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OPINION

Engineering bacterial biocatalysts for the degradation of phthalic acid esters

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Abstract

Phthalic acid esters (PAEs) are synthetic diesters derived from *o*-phthalic acid, commonly used as plasticizers. These compounds pose significant environmental and health risks due to their ability to leach into the environment and act as endocrine disruptors, carcinogens, and mutagens. Consequently, PAEs are now considered major emerging contaminants and priority pollutants. Microbial degradation, primarily by bacteria and fungi, offers a promising method for PAEs bioremediation. This article highlights the current state of microbial PAEs degradation, focusing on the major bottlenecks and associated challenges. These include the identification of novel and more efficient PAE hydrolases to address the complexity of PAE mixtures in the environment, understanding PAEs uptake mechanisms, characterizing novel *o*-phthalate degradation pathways, and studying the regulatory network that controls the expression of PAE degradation genes. Future research directions include mitigating the impact of PAEs on health and ecosystems, developing biosensors for monitoring and measuring bioavailable PAEs concentrations, and valorizing these residues into other products of industrial interest, among others.

Phthalic acid esters (PAEs) are a group of synthetic diesters derived from *o*-phthalic acid (1,2-benzenedicarboxylic acid, PA). They are widely utilized in various industrial and consumer applications, including paints, adhesives, cosmetics, fragrances, and toiletries, where they function as solvents, fixatives, and fragrance carriers (Qiao et al., 2024). The primary use of PAEs, however, is as plasticizers which enhance the plasticity, flexibility, and mechanical strength of plastics. PAEs can constitute 10%–60% of the plastic weight, such as up to 50% in flexible polyvinyl acetates (Gómez-Hens & Aguilar-Caballos, 2003). Annually, more than 8 million tons of plasticizers are utilized, with PAEs comprising approximately 70% of this total (Giuliani et al., 2020; Ren et al., 2018). To date, over sixty varieties of PAEs have been produced and consumed in plastic manufacturing (Wang et al., 2021). PAEs are characterized by their diverse side chains, which range from simple and short, e.g., dimethyl phthalate (DMP), diethyl phthalate (DEP) or dibutyl phthalate (DBP), to longer and more complex ones, e.g., di-(2-ethylhexyl) phthalate (DEHP) (Ren et al., 2018; Wang et al., 2021).

Since PAEs are linked to plastics through non-covalent bonds, they can be easily released into the environment, including drinking water, marine water, soil, air, where they become ubiquitous pollutants and pose a potential threat to animal and human health (Li et al., 2016). It is well known that PAEs act as endocrine disruptors, i.e., they interfere with hormonal signalling pathways causing adverse reproductive outcomes. They are also recognized as carcinogens and mutagens. Moreover, PAEs have been linked to other

serious health issues, such as abnormal lipid metabolism, childhood obesity, immune response interference, and neuropsychological disorders (Gao et al., 2018; Tian et al., 2022). The contamination levels of PAEs in soil have gradually increased to several milligrams per kilogram due to their rising production and extensive use in agricultural activities, such as plastic coating and insecticide application (Gao et al., 2018; Lü et al., 2018). Crops like rice, wheat and sorghum can uptake and accumulate PAEs from polluted water and soil, making dietary intake a major route of human exposure to PAEs (Dong, Guo, et al., 2019; Giuliani et al., 2020). Recent studies have also examined the effects of PAEs on microbial community structure. Exposure to high levels (i.e., 20–40 mg/L) of DMP significantly inhibited planktonic bacterial activity by disrupting cell permeability, inducing the production of intracellular reactive oxygen species (Wang, Wang, You, Xu, Lv, Liu, Chen, Shi, & Wang, 2019). Furthermore, environmentally relevant levels of PAEs can promote microbial biofilm formation, complicating traditional antibiotic treatments and increasing their cost (Wang et al., 2022). Due to these concerns, the United States Environmental Protection Agency has listed six types of PAEs, including DMP, DEP, DBP, benzyl butyl phthalate (BBP), dioctyl phthalate (DOP) and DEHP, as emerging contaminants and priority pollutants (Hu et al., 2021; Kashyap & Agarwal, 2018; Qiao et al., 2024).

Given the serious pollution and environmental risks posed by PAEs, it is urgent to develop effective strategies for their removal. The high hydrophobicity of PAEs makes them stable and recalcitrant to efficient degradation through abiotic treatments such as: (i) coagulation/flocculation (Dong, Huang, et al., 2019); (ii) advanced oxidation processes, including photocatalysis (Chan et al., 2007; Lertsirisopon et al., 2009), ultraviolet irradiation (Xu et al., 2007), and electro-Fenton process (Yang et al., 2020); and (iii) adsorption onto activated carbon or other porous materials such as active coke, carbon molecular sieves, carbon fabrics, ion exchange resins, alumina, or biochar (Gan et al., 2019). However, microorganisms, primarily bacteria and fungi, can effectively degrade PAEs and provide environmentally friendly, cheap, and more efficient remediation options (Das et al., 2021; Ren et al., 2018; Xu et al., 2020).

MICROBIAL DEGRADATION OF PAEs

Since the first report on microbial metabolism of PAEs (Engelhardt et al., 1975), over 80 PAE-degrading bacterial strains from 36 genera, predominantly *Pseudomonas*, *Comamonas*, *Sphingomonas*, *Gordonia*, *Rhodococcus*, and *Bacillus*, have been isolated from various ecosystems like soil, water, sediments, plant tissues, and animal gastrointestinal tracts (Qiao et al., 2024). Some of these strains can tolerate PAEs concentrations of up to 500–2000 mg/L. Although Gram-negative bacteria tend to degrade low molecular weight PAEs (with side chains of C1–C4), Gram-positive bacteria have a broader substrate spectrum and they are capable of degrading both low and high molecular weight PAEs (with side chains of C5 or more) (Huang et al., 2019; Qiao et al., 2024). Additionally, Gram-positive and Gram-negative strains may form natural consortia for remediation of PAEs contamination (He et al., 2013; Lu et al., 2020). Some fungi, e.g., *Fusarium culmorum* or *Pleurotus ostreatus*, as well as marine algae like *Dunaliella salina*, have also been reported to mineralize PAEs (Ahuactzin-Pérez et al., 2016, 2018; Chi et al., 2019).

