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Review article

# Bacteria-driven phthalic acid ester biodegradation: Current status and emerging opportunities

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ABSTRACT

The extensive use of phthalic acid esters (PAEs) has led to their widespread distribution across various environments. As PAEs pose significant threats to human health, it is urgent to develop efficient strategies to eliminate them from environments. Bacteria-driven PAE biodegradation has been considered as an inexpensive yet effective strategy to restore the contaminated environments. Despite great advances in bacterial culturing and sequencing, the inherent complexity of indigenous microbial community hinders us to mechanistically understand *in situ* PAE biodegradation and efficiently harness the degrading power of bacteria. The synthetic microbial ecology provides us a simple and controllable model system to address this problem. In this review, we focus on the current progress of PAE biodegradation mediated by bacterial isolates and indigenous bacterial communities, and discuss the prospective of synthetic PAE-degrading bacterial communities in PAE biodegradation research. It is anticipated that the theories and approaches of synthetic microbial ecology will revolutionize the study of bacteria-driven PAE biodegradation and provide novel insights for developing effective bioremediation solutions.

## 1. Introduction

Phthalic acid esters (PAEs) are the typical synthetic organic compounds mainly produced from phthalic anhydride and appropriate alcohol (Graham, 1973). Since the first production in 1920 s, PAEs have been widely used as plasticizers in the manufacture and processing of plastic goods, such as food wrappings, children's toys, medical devices, and agricultural films (Kang et al., 2012; Kimber and Dearman, 2010). An estimate of 7.5 million tons of plasticizers is annually used around the world, of which PAEs account for 60–65% of the total consumption (related data available at https://www.plasticisers.org/plasticiser/ortho-phthalates/; https://ihsmarkit.com/products/plasticizers-chemical-economics-handbook.html). As no suitable alternatives have been yet found so far, the production and consumption of PAEs are expected to remain high in the future (Chiellini et al., 2013; Gao et al., 2018). The production and use of plastics contribute to the PAE leaching and migration into water, solid, air and dust systems. A quantitative survey (Fig. 1, Table S1) indicates that the maximal concentration ranges of PAE residues in water, solid, air and dust systems are 0.02–2016 μg/L (Fang et al., 2009; Li et al., 2019a,b), 10.4–111000 μg/kg (Meng et al., 2014; Zhao et al., 2018c), 10.3–143000 ng/m$^3$ (Wang et al., 2008; Zeng et al., 2010), and 10.1–23300 μg/g (Sun et al., 2017; Wang et al., 2017),

*Abbreviations:* BBP, Butyl benzyl phthalate; DMP, Dimethyl phthalate; DEP, Diethyl phthalate; DBP, Di-n-butyl phthalate; DiBP, Diisobutyl phthalate; DEHP, Di(2-ethylhexyl) phthalate; DOP, Di-n-octyl phthalate; DiOP, Diisooctyl phthalate; DnPrP, Di-n-propyl phthalate; DnPeP, Di-n-pentyl phthalate; DnNP, Di-n-nonyl phthalate; DiNP, Diisononyl phthalate; DnDP, Di-n-decyl phthalate; DiDP, Diisodecyl phthalate; BMEP, Bis-(2-methoxyethyl) phthalate; DAlP, Diallyl phthalate; DBEP, Bis-(2-n-butoxyethyl) phthalate; DEEP, Bis-(2-ethoxyethyl) phthalate; DCHP, Dicyclohexyl phthalate.

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respectively. The peak of PAE concentrations often occurs in common contaminated water sources, such as landfill leachate (1.32–2106 μg/L), wastewater treatment plants (WWTPs) influent (1.2–1519 μg/L) and sewage sludge (39–111000 μg/kg) (Fig. 1, Table S2). It is worth noting that most of the hydrophobic PAE concentrations far exceeded its water solubility, which is due to the fact that the existing measurements failed to distinguish absorbed PAEs by dissolved organic matter from truly dissolved PAEs (Bauer and Herrmann, 1998; Chiou et al., 1986). A more striking indication of widespread PAE occurrence is that PAE residues are detected in drinking water, and a long-term exposure of PAEs may raise dysfunctions of human reproductive, nervous and immune systems (Andersen et al., 2018; Benjamin et al., 2017; Matsumoto et al., 2008). As a consequence, the United States Environmental Protection Agency (USEPA), the European Union (EU) and the Chinese National Environmental Monitoring Center (CNEMC) have listed six PAE compounds as the priority pollutants, including dimethyl phthalate (DMP), benzyl butyl phthalate (BBP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), di-n-octyl phthalate (DOP) and di(2-ethylhexyl) phthalate (DEHP) (Gao et al., 2017; Net et al., 2015). Of them, the most prevalent pollutants in the contamination sources and related environmental matrices are DEHP and DBP, with 135 and 130 positive samples out of the total 144 samples, respectively (Fig. 1, Table S2).

To protect environment and human health, researchers have developed many PAE remediation strategies, such as physical adsorption, chemical coagulation/flocculation, advanced oxidation and bacterial biodegradation (Benjamin et al., 2015; Gao and Wen, 2016; Rivera-Utrilla et al., 2012). Among them, bacteria-driven biodegradation has been considered as the most promising strategy, and its prominent advantages include the complete degradation of pollutants, lower treatment cost, greater safety and less environment disturbance (Fenner et al., 2013; Staples et al., 1997). Currently, great advances in isolation and cultivation technology have made more and more PAE-degrading pure cultures available in the lab, and their degradation characteristics, molecular bases and lab-scale bioremediation applications have been widely studied (Jin et al., 2013; Kumar et al., 2017; Wang et al., 2012; Zhao et al., 2017; Zhao et al., 2019). Numerous studies have also showed that the PAE metabolism in the natural environments is accompanied by the joint efforts of various indigenous bacteria (Gu et al., 2005; Liang et al., 2008). However, the high complexity of indigenous bacterial communities prevents us far from molecular bases of *in situ* PAE biodegradation processes (Zhu et al., 2020), and hampers the development of effective bioremediation applications at actual contaminated sites (Lovley, 2003). Therefore, a comprehensive understanding of indigenous bacterial community in the PAE-contaminated environments is highly required and valuable.

Recently, synthetic microbial ecology has been rapidly developed and attracted researchers' great interests due to its potential to address fundamental ecological issues surrounding natural or human microbiota (De Roy et al., 2014; Großkopf and Soyer, 2014). With the reduced community complexity and increased experimental controllability, many synthetic microbial communities have been built to identify the ecological forces governing the assembly, stability and function of

