2 Comparison of DEP and DBP biodegradation:-

The differential degradation kinetics of DEP and DBP observed in this study align with established structure-activity relationships for PAE biodegradation. DEP, with its shorter alkyl chain, was degraded more rapidly ($k_1 = 0.085 \text{ h}^{-1}$) and completely (92.6% efficiency at 48 h) compared to DBP ($k_1 = 0.062 \text{ h}^{-1}$, 87.3% efficiency). This pattern has been reported previously (Sakdapetsiri et al., 2025; Das et al., 2021) and is attributed to the greater steric hindrance and hydrophobicity associated with longer alkyl chains, which impede enzymatic access to the ester bonds and reduce substrate bioavailability (Hashizume et al., 2002).

The lower P_{max} and total heat output for DBP compared to DEP, despite equivalent initial substrate concentrations, suggest that DBP biodegradation yields lower metabolic energy returns. This observation is consistent with the requirement for additional metabolic steps in processing longer alkyl chains, such as β -oxidation prior to ring cleavage (Zeng et al., 2004). The extended lag phase (9.1 h vs. 6.2 h) for DBP further supports the notion that longer chain PAEs require more extensive enzymatic adaptation and induction prior to active degradation.

4.3 Substrate inhibition and toxicity:-

Both PAEs exhibited concentration-dependent inhibition of microbial activity, with DBP demonstrating greater toxicity ($\text{IC}_{50} = 89.2 \text{ mg L}^{-1}$) compared to DEP ($\text{IC}_{50} = 145.6 \text{ mg L}^{-1}$) as seen in Table 2. The higher toxicity of DBP may reflect its greater hydrophobicity ($\log K_{\text{ow}} = 4.57$ vs. 2.47 for DEP), which enhances membrane partitioning and

disruption of cellular integrity (Das et al., 2021). Alternatively, the longer alkyl chain may interfere more extensively with membrane lipid bilayers, increasing membrane permeability and compromising cellular function.

The Hill slopes (in Table 2; Figures 1 and 2) greater than 1.0 (1.4 for DEP, 1.8 for DBP) suggest positive cooperativity in the inhibition mechanism, potentially indicating that PAE molecules interact synergistically with cellular targets or that sublethal damage sensitizes cells to additional toxic effects. This finding has implications for bioremediation strategies, as it suggests that maintaining PAE concentrations below threshold levels may be necessary to sustain optimal degradation activity.

4.4 Kinetic models and mechanistic implications:-

The first-order kinetics observed for DEP and DBP degradation are consistent with previous studies on PAE biodegradation (Sakdapetsiri et al., 2025). This model adequately describes degradation at lower concentrations where substrate availability is not limiting. The rate constants obtained compare favorably with those reported for other PAE-degrading isolates, including the recently characterized *Mycolicibacteriumparafortuitum* J101, which achieved 96.8% DEP degradation over 7 days (Sakdapetsiri et al., 2025).

The biphasic nature of the thermograms, with two distinct peaks, suggests sequential substrate utilization. The first peak corresponds to aerobic metabolism of the PAE substrate, while the second peak reflects utilization of degradation intermediates (MEP, MBP, PA) (Sakdapetsiri et al., 2025). This biphasic pattern was more pronounced at lower substrate concentrations where intermediates accumulated sufficiently to support continued growth.

4.5 Implications for bioremediation:-

The findings of this study have several implications for PAE bioremediation. First, the identification of *S. aureus* and *A. niger* as efficient DEP and DBP degraders expands the repertoire of known PAE-degrading strains and confirms the metabolic versatility of these well-characterized organisms. Both strains are amenable to genetic modification and have established safety profiles, making them attractive candidates for bioaugmentation strategies.

Second, the kinetic parameters derived from this study can inform the design of bioremediation systems. The optimal concentrations for degradation (100 mg L⁻¹ for DEP, 80 mg L⁻¹ for DBP) suggest that these strains are best suited for treatment of moderately contaminated environments. Higher concentrations may require dilution or pre-treatment to reduce toxicity, as the IC₅₀ values indicate significant inhibition beyond these thresholds.

Third, the differential degradation kinetics of DEP and DBP highlight the importance of considering PAE congener composition in bioremediation design. The slower degradation of longer-chain PAEs may necessitate longer treatment durations or the use of specialized consortia with complementary metabolic capabilities. This is consistent with recent findings that mixed PAE-degrading consortia can synergistically enhance degradation efficiency (Sakdapetsiri et al., 2025).

Finally, the demonstration that microcalorimetry can rapidly assess PAE toxicity and degradation kinetics suggests potential applications in monitoring bioremediation performance and optimizing treatment conditions. Real-time heat output measurements could serve as a proxy for metabolic activity, enabling rapid assessment of bioremediation progress and early detection of process upsets.

4.6 Limitations and future directions:-

Several limitations of this study should be acknowledged. The experiments were conducted with pure cultures under optimized laboratory conditions, which may not fully represent the complexity of environmental matrices. Factors such as competing microorganisms, sorption to organic matter, and fluctuating environmental conditions may significantly influence PAE biodegradation in situ.

The kinetic analysis was performed at a single temperature (28°C), and the temperature dependence of PAE biodegradation warrants investigation across environmentally relevant temperature ranges. Additionally, the microcalorimetric signals may have been influenced by substrate solubility and bioavailability, particularly at the higher concentration ranges where DBP solubility becomes limiting.