Since alkyl side chains of PAEs contribute significantly to their toxicity and hydrophobicity, removing these side chains is a critical step for PAEs detoxification. This can occur through at least four mechanisms: (i) β-oxidation, (ii) transesterification; (iii) demethylation, and (iv) ester bond hydrolysis. Although β-oxidation, transesterification and demethylation reactions aim to shorten the alkyl side chains, thereby reducing steric hindrance in subsequent enzymatic steps, ester bond hydrolysis is the most common and efficient method for removing these side chains (Jianlong et al., 2000; Ren et al., 2018). Common enzymes capable of ester bond hydrolysis include lipases, esterases, cutinases and α/β hydrolases (Lai et al.2023; Ren et al., 2018). However, these enzymes exhibit different substrate specificities and cannot hydrolyse all kinds of side chain ester bonds of PAEs. In general, PAEs with longer alkyl side chains and complex structures are more challenging to degrade. The two ester bonds of PAEs are typically hydrolysed stepwise with the combination of type I enzymes (diesterases that hydrolyse only one ester bond of PAEs) and type II enzymes (monoesterases that hydrolyse the remaining ester bond of the phthalate mono-ester). Type III enzymes, however, possess both activities and can hydrolyse both ester bonds of PAEs (Bhattacharyya et al., 2022; Chen et al., 2023; Huang et al., 2019; Lai et al., 2023; Ren et al., 2018). Several genes encoding PAE hydrolases have been identified and cloned from bacteria through (meta)genomic library mining or genome sequence analyses (Chen et al., 2021; Xu et al., 2020; Yan et al., 2021; Zhao et al., 2023). Structural and functional studies based on molecular modelling, docking assays and site-directed mutagenesis have identified key catalytic residues in PAE hydrolases (Chen et al., 2023; Fan, Guo, et al., 2023; Huang et al., 2023; Qiu et al., 2021; Ren et al., 2019). So far, most reported PAE hydrolases belong to hydrolases family IV, sharing significant amino acid sequence identity with mammalian hormone-sensitive lipases. These hydrolases are characterized by conserved motifs such as the oxygen anion hole (HGGG), the catalytic triad (Ser-His-Asp), and the GDSAG motif where the catalytic Ser is located (Bhattacharyya et al., 2022; Lai et al., 2023). A distinctive

catalytic mechanism involving Thr-Ser residues has also been proposed (Du et al., 2021). Other PAE hydrolases that cluster into hydrolase families II, V, VI, VII and VIII have been less studied (Fan, Li, et al., 2023; Sarkar et al., 2020; Xu et al., 2020, 2021).

After the action of PAE hydrolases, the alkyl side chains are removed as corresponding alcohols, leaving *o*-phthalic acid (PA) as the aromatic moiety (Figure 1A). PA mineralization can follow several degradation pathways. Typically, PA degradation involves oxidation and decarboxylation steps catalysed by phthalate 4,5- or 3,4-dioxygenases (*pht* genes), forming of the central intermediate protocatechuic acid, which can then be further metabolized via either extradiol (*lig* and *pra* genes) or intradiol (*pca* genes) ring cleavage pathways (Bhattacharyya et al., 2022; Hu et al., 2021; Ren et al., 2018; Zhao et al., 2023) (Figure 1A). However, in some organisms, PA degradation involves the formation of 2,3-dihydroxybenzoate by the action of a phthalate 2,3-dioxygenase (Kasai et al., 2019). Additionally, an oxygen-independent PA decarboxylation mechanism has been described, generating benzoyl-CoA that can be further mineralized through either aerobic (box pathway) or anaerobic (bzd pathway) mechanisms (Boll et al., 2020) (Figure 1A). Other PA degradation mechanisms, such as decarboxylation to benzoate or salicylate, have been suggested, although the enzymes involved have not yet been characterized (Chen et al., 2021; Hu et al., 2022; Wright et al., 2020; Xu et al., 2020) (Figure 1A). Interestingly, in most bacterial