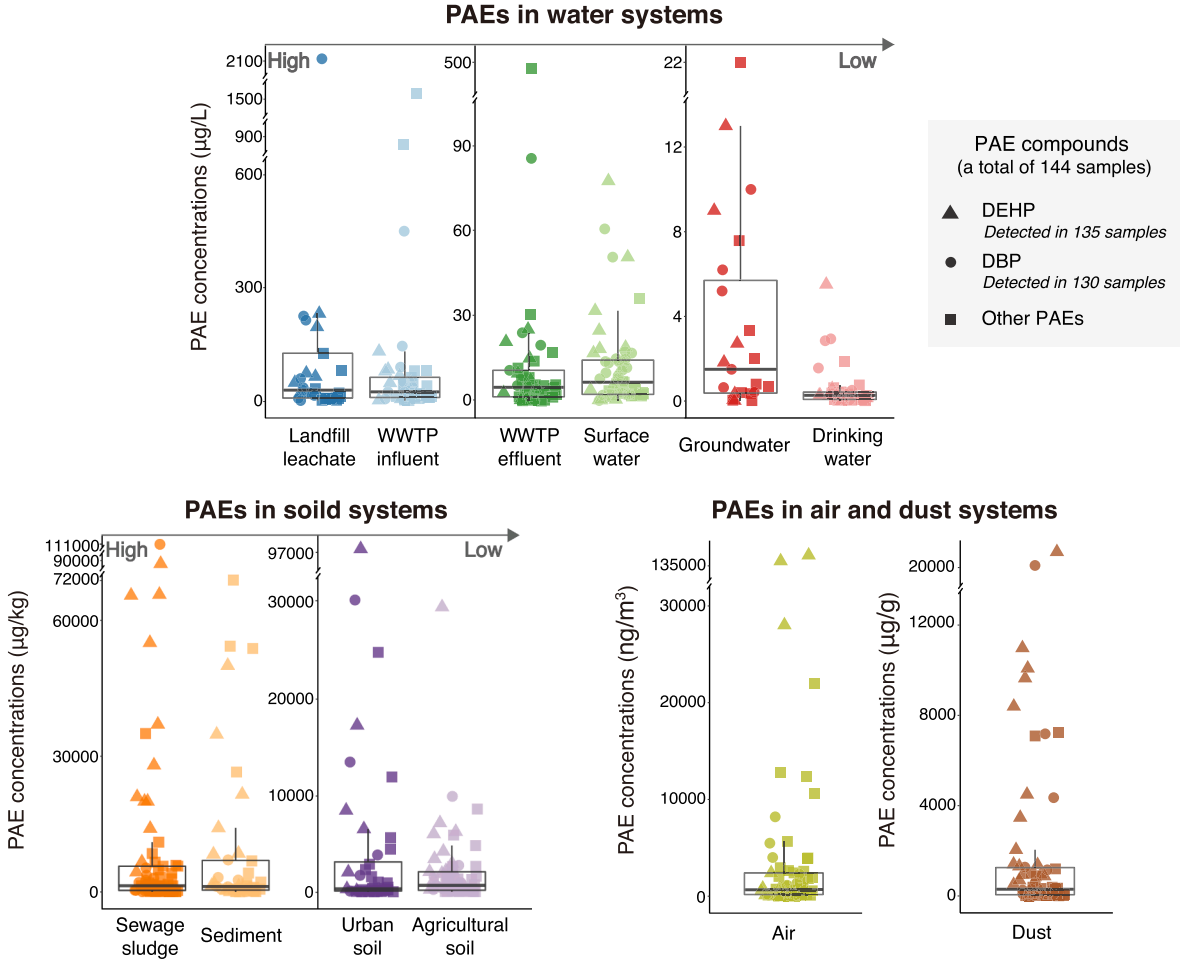



**Fig. 1.** Box-plot of PAE occurrence in polluted sources and environmental compartments. Data summarizes the maximal concentrations of 13 types of frequently encountered PAEs from 108 publications. The details of PAE concentrations (both maximum and average) are included in Table S1. The two most frequently detected PAEs (DBP and DEHP) are listed in Table S2. DBP: di-n-butyl phthalate; DEHP: di-2-ethylhexyl phthalate.

natural or human microbiota (Che and Men, 2019; Vorholt et al., 2017; Vrancken et al., 2019). By analyzing replicate synthetic communities and constructing predictive and mechanistic models, researcher uncovered fundamental principles of controlling microbial community functioning and dynamics (D'Hoe et al., 2018; Mee et al., 2014; Venturelli et al., 2018). In addition, synthetic microbial communities held a great promise in energy production, medicine synthesis and environmental governance (McCarty and Ledesma-Amaro, 2019; Qian et al., 2020). Because of community robustness and division of labor among synthetic community members, researchers could manipulate and optimize artificially-assembled microbial communities to accomplish complex tasks, such as electricity generation, anti-cancer drug

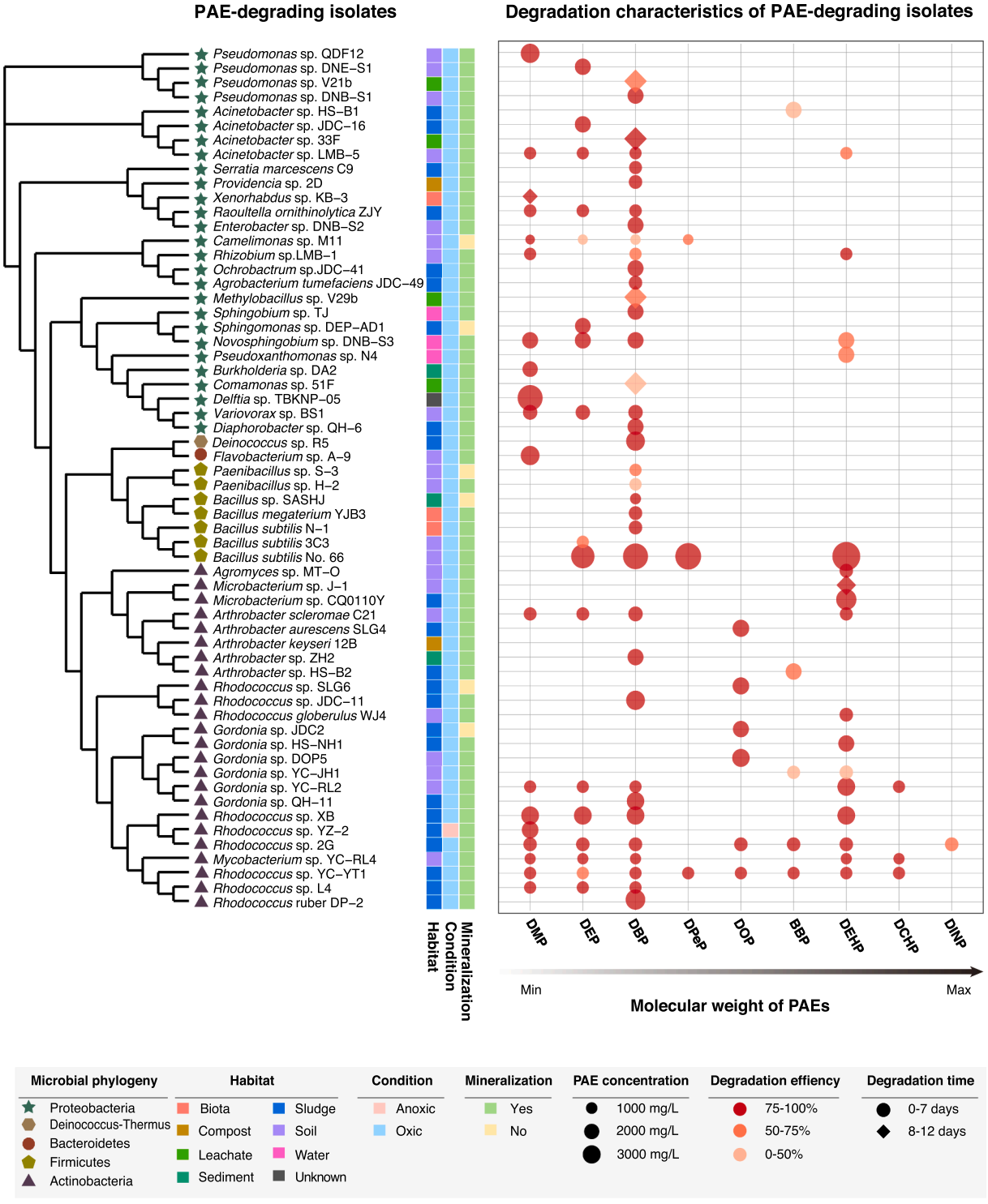



**Fig. 2.** Phylogenetic analyses and degradation characteristics of typical PAE-degrading isolates. The phylogenetic tree was built based on 16S rRNA gene sequences (>500 bp) covering 60 typical PAE-degrading isolates (the corresponding NCBI accession numbers are shown in Table S3). Phylogeny in this tree corresponds to 1000 bootstrap replicates. Detailed information on the degradation characteristics of 60 PAE-degrading isolates is also included in Table S3. DMP: dimethyl phthalate; BBP: benzyl butyl phthalate; DOP: di-n-octyl phthalate; DEP: diethyl phthalate; DBP: di-n-butyl phthalate; DEHP: di-2-ethylhexyl phthalate; DCHP: dicyclohexyl phthalate; DPrP: di-n-propyl phthalate; DINP: diisononyl phthalate.