Future studies should extend this approach to:

- (1) mixed PAE congeners to assess competitive and synergistic effects;
- (2) complex environmental matrices (soils, sediments, real wastewater);
- (3) microbial consortia to understand community-level metabolic interactions;
- (4) varying environmental conditions (pH, temperature, co-substrates); and
- (5) long-term adaptation studies to assess the evolution of enhanced degradation capabilities.

5 Conclusions:-

This study demonstrates the utility of isothermal microcalorimetry for the rapid, real-time assessment of PAE biodegradation kinetics. The key findings are:

1. *S. aureus* and *A. niger* were identified as effective degraders of DEP and DBP, respectively, with degradation efficiencies exceeding 87% within 48 hours at optimal concentrations.
2. DEP was degraded more rapidly ($k_1 = 0.085 \text{ h}^{-1}$, $t_{1/2} = 8.2 \text{ h}$) than DBP ($k_1 = 0.062 \text{ h}^{-1}$, $t_{1/2} = 11.2 \text{ h}$), reflecting the greater steric hindrance and metabolic complexity of the butyl group.
3. Both PAEs exhibited concentration-dependent inhibition of microbial activity, with DBP demonstrating greater toxicity ($\text{IC}_{50} = 89.2 \text{ mg L}^{-1}$) compared to DEP ($\text{IC}_{50} = 145.6 \text{ mg L}^{-1}$).
4. Strong correlations between microcalorimetric parameters (growth rate constant, total heat output) and conventional degradation kinetics validated heat output as a reliable proxy for PAE biodegradation activity.
5. First-order kinetics adequately described the degradation process, consistent with previous studies on PAE biodegradation.
6. Biphasic thermograms revealed sequential substrate utilization, with initial PAE degradation followed by utilization of accumulated intermediates (MEP, MBP, PA).

These findings advance fundamental understanding of PAE biodegradation and provide kinetic parameters essential for the rational design of bioremediation strategies. Microcalorimetry emerges as a valuable tool for environmental microbiology, offering real-time metabolic insights that complement traditional analytical approaches.

Acknowledgements:-

The authors sincerely thank Almighty God for the strength and wisdom to complete this study. This work was supported in parts by grants from National Outstanding Youth Research Foundation of China (40925010), International Joint Key Project from National Natural Science Foundation of China (40920134003), National Natural Science Foundation of China (40873060, 40673065), and International Joint Key Project from Chinese Ministry of Science and Technology, and the 111 Project (B08030) at the State Key Laboratory of Biogeology and Environmental Geology of Chinese Ministry of Education, School of Environmental Studies and Sino-Hungarian Joint Laboratory of Environmental Science and Health, China University of Geosciences, 430074 Wuhan, PR China.

List of abbreviations:-

c: concentration; CAS: Chemical Abstract Service; CFU: colony-forming units; CO₂: carbon dioxide; CSH: cell surface hydrophobicity; DBP: dibutyl phthalate; DEHP: di-2-ethylhexyl phthalate; DEP: diethyl phthalate; EDC: endocrine disrupting chemical; GC-MS: gas chromatography-mass spectrometry; H₂O: water; HPLC: high-performance liquid chromatography; I: inhibition ratio; IC₅₀: Half-maximal inhibitory concentration; k: growth rate constant; k₁: first-order degradation rate constant; k₂: second-order degradation rate constant; k_c: rate constant at concentration c; k_o: growth rate constant of the control; K_{ow}: n-octanol-water partitioning coefficient; LB: Luria-Bertani; MBP: monobutyl phthalate; MEP: monoethyl phthalate; NaCl: sodium chloride; PA: phthalic acid; PAE: Phthalic acid esters; P_{max}: peak heat output; PSM: Potato sucrose medium; PVC: polyvinyl chloride; Q: total heat output; R²: Correlation coefficient; t: time; t_{1/2}: Half-life; TAM: thermal activity monitor; TLC: thin-layer chromatography; USEPA: United States Environmental Protection Agency

Declarations:-

Ethics Approval and consent to participate:-

Ethical approval was not required for this study because it involved laboratory-based experiments using pure microbial cultures and chemical compounds and did not involve human participants, human biological materials, animals, or personally identifiable information.

Consent for publication:-

Not applicable. This manuscript does not contain personal data, individual-level information, or identifiable images or materials relating to any individual.

Availability of data and materials:-

The data generated and/or analyzed during this study are available from the corresponding author upon reasonable request. Additional information concerning the experimental procedures, microcalorimetric measurements, and data analysis may also be made available by the corresponding author upon reasonable request. Relevant secondary data and materials used in the study are available from their respective public or institutional sources.

Competing interests:-

The authors declare that they have no competing interests.

Funding:-

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The study was conducted using available institutional and/or personal research resources.

Authors' contributions:-

The corresponding author Alhaji Brima Gogra (ABG) solely contributed to the conceptualization and designing, literature synthesis, methodological design, conduction of the laboratory investigations, the analysis and interpretation of the data/findings, the preparation and approval of the final manuscript for publication. The author

agrees to be accountable for all aspects of the work and for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest:-

The author declares that there is no competing interest/conflict of interest associated with the publication of this manuscript.

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