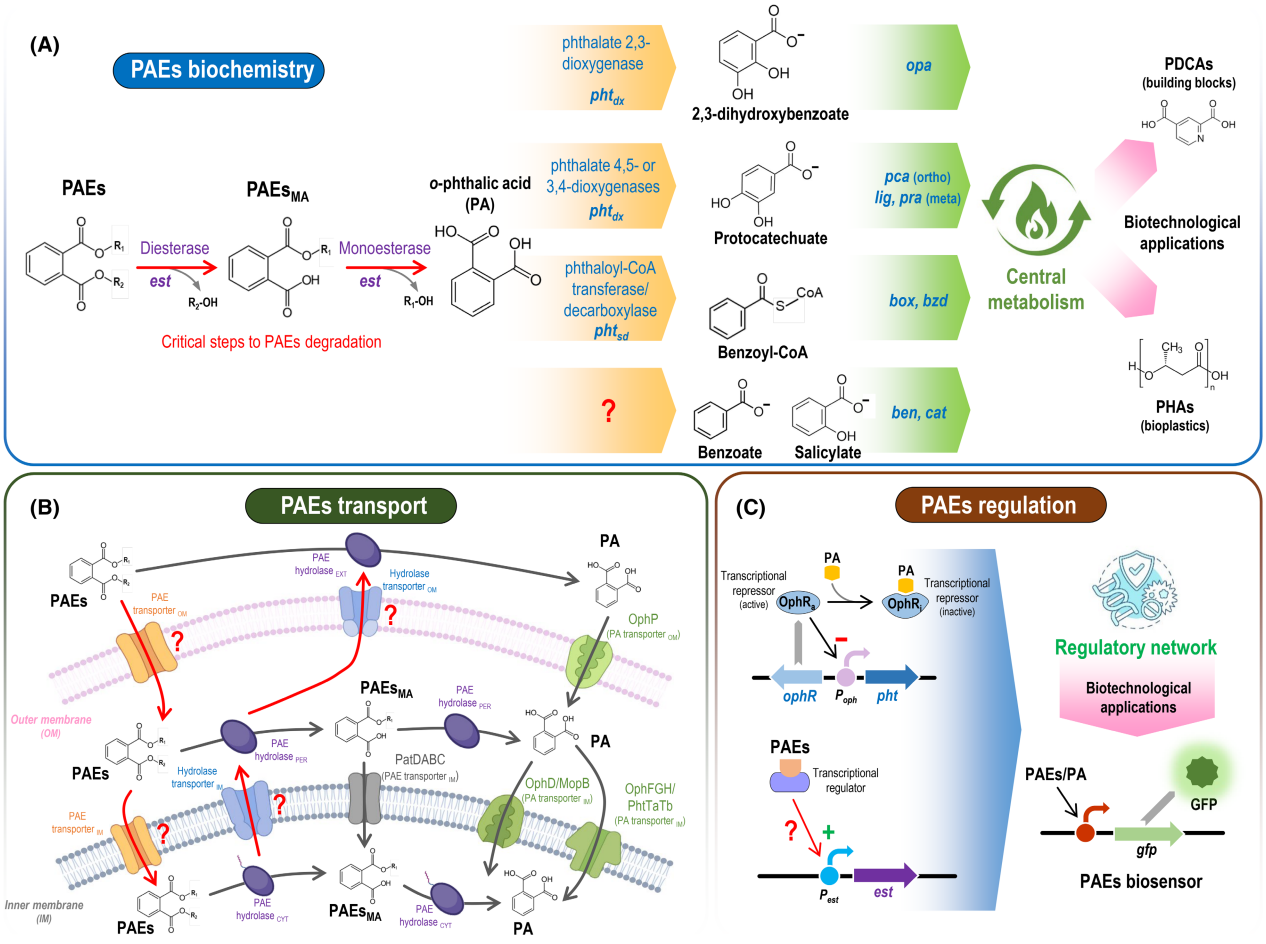

FIGURE 1 Metabolism of PAEs in bacteria. PAEs refers to phthalic acid diester; PAEs$_{MA}$ refers to phthalic acid monoester. (A) PAEs biochemistry. Degradation pathways of PAEs and further valorization to added-value products such as polyhydroxyalkanoates (PHAs) or building blocks, e.g., pyridine dicarboxylic acids (PDCAs). The enzymes and genes involved in the PAEs metabolism are shown in blue. Red arrows represent bottlenecks. Question marks represent unknown enzymatic steps. Brown arrows, enzymes that convert PA into a central intermediate; green arrows, enzymes that convert central intermediates into tricarboxylic acid cycle intermediates (central metabolism); pink arrows, conversion into PHAs or building blocks. (B) PAEs transport through the outer membrane (OM) and inner membrane (IM). Purple ovals represent PAE hydrolases and the subscripts indicate location: CYT, cytoplasmic; PER, periplasmic; EXT, extracellular. Several transporters and their names are indicated: Blue, PAE hydrolase transporters; orange, PAE transporters; grey, PAE$_{MA}$ transporters; green, PA transporters. Red arrows and question marks indicate that the corresponding transporters have not been yet characterized. (C) PAEs regulation. Scheme of the OphR-mediated regulation of *pht* genes in some Gram-positive bacteria. Symbols + and – indicate transcriptional activation or repression, respectively. Red arrow and question mark represent the still unknown regulation of genes encoding PAE hydrolases. PAEs/PA-responding regulatory circuits will facilitate the development of whole-cell biosensors. GFP, green fluorescent protein.

genomes there is no physical association between the genes involved in PA degradation and those encoding PAE hydrolases (Ren et al., 2018), hence suggesting different evolutionary paths for these two enzymatic modules.

As excellent reviews on the molecular bases of PAEs degradation are already available (Benjamin et al., 2015; Bhattacharyya et al., 2022; Boll et al., 2020; Hu et al., 2021; Ren et al., 2018), we will focus on summarizing the major bottlenecks and challenges in bacterial PAEs removal.

BOTTLENECKS AND CHALLENGES IN PAEs DEGRADATION

Several factors hinder the efficiency of microbial-based PAEs decontamination strategies. These include the complexity of PAE mixtures in environmental samples, the limited understanding on microbial degradation pathways and the uptake processes, and the lack of knowledge on the regulatory mechanisms that govern the expression of the genes involved in PAEs degradation. Addressing these issues is crucial for improving the efficiency and feasibility of bioremediation approaches.

PAEs catabolism

The removal of the alkyl side chains by PAE hydrolases is critical for the detoxification and degradation of PAEs. In the natural environment, PAEs often exist as complex mixtures of esters with varying chain lengths, which makes their complete removal challenging for microbial activities that tend to be highly specific rather than broad-spectrum (Hu et al., 2021). Moreover, the catalytic activity of certain native PAE hydrolases is low, creating a bottleneck in the development of efficient biodegradation processes. Therefore, identifying novel and more efficient PAE hydrolases, and/or improving the de-esterification efficiency of currently known hydrolases, is a major challenge in bacterial PAEs degradation (Huang et al., 2020; Lai et al., 2023) (Figure 1A).

Regarding further metabolic steps, the identification and characterization of unknown pathways that convert PA into intermediates other than protocatechuate, such as benzoate or salicylate, present additional challenges in microbial PAEs metabolism (Figure 1A).

PAEs bioavailability and uptake

Water solubility and bioavailability of PAEs constitute significant bottlenecks in PAEs biodegradation. Thus, PAEs with longer alkyl side-chains typically exhibit longer half-lives in the environment due to their low water solubility, which leads to low PAEs concentrations that cannot sustain cell growth or induce the expression of catabolic enzymes (Gu et al., 2005; Liang et al., 2008). Hence, enhancing the biological mechanisms that could increase PAEs solubility becomes a major challenge.