production and toxin degradation (Liu et al., 2017; Wanapaisan et al., 2018; Zhou et al., 2015a,b). As a consequence, it is foreseeable that synthetic PAE-degrading communities are promising to improve our mechanistic insights into the bacteria-driven PAE biodegradation and enhance the efficiency of PAE bioremediation in the contaminated environments. In this review, we highlight the current progress of bacteria-driven PAE biodegradation mediated by individual bacteria and indigenous bacterial communities, and discuss the prospective of synthetic PAE-degrading bacterial communities for understanding bacteria-driven PAE biodegradation mechanisms at the polluted sites and their possible applications in bioremediation.

## 2. An overview of PAE biodegradation by bacterial isolates

In the past 50 years, a considerable number of PAE-degrading bacteria have been isolated from the PAE-polluted environments. These PAE-degrading isolates have provided indispensable foundations for studying the bacteria-driven PAE biodegradation and its underlying mechanisms. In this section, we outline the phylogenetic diversity, degradation characteristics, metabolic pathways and molecular mechanisms of these isolates, and discuss the feasibility and limitation of applying PAE-degrading bacteria in the environment.

### 2.1. Degradation characteristics and phylogenetic diversity of PAE-degrading bacterial isolates

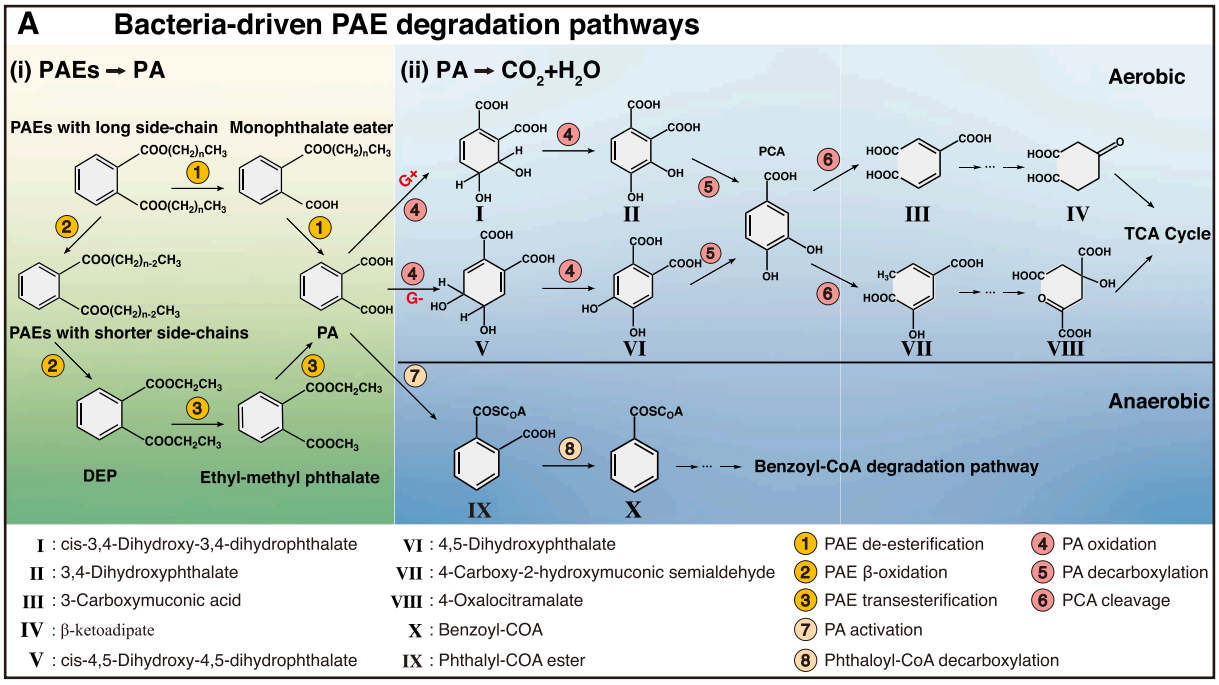
To the best of our knowledge, 60 isolates were reported to have the capacities of breaking down various PAEs (Liang et al., 2008; Ren et al., 2018), and they were sourced from diverse habitats, with 44 out of 60 isolated from sludge and soil (Fig. 2, Table S3). Biochemical analysis revealed that different isolates exhibited distinct degrading capacities. Isolates of genus *Rhodococcus* showed a wide range of available substrate and a high degradation efficiency (Fig. 2, Table S3). For instance, *Rhodococcus* sp. 2G was able to degrade seven types of PAE compounds within 5 days, and their degradation rates ranged from 70.8% to 95% (Zhao et al., 2018b). It was reported that *Bacillus subtilis* No. 66 and *Delftia* sp. TBKNP-05 could completely metabolize a high concentration of PAEs, while some isolates (e.g., *Paenibacillus* sp. S-3 and *Gordonia* sp. JDC-2) could not mineralize PAEs into $CO_2$ and $H_2O$ owing to the lack of genes responsible for metabolizing PAE intermediates (Jin et al., 2014; Quan et al., 2005; Wu et al., 2010; Zhang et al., 2018a,b). In addition, the PAE-degrading efficiency varied with molecular weight of PAEs. In comparison with high-molecular-weight PAEs (e.g., DOP, BBP, DEHP), low-molecular-weight PAEs (e.g., DMP, DEP, DBP) were easier to be metabolized by isolates, and DBP frequently detected in the environment could be degraded by 36 isolates at a degradation rates from 44% to 100% (Chen et al., 2015; Lu et al., 2009).

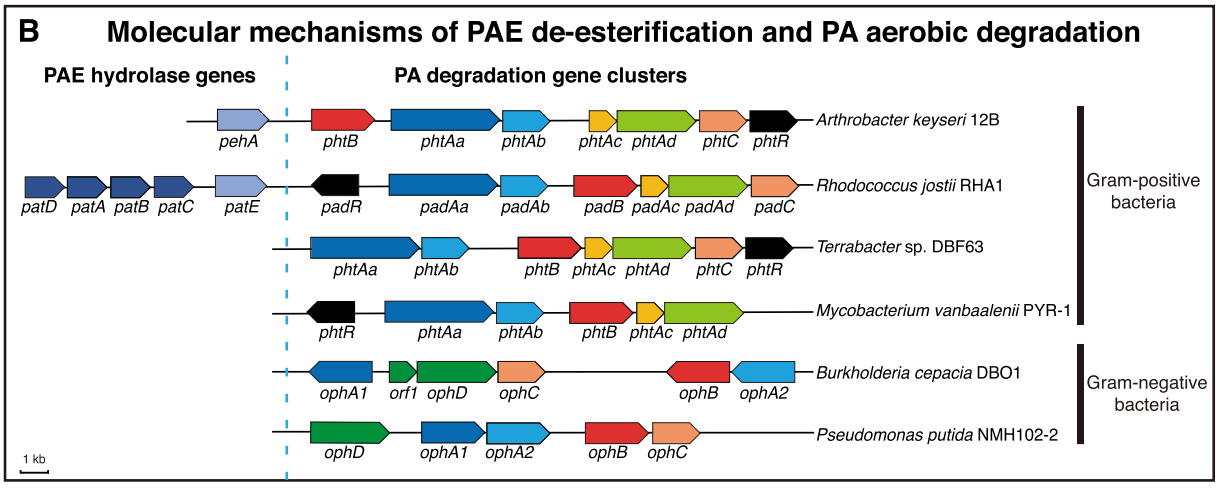
From the phylogenetic perspective, these 60 isolates spanning 31 genera belonged to a broad range of bacterial phyla: Proteobacteria (45%, 27 out of 60), Actinobacteria (40%), Firmicute (12%), Bacteroidetes (1.5%) and Deinococcus-Thermus (1.5%). Of the 31 genera represented, *Rhodococcus* constituted a large proportion of the reported isolates (15%), followed by *Gordonia* (10%), *Arthrobacter* (8%), and *Bacillus* (8%) (Fig. 2, Table S3). Theoretically, these reported isolates from six phyla were far from covering the overall diversity of PAE-degrading bacteria in the natural environments (Lewis et al., 2020; Song et al., 2019). To obtain more novel PAE degraders, researchers could take advantage of multi-omics approaches and function-based labeling techniques to link microbial species with functions, and then develop some targeted cultivation strategies based on membrane diffusion, microfluidic and cell sorting (Jiao et al., 2020; Lewis et al., 2020).

### 2.2. Metabolic pathways and molecular mechanisms of PAE-degrading bacterial isolates

Before bacteria metabolized PAEs, the transport of PAE into bacterial cell was a key process. This process was driven by an ABC transporter-type PAE transport system or a permease-type PAE transporter (Chang et al., 2009; Chang and Zylstra, 1999; Hara et al., 2010). After PAE entered bacterial cell, PAE biodegradation was activated and included two steps (Fig. 3A): (i) the transformation of PAEs into intermediate phthalic acid (PA), and (ii) the metabolism of PA into $CO_2$ and $H_2O$. The first step of biodegrading PAEs to PA involved β-oxidation, transesterification and de-esterification of side chains, which can be performed by most of the PAE-degrading bacteria (Boll et al., 2020; Cartwright et al., 2000; Sarkar et al., 2013). With the influence of oxygen availability, the second step of PA metabolism was divided into two parts: aerobic degradation and anaerobic degradation (Junghare et al., 2019). Under aerobic conditions, PA degradation comprised (i) the transformation of PA into protocatechuate (PCA), and (ii) the ring-cleavage of PCA (Fig. 3A). It is worth noting that the transformation of PA into PCA contained two pathways, which varied between Gram-positive (G+) bacteria and Gram-negative (G–) bacteria (Chang and Zylstra, 1998; Eaton and Ribbons, 1982). Unlike aerobic degradation, anaerobic PAE-degrading bacteria firstly transformed PA into phthalyl-CoA, and subsequently performed the decarboxylation to form benzoyl-CoA, which finally entered into the benzoyl-CoA degradation pathway (Junghare et al., 2016; Nozawa and Maruyama, 1988).

The screening and cloning approaches have enabled us to identify the genes and enzymes involved in PAE degradation pathways (Fig. 3B). As the initial step of PAE biodegradation, the hydrolysis of ester bonds was facilitated by the catalyzation of hydrolase. Many hydrolase genes have been identified in PAE-degrading bacteria, such as *pehA* in *Arthrobacter keyseri* 12B and *patE* in *Rhodococcus jostii* RHA1 (Eaton, 2001; Hara et al., 2010). Gene sequence analyses revealed that PAE hydrolases were distributed across five families of esterase (family IV, V, VII, VIII and one unknown family) and had different conserved sequence motifs (e.g., Clustal W2 and MEME) (Ren et al., 2018). Similarly, researchers have identified gene clusters that were involved in the catabolism of PA to PCA, which could be phylogenetically classified into two classes: (i) the gene cluster *pht* of G+ bacteria and (ii) the gene cluster *oph* of G– bacteria (Stingley et al., 2004). Gene cluster *pht* was firstly found in a 130 kb plasmid of G+ *Arthrobacter keyseri* 12B, and it consisted of *phtAaAbAcAd* encoding phthalate 3,4-dioxygenase, *phtB* encoding 3,4-dihydroxy-3,4-dihydrophthalate dehydrogenase, *phtC* encoding 3,4-Dihydroxyphthalate decarboxylase and a regulatory gene *phtR* with the sequence arrangement as *phtB-phtAaAbAcAd-phtC-phtR* in the plasmid (Fig. 3B). Although gene cluster *pht* was also cloned in *Rhodococcus jostii* RHA1, *Terrabacter* sp. DBF63 and *Mycobacterium vanbaalenii* PYR-1, their operons were different from *Arthrobacter keyseri* 12B in terms of gene arrangement and nucleic acid sequences (Habe et al., 2003; Hara et al., 2010; Stingley et al., 2004). In comparison with G+ bacteria, G– bacteria contained different genes/enzymes involved in the specialized phthalate oxidation process, where PA was transformed into *cis*-4,5-dihydroxy-4,5-dihydrophthalate, 4,5-dihydroxyphthalate and PCA in turn (Fig. 3A). Taking *Burkholderia cepacia* DBO1 as an example, researchers cloned the gene cluster *oph* (five structural genes and a nonfunctional permease gene) involved in the conversion of PA to PCA, and they were arranged in at least three transcriptional units based on orientation: *ophA1-ophDC-ophA2B* (Chang and Zylstra, 1998). *Pseudomonas putida* NMH102-2 contained a similar gene cluster *oph*, but it was different from *Burkholderia cepacia* DBO1 in terms of the operon structure (Nomura et al., 1992). In addition, gene cluster (*pcm* operon) for PCA *meta*-cleavage degradation was also identified in *Arthrobacter keyseri* 12B, and this gene cluster consisted of *pcmDECABF* encoding 4-oxalomesaconate hydratase, 4-oxalocitramalate aldolase, 2-pyrone-4,6-dicarboxylate hydrolase, protocatechuate 4,5-dioxygenase, 2-hydroxy-4-carboxymuconic semialdehyde dehydrogenase and oxidoreductase (Eaton, 2001). It was therefore concluded that PAE transformation in the aerobic condition contained more diverse metabolic pathways than that in the anaerobic condition, and PA catabolism genes were highly conserved. Overall, these studies provide novel