The uptake of PAEs into bacterial cells is critical for their degradation; however, it remains poorly understood (Qiao et al., 2024). Therefore, addressing this knowledge gap constitutes another major challenge in PAEs biodegradation (Figure 1B). PAEs tend to adsorb to the cell envelope of Gram-negative bacteria, mainly due to hydrophobic interactions with outer membrane lipids and proteins, which increases the ratio of unsaturated fatty acids and fluidity in the outer membrane (Lu et al., 2016; Luo et al., 2017). Scanning electron microscopy (SEM) has revealed morphological changes and damage to cell walls and membranes upon PAEs treatment of bacterial cells (Sun et al., 2022). In this sense, Gram-positive bacteria, which lack an outer membrane, seem to be more robust against PAEs compared to Gram-negative bacteria, possibly explaining why Gram-positive bacteria are more predominant in the degradation of PAEs, especially those with long side chains (Qiao et al., 2024). In addition to altering cell surface permeability, PAEs exposure induces the expression of genes involved in energy metabolism and ATP-binding cassette (ABC) transporters, suggesting that membrane proteins probably participate in bacterial PAEs uptake (Wang, Wang, You, Xu, Lv, Liu, Chen, & Shi, 2019). Although direct transport of PAEs by outer membrane proteins has not been reported, research on the transporters of hydrophilic PAEs analogous, such as long-chain fatty acid transport proteins (FadLs), TonB-dependent transporters (TBDTs), and OmpW-like outer membrane proteins, suggests that similar mechanisms could be involved in PAEs transport (Qiao et al., 2024). Regarding transport through the cytoplasmic membrane, only one ABC transport system of monoalkyl PAEs, i.e., the PatDABC multicomponent transport system from *Rhodococcus jostii* RHA1 (Figure 1B), has been reported, and it is encoded next to the *patE* gene encoding a monoalkyl PAE monoesterase (Hara et al., 2010). The identification and characterization of efficient PAE membrane transporters could provide alternative strategies to improve intracellular PAEs catabolism.

In contrast to PAEs uptake, there are several reports on PA uptake by bacterial cells. Thus, the OphP porin from *Burkholderia* sp. transports PA through the outer membrane (Chang et al., 2009). Several transport systems for the uptake of PA through the cytoplasmic membrane have been reported, and they span from monocomponent inner membrane MFS transporters, e.g., OphD and MopB (Chang et al., 2009; Saint & Romas, 1996) to multi-component ABC-type transporters, e.g., OphFGH and PatDABC (Chang et al., 2009;

Hara et al., 2010), and TAXI-TRAP transporters, e.g., PhtTaTb (Sanz et al., 2020) (Figure 1B). These PA uptake systems may play a crucial role in PAEs metabolism by bacteria that secrete hydrolases to the periplasm and/or extracellular medium (Wei et al., 2021; Wright et al., 2020), and they should be taken into account when designing biocatalysts for extracellular PAEs degradation (Figure 1B).

PAEs regulatory circuits

There is a complete lack of knowledge on the regulatory mechanisms controlling the expression of PAE hydrolases-encoding genes (Figure 1C). On the other hand, only a few regulatory genes controlling PA degradation pathways have been characterized, e.g., the OphR regulator that controls the *pht* genes for PA degradation (Choi et al., 2015). Characterizing the regulatory circuits that control gene expression could enable the development of new strategies to enhance gene expression, optimize degradation pathways, and engineer the first PAE biosensors. Therefore, identifying transcriptional regulators that recognize and respond to PAEs is a critical challenge that should be addressed in the coming years (Figure 1C).

FUTURE PERSPECTIVES

Current PAE hydrolases often suffer poor thermostability and low stability in the presence of organic solvents, and may not meet the demands for environmental (activity at low substrate concentrations) or industrial (high catalytic efficiency) applications. Consequently, mining of novel PAE hydrolases becomes crucial. Isolating new bacterial strains with enhanced abilities to tolerate high PAEs concentration and degrade a broader range of these pollutants than reported strains is essential for discovering both novel cellular biocatalysts and new enzymes for PAEs bioremediation. Genomic approaches, together with functional metagenomics and metaproteomics, offer promising strategies for discovering novel enzymes of environmental and industrial interest, which can expand the current portfolio of PAE hydrolases (Qiu et al., 2020; Zhu et al., 2022). On the other hand, protein engineering approaches can also be used to improve existing PAE hydrolases. The rational design of highly active mutants relies on a deep understanding of functional–structural relationships, which can be significantly enhanced by advances in computer-assisted modelling, molecular docking, molecular dynamics simulations, and machine learning methods (Xu et al., 2023). Future research should also focus on the detailed characterization of the catalytic mechanism of PAE hydrolases that have been poorly studied, i.e., hydrolases that belong to families II, V, VI, VII, and VIII. Conversely, error-prone PCR combined with adaptive laboratory evolution (ALE) experiments are successful strategies for evolving non-targeted mutant PAE hydrolases with novel biochemical properties, e.g., broader substrate range, higher catalytic efficiency with selected PAEs, enhance tolerance to wide range of temperature, pH, solvents, etc. (Lai et al., 2023). On the other hand, only a limited number of fungal PAE hydrolases, which belong to the lipase and cutinase families, have been characterized to date, and the majority lack a known primary structure and are glycosylated (Bhattacharyya et al., 2022). This suggests that fungal enzymes may possess biochemical properties distinct from bacterial hydrolases, e.g., higher stability, warranting further investigation in future research.

For industrial applications, engineering cascade reactions by combining type I (diesterases) and type II (monoesterases) hydrolases for the complete transformation of PAEs into PA, and exploring the effects of strain or enzyme immobilization on PAEs removal efficiency (Feng et al., 2021), should be further exploited. However, type III hydrolases, which can hydrolyse both phthalate diesters and monoesters, offer a more cost-effective solution with lower operational complexity compared to the combination of type I and type II enzymes, which may require distinct operational conditions. Despite this advantage, the catalytic mechanisms of type III enzymes are not yet well understood, and only a few have been characterized to date (Lai et al., 2023). Elucidating the key structural features of type III PAE hydrolases could be instrumental in improving their relatively low catalytic efficiency and/or engineering them into more efficient enzymes that can act on a broader substrate spectrum and over a wide range of temperature and pH.

To tackle the challenge posed by the considerable heterogeneity of PAEs in certain environments, designing synthetic bacterial consortia emerges as a promising strategy. Such microbial consortia could combine specialized strains that target both short-chain and long-chain PAEs, along with strains capable of efficiently degrading PA and the resulting alcohols. These consortia can be artificially constructed and optimized over time (Gu et al., 2005; Liu et al., 2023; Staples et al., 1997). With the aid of metabolic modelling-based analysis, e.g., FLYCOP (García-Jiménez et al., 2018), and by iterative design-build-test-learn cycles, synthetic PAE-degrading bacterial consortia can be optimized to enhance bioremediation strategies, such as strain selection for bioaugmentation and the choice of energy sources or electron acceptors for biostimulation (Hu et al., 2021). Although the exogenous addition of PAE-degrading bacteria into contaminated environments (Zhao et al., 2018) or in wastewater treatment reactors

(Zhang et al., 2018) represents a promising bioremediation strategy (bioaugmentation), this approach requires further optimization, particularly to enhance the long-term efficacy of the introduced bacteria, which may struggle to compete with native microorganisms in the environment.

The initial hydrolysis of PAEs may require either the uptake of these molecules into bacterial cells or the secretion of hydrolase enzymes outside of the bacterial cytoplasm. A comprehensive understanding of the molecular mechanisms involved in these processes is crucial for designing more efficient biocatalysts for PAEs degradation in bioreactors/waste treatment plants (Figure 1B). Currently, the membrane proteins responsible for PAEs uptake remain unidentified, highlighting the need for research to discover and engineer PAE transporters to enhance the intracellular metabolism of these contaminants. Alternatively, secreting PAE hydrolases to the bacterial surface or into the culture medium can facilitate de-esterification by enabling substrate-enzyme co-localization outside the cell. In this context, studies on the secretion and cellular localization of PAE hydrolases, particularly those with potential N-terminal signal peptides (Wei et al., 2021; Wright et al., 2020), are necessary. Engineering artificial secretion signals into cytoplasmic PAE hydrolases has been reported as a viable approach (Ding et al., 2020), and could further enhance PAEs degradation when combined with known PA transport systems (Chang et al., 2009; Sanz et al., 2020).

Given that the regulatory circuits controlling the expression of genes encoding PAE hydrolases remain unknown, it is crucial to urgently identify and characterize regulators capable of recognizing PAEs, as well as elucidate the mechanisms by which they govern their corresponding transcriptional promoters (Figure 1C). These studies will enable the optimization of synthetic DNA modules for high gene expression levels, and pave the way for developing the first generation of whole-cell biosensors capable of monitoring and measuring bioavailable PAEs concentrations in environmental samples (Figure 1C).

Engineering efficient PAEs degradation pathways into microbial biocatalysts of industrial relevance, such as *Pseudomonas putida*, *Cupriavidus necator*, and *Corynebacterium glutamicum*, could facilitate the conversion and upcycling of these toxic compounds into valuable products like bioplastics (Figure 1A) (Sanz et al., 2020). However, to create robust biocatalysts for PAEs removal and valorization, it is crucial to determine and integrate the global responses of these bacterial cells to PAEs using omic techniques. This information should be incorporated into genome-scale metabolic models to identify and mitigate the adverse physiological effects of PAEs on the host cells.

Although microbial degradation and upcycling of PAEs are promising green and environmentally friendly approaches, assessing the upscaling and economic feasibility of these technologies is needed and it should be subject of future research (Tran et al., 2022).

In summary, addressing the challenges posed by PAEs requires a concerted interdisciplinary effort involving scientists, engineers, and policymakers. By fostering collaboration across these fields, we can effectively tackle the issues associated with these emerging pollutants and protect public health and ecological well-being for current and future generations.

AUTHOR CONTRIBUTIONS

Gonzalo Durante-Rodríguez: Writing – original draft; writing – review and editing; supervision; conceptualization. **Sofía de Francisco-Polanco:** Writing – original draft; writing – review and editing. **Unai Fernández-Arévalo:** Writing – original draft; writing – review and editing. **Eduardo Díaz:** Funding acquisition; writing – original draft; writing – review and editing; supervision; conceptualization.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

Ahuactzin-Pérez, M., Tlecuitl-Beristain, S., García-Dávila, J., González-Pérez, M., Gutiérrez-Ruíz, M.C. & Sánchez, C. (2016) Degradation of di(2-ethyl hexyl) phthalate by *Fusarium culmorum*: kinetics, enzymatic activities and biodegradation pathway based on quantum chemical modeling pathway based on quantum chemical modeling. *Science of the Total Environment*, 566–567, 1186–1193.

Ahuactzin-Pérez, M., Tlecuitl-Beristain, S., García-Dávila, J., Santacruz-Juárez, E., González-Pérez, M., Gutiérrez-Ruíz, M.C. et al. (2018) A novel biodegradation pathway of the endocrine-disruptor di(2-ethyl hexyl) phthalate by *Pleurotus ostreatus* based on quantum chemical investigation. *Ecotoxicology and Environmental Safety*, 147, 494–499.

Benjamin, S., Pradeep, S., Sarath Josh, M., Kumar, S. & Masai, E. (2015) A monograph on the remediation of hazardous phthalates. *Journal of Hazardous Materials*, 298, 58–72.

Bhattacharyya, M., Basu, S., Dhar, R. & Dutta, T.K. (2022) Phthalate hydrolase: distribution, diversity and molecular evolution. *Environmental Microbiology Reports*, 14, 333–346.

Boll, M., Geiger, R., Junghare, M. & Schink, B. (2020) Microbial degradation of phthalates: biochemistry and environmental implications. *Environmental Microbiology Reports*, 12, 3–15.

Chan, C.M., Wong, K.H., Chung, W.K., Chow, T.S. & Wong, P.K. (2007) Photocatalytic degradation of di(2-ethylhexyl)phthalate adsorbed by chitin a. *Water Science and Technology*, 56, 125–134.