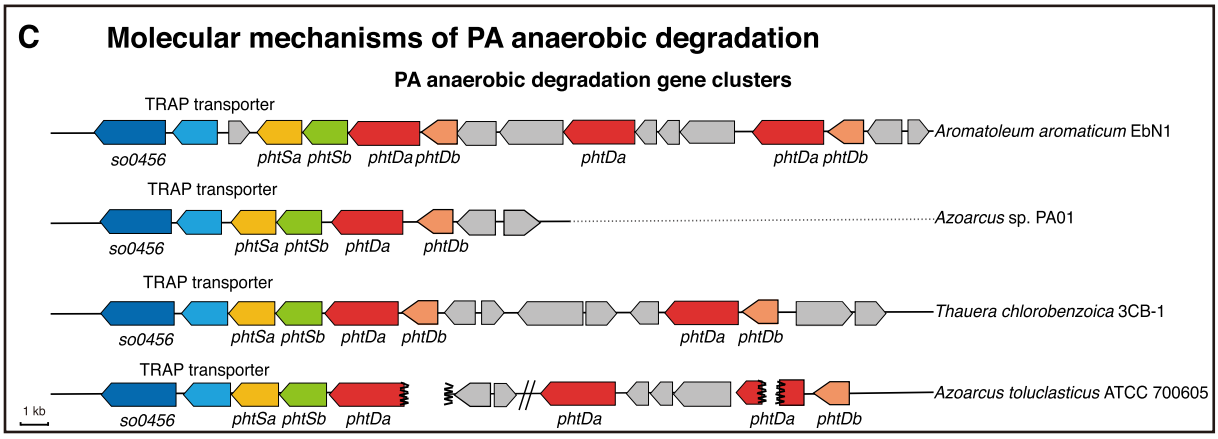




**Fig. 3.** An overview of PAE degradation pathways and related molecular mechanisms. (A) Bacteria-driven PAE degradation pathways. (B) Molecular mechanisms of PAE degradation under the aerobic condition. (C) Molecular mechanisms of PAE degradation under the anaerobic condition. *patDABC*, ABC transporter; *pehA* or *patE*, putative phthalate ester hydrolase gene; *phtAαAbAcAd*, phthalate 3,4-dioxygenase gene; *phtB*, 3,4-dihydroxy-3,4-dihydrophthalate dehydrogenase gene; *phtC*, 3,4-Dihydroxyphthalate decarboxylase gene; *phtR*, the transcriptional regulator; *ophA1*, phthalate dioxygenase reductase gene; *ophA2*, phthalate dioxygenase; *ophB*, 4,5-dihydro-4,5-dihydroxyphthalate dehydrogenase gene; *ophC*, 4,5-dihydroxyphthalate decarboxylase gene; *ophD* and *orf1*, putative phthalate transporter; *so0456*, periplasmic binding protein gene; *phtSa*, type III CoA transferase subunit; *phtSb*, type III CoA transferase subunit; *phtDa*, phthalyl-CoA decarboxylase gene; *phtDb*, Ubix-like protein gene.

insights into the mechanisms of PAE biodegradation at the biochemical or molecular level, and lay a theoretical basis for our follow-up study on bacteria-mediated PAE biodegradation.

So far, 16 bacteria that could effectively degrade PAEs have been sequenced, and their genomic information was summarized in Table 1. Through comparative genomic analysis, a large number of genes or gene clusters regulating primary PAE degradation reactions were identified in different PAE-degrading isolates, such as hydrolase genes, PA-degrading gene clusters (*pht* (G+) and *oph* (G–)) and PCA degradation gene clusters (*pca*). Meanwhile, the whole genomic sequencing also contributed to the discovery of a previously unknown gene cluster catalyzing β-oxidation, benzoic acid (BA) degradation and PA anaerobic degradation (Table 1). For example, Wright et al. (2020) for the first time deciphered molecular mechanisms of β-oxidation by analyzing the genome of PAE-degrading bacteria–*Halomonas* sp. ATBC28, and the identified catalytic enzymes include alcohol dehydrogenase (Enzyme ID number: 1239), aldehyde dehydrogenase (1238), fatty acid-CoA ligase (1456, 4867), acyl-CoA dehydrogenase (1457, 4872), enoyl-CoA hydratase (1458, 4870) and beta-ketoadipyl CoA thiolase (4908). Similarly, Zhao et al. (2018b) identified BA degradation gene cluster from the annotated genome of

**Table 1**
Overview of the PAE-degrading bacteria with sequenced genomes and functional genes or gene clusters.

| Species name | Origin | Degrading substrate | Identified PAE-degrading genes or gene clusters | GenBank ID | Ref. |
|---|---|---|---|---|---|
| **PAE → PA (PAE β-oxidation and de-esterification)** | | | | | |
| *Rhodococcus* sp. 2G | Activated sludge | DMP; DBP; DEP; BBP; DEHP; DOP | hydrolase gene | CP018063; CP018064 | (Zhang et al., 2018a,b,c) |
| *Gordonia* sp. QH-12 | Activated sludge | DMP; DBP; DEP; DEHP; DOP | hydrolase gene | JPYZ00000000 | (Jin et al., 2016a) |
| *Mycobacterium* sp. DBP42 | Marine plastic debris | DBP; DEHP | hydrolase gene; β-oxidation gene | VCMY00000000 | (Wright et al., 2020) |
| *Halomonas* sp. ATBC28 | Marine plastic debris | DBP; DEHP | hydrolase gene; β-oxidation gene | VCQL00000000 | (Wright et al., 2020) |
| *Paracoccus kondratievae* 0001 | Fermentation starter | DMP; DEP; DBP; DEHP | hydrolase gene | CP045072; CP045073; CP045074 | (Xu et al., 2020) |
| *Sphingobium yanoikuyae* TJ | Seawater | DBP | hydrolase gene | LSVG00000000 | (Jin et al., 2016b) |
| *Rhodococcus* sp. HS-D2 | River sediment | BBP | hydrolase gene | LUTX00000000 | (Zhang et al., 2018a,b,c) |
| *Curvibacter* sp. PAE-UM | River sediment | DMP; DBP; DEP; BBP; DEHP | hydrolase gene | LKCX00000000 | (Ma et al., 2016) |
| *Thiobacimonas* sp. D13 | Deep-sea sediment | DMP; DBP; DEP; DEHP | hydrolase gene | RAPJ00000000 | (Liu et al., 2019) |
| *Rhodococcus ruber* YC-YT1 | Sediment | DEHP; BBP; DEP; DMP; DOP; DCHP; DPrP | hydrolase gene | CP023712; CP023713; CP023714 | (Yang et al., 2018) |
| *Gordonia alkanivorans* YC-RL2 | Soil | DMP; DBP; DEP; DEHP; DCHP | hydrolase gene | CP027114; CP027115 | (Nahurira et al., 2019) |
| *Gordonia* sp. YC-JH1 | Soil | DOP; BBP; DEHP; DCHP | hydrolase gene | CP025435; CP025436 | (Fan et al., 2018) |
| *Mycobacterium* sp. YC-RL4 | Soil | DMP; DEP; DBP; DEHP; DCHP | hydrolase gene | CP015596; CP015597 | (Ren et al., 2017) |
| **PA → PCA (PA oxidation and decarboxylation); Aerobic condition** | | | | | |
| *Mycobacterium* sp. DBP42 | Marine plastic debris | DBP; DEHP | gene cluster *pht* | VCMY00000000 | (Wright et al., 2020) |
| *Halomonas* sp. ATBC28 | Marine plastic debris | DBP; DEHP | gene cluster *pht* | VCQL00000000 | (Wright et al., 2020) |
| *Sphingobium yanoikuyae* TJ | Seawater | DBP | gene cluster *oph* | LSVG00000000 | (Jin et al., 2016b) |
| *Rhodococcus* sp. HS-D2 | River sediment | BBP | gene cluster *pht* | LUTX00000000 | (Zhang et al., 2018a,b,c) |
| *Curvibacter* sp. PAE-UM | River sediment | DMP; DBP; DEP; BBP; DEHP | gene cluster *oph* | LKCX00000000 | (Ma et al., 2016) |
| *Thiobacimonas* sp. D13 | Deep-sea sediment | DMP; DBP; DEP; DEHP | gene cluster *oph* | RAPJ00000000 | (Liu et al., 2019) |
| *Sphingobium yanoikuyae* SHJ | Sediment | DEP; DMP; DBP | gene cluster *oph* | CP020925; CP020926 | (Wang et al., 2018) |
| *Rhodococcus ruber* YC-YT1 | Sediment | DEHP; BBP; DEP; DMP; DOP; DCHP; DPrP | gene cluster *pht*; | CP023712; CP023713; CP023714 | (Yang et al., 2018) |
| *Comamonas* sp. E6 | Soil | — | gene cluster *oph* | BBXH00000000 | (Shimodaira et al., 2015) |
| *Gordonia* sp. YC-JH1 | Soil | DOP; BBP; DEHP; DCHP | gene cluster *pht* | CP025435; CP025436 | (Fan et al., 2018) |
| **PCA → $CO_2$ + $H_2O$ (PCA cleavage); Aerobic condition** | | | | | |
| *Rhodococcus* sp. 2G | Activated sludge | DMP; DBP; DEP; BBP; DEHP; DOP | gene cluster *pca* | CP018063; CP018064 | (Zhao et al., 2018) |
| *Mycobacterium* sp. DBP42 | Marine plastic debris | DBP; DEHP | gene cluster *pca* | VCMY00000000 | (Wright et al., 2020) |
| *Halomonas* sp. ATBC28 | Marine plastic debris | DBP; DEHP | gene cluster *pca* | VCQL00000000 | (Wright et al., 2020) |
| *Curvibacter* sp. PAE-UM | River sediment | DMP; DBP; DEP; BBP; DEHP | gene cluster *pca* | LKCX00000000 | (Ma et al., 2016) |
| *Thiobacimonas* sp. D13 | Deep-sea sediment | DMP; DBP; DEP; DEHP | gene cluster *pca* | RAPJ00000000 | (Liu et al., 2019) |
| *Gordonia* sp. YC-JH1 | Soil | DOP; BBP; DEHP; DCHP | gene cluster *pca* | CP025435; CP025436 | (Fan et al., 2018) |
| **BA → catechol (BA oxidation and dehydrogenation); Aerobic condition** | | | | | |
| *Rhodococcus* sp. 2G | Activated sludge | DMP; DBP; DEP; BBP; DEHP; DOP | BA degradation gene cluster | CP018063; CP018064 | (Zhao et al., 2018) |
| *Rhodococcus ruber* YC-YT1 | Sediment | DEHP; BBP; DEP; DMP; DOP; DCHP; DPrP | BA degradation gene cluster | CP023712; CP023713; CP023714 | (Yang et al., 2018) |
| *Gordonia alkanivorans* YC-RL2 | Soil | DMP; DBP; DEP; DEHP; DCHP | BA degradation gene cluster | CP027114; CP027115 | (Nahurira et al., 2019) |
| *Mycobacterium* sp. YC-RL4 | Soil | DMP; DEP; DBP; DEHP; DCHP | BA degradation gene cluster | CP015596; CP015597 | (Ren et al., 2017) |
| **PA → $CO_2$ + $H_2O$ (PA activation and decarboxylation); Anaerobic condition** | | | | | |
| *Azoarcus* sp. PA01T | Activated sludge | PA | PA anaerobic degradation gene cluster | LARU00000000 | (Junghare et al., 2015) |

Note: BBP, Butyl benzyl phthalate; DMP, Dimethyl phthalate; DEP, Diethyl phthalate; DBP, Di-n-butyl phthalate; DEHP, Di(2-ethylhexyl) phthalate; DOP, Di-n-octyl phthalate; DCHP, Dicyclohexyl phthalate; DPrP, Di-n-propyl phthalate; PA, Phthalic acid; BA, Benzoic acid.

*Rhodococcus* sp. 2G, with four *xyl* genes (*xylL*, *xylZ*, *xylY*, and *xylX*) and one *MFS* transporter gene. Moreover, Junghare et al. (2016) filled the knowledge gap about molecualr mechanism of PA anaerobic degradation based on the genome information of anaerobic strain *Azoarcus* sp. PA01T, and they found that the gene cluster of PA anaerobic degradation included *so0456* encoding periplasmic binding protein, *phtSa* encoding type III CoA transferase subunit, *phtSb* encoding type III CoA transferase subunit, *phtDa* encoding phthalyl-CoA decarboxylase, and *phtDb* encoding Ubix-like protein (Fig. 3C). With the popularity of high-throughput sequencing technologies, more PA anaerobic degradation gene clusters have been identified from the anaerobic bacterial genomes, and the corresponding functional gene sequences have been found to share a high degree of homology (Ebenau-Jehle et al., 2016). Despite the growing number of novel PAE degradation genes identified by genome sequencing, comprehensive sequence analysis and functional validation of these novel genes are still needed.

### 2.3. Bioaugmentation with PAE-degrading bacterial isolates

The physiological and biochemical characterization of PAE-degrading bacteria had substantially contributed to bioaugmentation applications in the PAE-polluted activated sludge and soil (Liang et al., 2008). *Arthrobacter* sp. SLG-4 was identified as a strong DOP degrader in an activated sludge, and its inoculation could shorten the start-up of a sequential batch reactor (SBR) and increase DOP degradation rate from 45% to 80% (Zhang et al., 2018a). Roslev et al. (1998) observed that the mineralization rate of [$^{14}$C] DEHP to $^{14}CO_2$ increased four-fold after the inoculation of DEHP-degrading strain SDE mixed with sludge and soil samples. Also, the exogenous addition of DEHP degrading bacteria (e.g., *Microbacterium* sp. J-1 and *Rhodococcus* sp. 2G) into soil pots not only enhanced the removal of soil DEHP pollution, but also reduced the bioaccumulation of DEHP in vegetables (Zhao et al., 2019; Zhao et al., 2017). However, most of the reported bioaugmentation applications were performed under laboratory conditions, and only a few studies initiated the applications of bioaugmentation in the PAE contaminated environment (Ren et al., 2016; Zhao et al., 2018a). In a study of bioaugmentation with the DEHP-degrading strain (*Rhodococcus* sp. ACSL-4) in a wastewater treatment reactor, Zhang et al. (2018b) observed that bioaugmentation significantly increased the DEHP degradation efficiency from 34.2% to 74.3%. In contrast, Liao et al. (2010) carried out a bioaugmentation practice in the activated sludge, and they found that DBP-degrading bacteria (*P. stutzeri*) could not survive after only 5 days of incubation. This failure primarily resulted from the ecological competitions between inoculated bacteria and indigenous microorganisms (Hu et al., 2015; Liao et al., 2010). Therefore, bioaugmentation strategy serving as a promising bioremediation technology needs to be further improved, especially concerning the long-term efficacy of the inoculated bacteria in the contaminated environments and underlying side effects of bioaugmentation for natural biological activity and integrity. In order to increase the feasibility of bioaugmentation applications, a systematical understanding of the diversity, function, interaction and dynamics of indigenous bacterial communities responsible for *in situ* PAE biodegradation is urgent.

## 3. Indigenous bacterial communities for *in situ* PAE biodegradation

Indigenous bacterial communities in the PAE-polluted environments play a vital role in the PAE biodegradation, yet little is known about their diversity, interactions and dynamics during the PAE biodegradation process. This knowledge gap involves three central questions: (i) who are the key degraders involved in PAE biodegradation; (ii) what are the key genes/enzymes responsible for PAE biodegradation processes; and (iii) how microbes interact to complete the whole process of PAE biodegradation. The answers to the above questions are essential to design bioremediation strategies for the contaminated sites. In this section, we mainly review the response of indigenous bacterial communities to PAE exposure, with a focus on the taxonomical diversity, composition and function of indigenous bacterial communities.