Chang, H.K., Dennis, J.J. & Zylstra, G.J. (2009) Involvement of two transport systems and a specific porin in the uptake of phthalate by *Burkholderia* spp. *Journal of Bacteriology*, 191, 4671–4673.

Chen, F., Chen, Y., Chen, C., Feng, L., Dong, Y., Chen, J. et al. (2021) High-efficiency degradation of phthalic acid esters (PAEs) by *Pseudarthrobacter defluvii* E5: performance, degradative pathway, and key genes. *Science of the Total Environment*, 794, 148719.

Chen, Y., Wang, Y., Xu, Y., Sun, J., Yang, L., Feng, C. et al. (2023) Molecular insights into the catalytic mechanism of plasticizer degradation by a monoalkyl phthalate hydrolase. *Communications Chemistry*, 6, 45.

Chi, J., Li, Y. & Gao, J. (2019) Interaction between three marine microalgae and two phthalate acid esters. *Ecotoxicology and Environmental Safety*, 170, 407–411.

Choi, K.Y., Kang, B.S., Nam, M.H., Sul, W.J. & Kim, E. (2015) Functional identification of OphR, an IclR family transcriptional regulator involved in the regulation of the phthalate catabolic operon in *Rhodococcus* sp. strain DK17. *Indian Journal of Microbiology*, 55, 313–318.

Das, M.T., Kumar, S.S., Ghosh, P., Shah, G., Malyan, S.K., Bajar, S. et al. (2021) Remediation strategies for mitigation of phthalate pollution: challenges and future perspectives. *Journal of Hazardous Materials*, 409, 124496.

Ding, J., Zhou, Y., Wang, C., Peng, Z., Mu, Y., Tang, X. et al. (2020) Development of a whole-cell biocatalyst for diisobutyl phthalate degradation by functional display of a carboxylesterase on the surface of *Escherichia coli*. *Microbial Cell Factories*, 19, 1–11.

Dong, C.-D., Huang, C.P., Nguyen, T.-B., Hsiung, C.-F., Wu, C.-H., Lin, Y.-L. et al. (2019) The degradation of phthalate esters in marine sediments by persulfate over iron–cerium oxide catalyst. *Science of the Total Environment*, 696, 133973.

Dong, W., Guo, R., Sun, X., Li, H., Zhao, M., Zheng, F. et al. (2019) Assessment of phthalate ester residues and distribution patterns in baijiu raw materials and baijiu. *Food Chemistry*, 283, 508–516.

Du, H., Hu, R.W., Zhao, H.M., Huang, H.B., Xiang, L., Liu, B.L. et al. (2021) Mechanistic insight into esterase-catalyzed hydrolysis of phthalate esters (PAEs) based on integrated multispectroscopic analyses and docking simulation. *Journal of Hazardous Materials*, 408, 124901.

Engelhardt, G., Wallnöfer, P.R. & Hutzlnger, O. (1975) The microbial metabolism of di-n-butyl phthalate and related dialkyl phthalates. *Bulletin of Environmental Contamination and Toxicology*, 13, 342–347.

Fan, S., Guo, J., Han, S., Du, H., Wang, Z., Fu, Y. et al. (2023) A novel and efficient phthalate hydrolase from *Acinetobacter* sp. LUNF3: molecular cloning, characterization and catalytic mechanism. *Molecules*, 28, 6738.

Fan, S., Li, C., Guo, J., Johansen, A., Liu, Y., Feng, Y. et al. (2023) Biodegradation of phthalic acid esters (PAEs) by *Bacillus* sp. LUNF1 and characterization of a novel hydrolase capable of catalyzing PAEs. *Environmental Technology and Innovation*, 32, 103269.

Feng, N.X., Feng, Y.X., Liang, Q.F., Chen, X., Xiang, L., Zhao, H.M. et al. (2021) Complete biodegradation of di-*n*-butyl phthalate (DBP) by a novel *Pseudomonas* sp. YJB6. *Science of the Total Environment*, 761, 143208.

Gan, L., Zhong, Q., Geng, A., Wang, L., Song, C., Han, S. et al. (2019) Cellulose derived carbon nanofiber: a promising biochar support to enhance the catalytic performance of $CoFe_2O_4$ in activating peroxymonosulfate for recycled dimethyl phthalate degradation. *Science of the Total Environment*, 694, 133705.

Gao, D., Li, Z., Wang, H. & Liang, H. (2018) An overview of phthalate acid ester pollution in China over the last decade: environmental occurrence and human exposure. *Science of the Total Environment*, 645, 1400–1409.

García-Jiménez, B., García, J.L. & Nogales, J. (2018) FLYCOP: metabolic modeling-based analysis and engineering microbial communities. *Bioinformatics*, 34, i954–i963.

Giuliani, A., Zuccarini, M., Cichelli, A., Khan, H. & Reale, M. (2020) Critical review on the presence of phthalates in food and evidence of their biological impact. *International Journal of Environmental Research and Public Health*, 17, 5655.

Gómez-Hens, A. & Aguilar-Caballos, M.P. (2003) Social and economic interest in the control of phthalic acid esters. *Trends in Analytical Chemistry*, 22, 847–857.

Gu, J.D., Li, J. & Wang, Y. (2005) Biochemical pathway and degradation of phthalate ester isomers by bacteria. *Water Science and Technology*, 52, 241–248.

Hara, H., Stewart, G.R. & Mohn, W.W. (2010) Involvement of a novel ABC transporter and monoalkyl phthalate ester hydrolase in phthalate ester catabolism by *Rhodococcus jostii* RHA1. *Applied and Environmental Microbiology*, 76, 1516–1523.

He, Z., Xiao, H., Tang, L., Min, H. & Lu, Z. (2013) Biodegradation of di-*n*-butyl phthalate by a stable bacterial consortium, HD-1, enriched from activated sludge. *Bioresource Technology*, 128, 526–532.

Hu, R., Zhao, H., Xu, X., Wang, Z., Yu, K., Shu, L. et al. (2021) Bacteria-driven phthalic acid ester biodegradation: current status and emerging opportunities. *Environment International*, 154, 106560.

Hu, T., Yang, C., Hou, Z., Liu, T., Mei, X., Zheng, L. et al. (2022) Phthalate esters metabolic strain *Gordonia* sp. GZ-YC7, a potential soil degrader for high concentration di-(2-ethylhexyl) phthalate. *Microorganisms*, 10, 641.

Huang, H., Xu, Y., Lin, M., Li, X., Zhu, H., Wang, K. et al. (2023) Complete genome sequence of *Acinetobacter indicus* and identification of the hydrolases provides direct insights into phthalate ester degradation. *Food Science and Biotechnology*, 33, 103–113.

Huang, H., Zhang, X.Y., Chen, T.L., Zhao, Y.L., Xu, D.S. & Bai, Y.P. (2019) Biodegradation of structurally diverse phthalate esters by a newly identified esterase with catalytic activity toward di(2-ethylhexyl) phthalate. *Journal of Agricultural and Food Chemistry*, 67, 8548–8558.

Huang, L., Meng, D., Tian, Q., Yang, S., Deng, H., Guan, Z. et al. (2020) Characterization of a novel carboxylesterase from *Bacillus velezensis* SYBC H47 and its application in degradation of phthalate esters. *Journal of Bioscience and Bioengineering*, 129, 588–594.

Jianlong, W., Lujun, C., Hanchang, S. & Yi, Q. (2000) Microbial degradation of phthalic acid esters under anaerobic digestion of sludge. *Chemosphere*, 41, 1245–1248.

Kasai, D., Iwasaki, T., Nagai, K., Araki, N., Nishi, T. & Fukuda, M. (2019) 2,3-dihydroxybenzoate *meta*-cleavage pathway is involved in *o*-phthalate utilization in *Pseudomonas* sp. strain PTH10. *Scientific Reports*, 9, 1253.

Kashyap, D. & Agarwal, T. (2018) Concentration and factors affecting the distribution of phthalates in the air and dust: a global scenario. *Science of the Total Environment*, 635, 817–827.

Lai, J., Huang, H., Lin, M., Xu, Y., Li, X. & Sun, B. (2023) Enzyme catalyzes ester bond synthesis and hydrolysis: the key step for sustainable usage of plastics. *Frontiers in Microbiology*, 13, 1113705.

Lertsirisopon, R., Soda, S., Sei, K. & Ike, M. (2009) Abiotic degradation of four phthalic acid esters in aqueous phase under natural sunlight irradiation. *Journal of Environmental Sciences*, 21, 285–290.

Li, C., Chen, J., Wang, J., Han, P., Luan, Y., Ma, X. et al. (2016) Phthalate esters in soil, plastic film, and vegetable from greenhouse vegetable production bases in Beijing, China: concentrations, sources, and risk assessment. *Science of the Total Environment*, 568, 1037–1043.

Liang, D.W., Zhang, T., Fang, H.H.P. & He, J. (2008) Phthalates biodegradation in the environment. *Applied Microbiology and Biotechnology*, 80, 183–198.

Liu, T., Ning, L., Mei, C., Li, S., Zheng, L., Qiao, P. et al. (2023) Synthetic bacterial consortia enhanced the degradation of mixed priority phthalate ester pollutants. *Environmental Research*, 235, 116666.

Lü, H., Mo, C.H., Zhao, H.M., Xiang, L., Katsoyiannis, A., Li, Y.W. et al. (2018) Soil contamination and sources of phthalates and its health risk in China: a review. *Environmental Research*, 164, 417–429.

Lu, M., Jiang, W., Gao, Q., Zhang, M. & Hong, Q. (2020) Degradation of dibutyl phthalate (DBP) by a bacterial consortium and characterization of two novel esterases capable of hydrolyzing PAEs sequentially. *Ecotoxicology and Environmental Safety*, 195, 110517.

Lu, T., Xue, C., Shao, J., Gu, J.-D., Zeng, Q. & Luo, S. (2016) Adsorption of dibutyl phthalate on *Burkholderia cepacia*, minerals, and their mixtures: behaviors and mechanisms. *International Biodeterioration & Biodegradation*, 114, 1–7.

Luo, S., Li, L., Chen, A., Zeng, Q., Xia, H. & Gu, J.D. (2017) Biosorption of diethyl phthalate ester by living and nonliving *Burkholderia cepacia* and the role of its cell surface components. *Chemosphere*, 178, 187–196.

Qiao, P., Ying, T., Gu, M., Zhu, J., Mei, C., Hu, T. et al. (2024) Assimilation of phthalate esters in bacteria. *Applied Microbiology and Biotechnology*, 108, 276.

Qiu, J., Yang, H., Shao, Y., Li, L., Sun, S., Wang, L. et al. (2021) Enhancing the activity and thermal stability of a phthalate-degrading hydrolase by random mutagenesis. *Ecotoxicology and Environmental Safety*, 209, 111795.

Qiu, J., Zhang, Y., Shi, Y., Jiang, J., Wu, S., Li, L. et al. (2020) Identification and characterization of a novel phthalate-degrading hydrolase from a soil metagenomic library. *Ecotoxicology and Environmental Safety*, 190, 110148.

Ren, L., Lin, Z., Liu, H. & Hu, H. (2018) Bacteria-mediated phthalic acid esters degradation and related molecular mechanisms. *Applied Microbiology and Biotechnology*, 102, 1085–1096.

Ren, L.Q., Chang, T.T., Ren, D.P., Zhou, Y. & Ye, B.C. (2019) Rational design to improve activity of the Est3563 esterase from *Acinetobacter* sp. LMB-5. *Enzyme and Microbial Technology*, 131, 109331.

Saint, C.P. & Romas, P. (1996) 4-Methylphthalate catabolism in *Burkholderia (pseudomonas) cepacia* Pc701: a gene encoding a phthalate-specific permease forms part of a novel gene cluster. *Microbiology*, 142, 2407–2418.