### 3.1. Community-wide response of indigenous bacterial taxonomic and functional attributes to PAE pollution

16S ribosomal RNA (16S rRNA) gene amplicon and metagenomic sequencing technologies have enabled us to analyze the diversity, composition and function of indigenous bacterial communities from PAE-polluted environments (Zhu et al., 2020; Kolb et al., 2019; Song et al., 2019). Here we listed the previous studies regarding the community-wide response of indigenous bacterial community to PAEs across various environmental matrices (Table 2). Clearly, such response varied with the type and concentration of PAEs, as well as the composition of indigenous bacterial community (Gao et al., 2020; Wang et al., 2020; Wang et al., 2016a,b). For instance, the genera *Delftia, Pseudomonas, Flavobacterium,* and *Sphingobium* were significantly enriched in the activated sludge treated by DBP (1000 mg/L), while the abundance of genera *Gordonia*, *Achromobacter*, *Rhodococcus*, *Rhizobium*, and *Chryseobacterium* increased in the DEHP (1000 mg/L)-treated activated sludge (Wang et al., 2020). Even under the same concentration of DBP exposure, the changes of bacterial communities varied across different environmental matrices (Table 2). In the black soil, the abundance of *Novosphingobium, Sphingobium and Sphingopyxis* increased under the DBP (40 mg/L) exposure. However, the addition of DBP (40 mg/L) into brown soil led to the increased abundance of *Lysobacter*, *Streptomyces*, *Gemmatirosa*, *Sphingomonas* and *Bacillus* (Gao et al., 2020; Wang et al., 2016a,b).

Despite the variable background in these studies, reduced microbial diversity and simplified community composition were consistently observed in response to PAE exposure (Table 2). In a survey on the response of bacterial communities in the wheat rhizosphere soil to DEHP contamination, researchers found that DEHP could reduce bacterial richness (e.g., Shannon index and Chao1 index) and decreased the relative abundance of *Pontibacter* and *Brevundimonas* (Gao et al., 2020). The reduced microbial diversity and simplified community composition were also observed in the response of indigenous bacterial communities in biofilm, dust, and activated sludge to PAE exposure (Li, Y. et al., 2019; Velazquez et al., 2019; Wang et al., 2020). During the shift of microbial community composition, it is important to identify key members with a significantly increased abundance in indigenous bacterial communities as they may potentially participate in PAE biodegradation at polluted sites. For instance, Wang et al. (2015) found the abundance of *Arthrobacter* increased after the addition of DMP into soils and its abundance had a positive relationship with the DMP concentration. The role of *Arthrobacter* species in the degradation of DMP was further supported by a series of biochemical tests, which revealed that *Arthrobacter* QD 15–4 isolated from the same soil was able to use DMP as carbon and energy sources for growth in the laboratory (Wang et al., 2019).

Compared to 16S rRNA gene amplicon sequencing, metagenomic sequencing analysis could provide more functional informations regarding the response of indigenous PAE-degrading bacterial community to PAE exposure, moving beyond the community diversity and structure analysis (Xu et al., 2018; Zhu et al., 2020). For instance, Zhu et al. (2020) found that the abundance of genes encoding hydrolase, esterase, cytochrome P450, lipase, carboxyl-esterase, laccase and cutinase in indigenous bacterial communities increased under DEHP (1000 mg/kg) pollution. Their corresponding taxonomic annotation indicated that these elevated functional genes were from genera *Gordonia*, *Rhodococcus*, *Nocardia*, *Mycobacterium*, *Nocardioides* and *Pimelobacter* in the order *Actinomycetales,* and genera *Lysobacter* and *Sphingobium* in the class *β-Proteobacteria*, suggesting that these bacteria may jointly participate in DEHP degradation. By further incorporating taxonomic information with DEHP metabolic pathways, Zhu et al. inferred that the genera *Nocardioides*, *Gordonia*, *Rhodococcus*, *Nocardia*,

**Table 2**
Responses of indigenous bacterial communities to PAE exposure in different environmental matrices.

| Environmental matrices | PAE type (concentrations) | Variations of microbial community | Variations of PAE-degrading taxa and genes | Community analysis approach | Ref. |
|---|---|---|---|---|---|
| Black soil | DMP (5; 10; 20; 40 mg/kg) | Alpha diversity and richness decreased; beta diversity changed | The relative abundance of *Novosphingobium*, *Sphingobium*, *Sphingopyxis* increased | 16S rRNA amplicon sequencing | (Wang et al., 2015) |
| Black soil | DBP (5; 10; 20; 40 mg/kg) | Alpha diversity and richness decreased; beta diversity changed | The relative abundance of *Novosphingobium, Sphingobium, Sphingopyxis, Arthrobacter, Sphingomonas, Rhodococcus* increased | 16S rRNA amplicon sequencing | (Wang et al., 2016a,b) |
| Vegetable soil | DMP; DEP; DBP; DEHP; DCHP; DOP; BBP; DiBP; BMPP; DEEP; DHXP; DBEP (756–1590 μg/kg) | Alpha diversity and richness decreased; beta diversity changed and community structure changed | The relative abundance of *Rhodanobacter, Dokdonella* increased | 16S rRNA amplicon sequencing | (Zhou et al., 2020a,b) |
| Agricultural soil | DBP (2; 10; 20 mg/kg) | Bacterial community structure changed | — | 16S rRNA amplicon sequencing | (Cheng et al., 2018) |
| Soil | DBP (50; 200; 500 mg/kg) | Alpha diversity and richness decreased; beta diversity changed and community structure changed | The relative abundance of *Delftia, Rhodococcus* and *Comamonas* increased | 16S rRNA amplicon sequencing | (Kong et al., 2018) |
| Soil | DEHP (200 mg/kg) | Beta diversity changed and community structure changed | The relative abundance of unclassified *Xanthomonadaceae, Dyella, Lysobacter, Ralstonia, Bradyrhizobium, Sphingomonas, Nocardia, Azoarcus, Nocardioides, Kribbella* increased | 16S rRNA amplicon sequencing | (Zhu et al., 2018) |
| Soil | DEHP (100; 1000 mg/kg) | Alpha diversity and richness decreased; beta diversity and community structure changed | The relative abundance of *Intrasporangiaceae, Arthrobacter, Blastococcus, Micromonospora, Marmoricoa, Nocardioides, Streptomyces, Gemmatimonas, Gemmatirosa, Gemmatimonadaceae* increased | 16S rRNA amplicon sequencing | (Zhu et al., 2019) |
| Soil | DBP (5; 10; 20; 40 mg/kg) | Bacterial community structure changed | The relative abundance of *Arthrobacter* and *Nocardioides* increased; *pht* gene cluster expression enhanced | Metagenomic sequencing | (Xu et al., 2018) |
| Soil | DEHP (1000 mg/kg) | Bacterial community structure changed | The relative abundance of *Pimelobacter, Nocardioides, Gemmatirosa, Gemmatimonas, Ramlibacter, Candidatus, Koribacter* and *Flavisolibacter* significantly increased; esterase/lipase/cytochrome P450 genes significantly enriched | Metagenomic sequencing | (Zhu et al., 2020) |
| Plant rhizosphere | DBP (10; 20; 40 mg/kg) DEHP (10; 20; 40 mg/kg) | Alpha diversity and richness decreased; beta diversity and community structure changed | The relative abundance of *Lysobacter, Streptomyces, Gemmatirosa, Sphingomonas, Bacillus* increased | 16S rRNA amplicon sequencing | (Gao et al., 2020) |
| Plant phyllosphere | DBP (320; 360; 400; 440 mg/L) | Alpha diversity and richness decreased; beta diversity and community structure changed | The relative abundance of *Paracoccus* and *Rhodococcus* significantly increased | 16S rRNA amplicon sequencing | (Pan et al., 2019) |
| Constructed wetland | DBP (500 μg/L) | Alpha diversity and richness decreased; beta diversity and community structure changed | The relative abundance of *Janthinobacterium, Flavobacterium* and *Curvularia* increased | 16S rRNA amplicon sequencing | (Li et al., 2020) |
| Biofilm | DEHP (1000 μg/L) | Alpha diversity and richness decreased; beta diversity and community structure changed | The relative abundance of *Acinetobacter* and *Bacillus* significantly increased | 16S rRNA amplicon sequencing | (Li et al., 2019) |
| Activated sludge | DBP (1000 mg/L) DEHP (1000 mg/L) | Bacterial community structure changed | The relative abundance of *Gordonia, Achromobacter, Rhodococcus, Rhizobium, Chryseobacterium, Delftia, Pseudomonas, Flavobacterium* and *Sphingobium* increased | 16S rRNA amplicon sequencing | (Wang et al., 2020) |
| Dust | DEHP (1041–10066 μg/g) | Alpha diversity and richness decreased; beta diversity and community structure changed | The relative abundance of *Bacillus, Corynebacterium jeddahense, Streptococcus, Peptoniphilus, Sphingomonas* and *Chryseobacterium* increased | 16S rRNA amplicon sequencing | (Velazquez et al., 2019) |