Sanz, D., García, J.L. & Díaz, E. (2020) Expanding the current knowledge and biotechnological applications of the oxygen-independent ortho-phthalate degradation pathway. *Environmental Microbiology*, 22, 3478–3493.

Sarkar, J., Dutta, A., Pal Chowdhury, P., Chakraborty, J. & Dutta, T.K. (2020) Characterization of a novel family VIII esterase EstM2 from soil metagenome capable of hydrolyzing estrogenic phthalates. *Microbial Cell Factories*, 19, 77.

Staples, C.A., Peterson, D.R., Parkerton, T.F. & Adams, W.J. (1997) The environmental fate of phthalate esters: a literature review. *Chemosphere*, 35, 667–749.

Sun, J., Rutherford, S.T., Silhavy, T.J. & Huang, K.C. (2022) Physical properties of the bacterial outer membrane. *Nature Reviews. Microbiology*, 20, 236–248.

Tian, M., Wu, S., Wang, Y.X., Liu, L., Zhang, J., Shen, H. et al. (2022) Associations of environmental phthalate exposure with male steroid hormone synthesis and metabolism: an integrated epidemiology and toxicology study. *Journal of Hazardous Materials*, 436, 129213.

Tran, H.T., Nguyen, M.K., Hoang, H.G., Hutchison, J.M. & Vu, C.T. (2022) Composting and green technologies for remediation of phthalate (PAE)-contaminated soil: current status and future perspectives. *Chemosphere*, 307, 135989.

Wang, C., Wang, Z., You, Y., Xu, W., Lv, Z., Liu, Z. et al. (2019) Response of *Arthrobacter* QD 15-4 to dimethyl phthalate by regulating energy metabolism and ABC transporters. *Ecotoxicology and Environmental Safety*, 174, 146–152.

Wang, H., Yu, P., Schwarz, C., Zhang, B., Huo, L., Shi, B. et al. (2022) Phthalate esters released from plastics promote biofilm formation and chlorine resistance. *Environmental Science & Technology*, 56, 1081–1090.

Wang, P., Gao, J., Zhao, Y., Zhang, M. & Zhou, S. (2021) Biodegradability of di-(2-ethylhexyl) phthalate by a newly isolated bacterium *Achromobacter* sp. RX. *Science of the Total Environment*, 755, 142476.

Wang, Z., Wang, C., You, Y., Xu, W., Lv, Z., Liu, Z. et al. (2019) Response of *Pseudomonas fluorescens* to dimethylphthalate. *Ecotoxicology and Environmental Safety*, 167, 36–43.

Wei, S.T.-S., Chen, Y.-L., Wu, Y.-W., Wu, T.-Y., Lai, Y.-L., Wang, P.-H. et al. (2021) Integrated multi-omics investigations reveal the key role of synergistic microbial networks in removing plasticizer di-(2-ethylhexyl) phthalate from estuarine sediments. *mSystems*, 6, e00358-21.

Wright, R.J., Bosch, R., Gibson, M.I. & Christie-Oleza, J.A. (2020) Plasticizer degradation by marine bacterial isolates: a proteogenomic and metabolomic characterization. *Environmental Science & Technology*, 54, 2244–2256.

Xu, R., Bao, Y., Li, M., Zhang, Y., Xi, L. & Guo, J. (2023) Computational insights into the allosteric modulation of a phthalate-degrading hydrolase by distal mutations. *Biomolecules*, 13, 443.

Xu, X.R., Li, H.B. & Gu, J.D. (2007) Photocatalytic reduction of hexavalent chromium and degradation of di-n-butyl phthalate in aqueous TiO_2 suspensions under ultraviolet light irradiation. *Environmental Technology*, 28, 1055–1061.

Xu, Y., Liu, X., Zhao, J., Huang, H., Wu, M., Li, X. et al. (2021) An efficient phthalate ester-degrading *Bacillus subtilis*: degradation kinetics, metabolic pathway, and catalytic mechanism of the key enzyme. *Environmental Pollution*, 273, 116461.

Xu, Y., Minhazul, K.A.H.M., Wang, X., Liu, X., Li, X., Meng, Q. et al. (2020) Biodegradation of phthalate esters by *Paracoccus kondratievae* BJQ0001 isolated from Jiuqu (baijiu fermentation starter) and identification of the ester bond hydrolysis enzyme. *Environmental Pollution*, 263, 114506.

Yan, Z., Ding, L., Zou, D., Qiu, J., Shao, Y., Sun, S. et al. (2021) Characterization of a novel carboxylesterase with catalytic activity toward di(2-ethylhexyl) phthalate from a soil metagenomic library. *Science of the Total Environment*, 785, 147260.

Yang, Z., Chen, H., Wang, J., Yuan, R., Wang, F. & Zhou, B. (2020) Efficient degradation of diisobutyl phthalate in aqueous solution through electro-Fenton process with sacrificial anode. *Journal of Environmental Chemical Engineering*, 8, 104057.

Zhang, K., Luo, H., Zhu, Z., Chen, W., Chen, J. & Mo, Y. (2018) Performance and microbial community structure of bioaugmentation in a sequencing batch reactor treating bis(2-ethylhexyl) phthalate wastewater at low temperature. *Journal of Environmental Engineering*, 144, 04018085.

Zhao, H.M., Hu, R.W., Chen, X.X., Chen, X.B., Lü, H., Li, Y.W. et al. (2018) Biodegradation pathway of di-(2-ethylhexyl) phthalate by a novel *Rhodococcus pyridinivorans* XB and its bioaugmentation for remediation of DEHP contaminated soil. *Science of the Total Environment*, 640-641, 1121–1131.

Zhao, Z., Liu, Y., Liu, C., Xu, Q., Song, M. & Yan, H. (2023) Whole-genome analysis of *Comamonas* sp. USTBZA1 for biodegrading diethyl phthalate. *3 Biotech*, 13, 329.

Zhu, B., Wang, D. & Wei, N. (2022) Enzyme discovery and engineering for sustainable plastic recycling. *Trends in Biotechnology*, 40, 22–37.

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