Note: BBP, Butyl benzyl phthalate; DMP, Dimethyl phthalate; DEP, Diethyl phthalate; DBP, Di-n-butyl phthalate; DEHP, Di(2-ethylhexyl) phthalate; DOP, Di-n-octyl phthalate; DCHP,Dicyclohexyl phthalate; DiBP, Di-isobutyl phthalate; BMPP, Bis (4-Methyl-2-pentyl) phthalate; DEEP, Bis(2-ethoxyethyl) phthalate; DHXP, Di-n-hexyl phthalate; DBEP, Bis (2-n-butoxyethyl) phthalate.

*Mycobacterium* and *Sphingobium* could be responsible for DEHP upstream degradation (i.e., DEHP to mono (2-ethylhexyl) phthalate (MEHP) to PA), while DEHP downstream degradation (i.e., PA to other metabolites) was mediated by the genera *Nocardioides*, *Pimelobacter*, *Mycobacterium*, *Nocardia* and *Azohydromonas*. Also, Xu et al. (2018) explored the impact of DBP on metabolic pathways of soil bacterial communities via metagenomic sequencing. The results revealed that the expressions of DBP-degrading genes (e.g., *peh*A, *pcm*A, *pht*Ab, *pht*B and *pht*C) were markedly increased when indigenous bacterial communities were exposed to DBP, which indicated that G+ bacteria could be mainly involved in DBP biodegradation in soil. Together, the above cultivation-independent investigations revealed that the genus of *Sphingomonas*, *Rhodococcus*, *Sphingobium*, *Arthrobacter*, *Bacillus* and *Flavobacterium* showed a positive response to PAE pollutants (Table 2), and generally agreed with the phylogeny of the cultured PAE-degrading isolates at the phylum level (Fig. 2).

Despite amplicon and metagenomic sequencing have been demonstrated to be the powerful methods for probing the structure and function of indigenous bacterial communities, it is hard to differentiate the genes/genomes of interest due to the vast diversity of microbial community in most ecosystems (Zhou et al., 2015a,b). Fortunately, this obstacle could be overcome by the integration of stable isotope probing (SIP) and high-throughput sequencing, which not only contributes to recover novel enzymes and operons, and also provides access to identify the specific bacteria participating in substrate metabolism of interest (Chen and Murrell 2010). For example, using the combinations of DNA-

SIP and 16S rRNA gene amplicon sequencing, Song et al. (2019) found that five novel OTUs affiliating to genera *Brevundimona*, *Singulisphaera*, and *Dyella* and classes *Spartobacteria* and *Ktedonobacteria* were most likely to participate into the *in situ* DEHP biodegradation, which facilitated our identification of *in situ* bacterial degraders.

### 3.2. Current status and challenges for understanding in situ interactions within PAE-degrading bacterial communities

Microorganisms often co-exist as a community in nature, where its members unidirectionally or bidirectionally interact with each other (Fuhrman, 2009; Little et al., 2008). On the basis of positive, negative, or no impacts on particular species involved, these interactions could be divided into six basal patterns: commensalism, competition, predation, parasitism, cooperation, amenalism or neutralism (Faust and Raes, 2012). Numerous studies showed that microbial interactions were closely related to community diversity and stability, as well as the driving force for the community function (e.g., element cycling, food fermentation and biofuels production) (Canon et al., 2020; Huang et al., 2020; Ivey et al., 2013; Ratzke et al., 2020; Zhou et al., 2020a,b). Importantly, microbial interactions played an important role in PAE biodegradation in the environments (Rodríguez Amor and Dal Bello, 2019; Tsoi et al., 2018). Gu et al. (2005) studied DMP degradation processes in mangrove sediments and found that the complete DMP degradation required a cooperation between *Comamonas* strains MPsc and *Rodococcus zopfii* MPpc. The similar phenomenon was also observed in a BBP-degrading bacterial consortium enriched from soil, where *Arthrobacter* sp. strain WY could deliver BBP metabolites (e.g., alcohols of BBP) to *Acinetobacter* sp. strain FW to cooperatively complete the BBP degradation (Chatterjee and Dutta, 2008). These studies highlighted the crucial role of bacteria interactions in the complete mineralization of PAEs. Although some studies have predicted the potential bacterial interactions based on community sequencing data, it is still unclear how PAE-degrading bacterial members co-exist to complete the entire process of PAE degradation in the environments. This was largely due to the complexity of microbial communities and a lack of effective bioinformatic tools (De Roy et al., 2014; Zengler and Palsson, 2012). For instance, Song et al. (2019) noted that the active DEHP degraders in soil had negative correlations with the dominant family *Oxalobacteraceae* through molecular ecological network analysis of 16S rRNA sequencing data, yet it is still difficult to determine the roles of active DEHP degraders in the community, or how they exchange substances to achieve DEHP degradation. With the aim of filling this knowledge gap, our current challenge is to identify the key PAE degraders within complex indigenous bacterial communities and decipher the interactions among community members.

## 4. Synthetic bacterial communities for PAE bioremediation

Synthetic microbial ecology is a young and fast-developing discipline, which aims to understand the relationship between microbial interactions and community-level properties by building artificial, simplified communities of specific functional features (Dolinšek et al., 2016; Widder et al., 2016). Because of the low complexity and high controllability, synthetic microbial community can be used as a simplified representation or simulation of complex microbial community, and enable us to detect the exchanged metabolites among community members. The theories and approaches of synthetic microbial ecology can help us advance fundamental principle learning and optimize microbial community function, thereby contributing to the development of biotechnological strategies (e.g., bioremediation or biopharming).

### 4.1. Diversity-based, model-guide construction of synthetic bacterial community for PAE degradation

At present, two main approaches could be used to design and build synthetic microbial communities — top-down and bottom-up (Großkopf and Soyer, 2014). The top-down approach starts from complex natural microbial communities and relies on the carefully selected environmental variables that force microbial communities to display the desired functions (Lawson et al., 2019). A good application of the top-down approach is that Kim et al. (2016) used a down-flow hanging sponge reactor and adjusted the organic loading rates to selectively enrich microbial consortia degrading soluble microbial products from wastewater. By monitoring the dynamics of this enriched community, they identified *Saprospiraceae* and *Flavobacteriales* as the key microbial members and predicted positive interactions between key members and their neighboring members such as *Geobacter* and *Azobacter*. In contrast, the bottom-up approach starts with microbial pure cultures of known physiology and genomic information, and creates desirable microbial communities with the help of the mathematical modeling and automation technology (Lawson et al., 2019). For instance, Chodkowski and Shade (2017) developed a synthetic community with the common environmental strains: *Burkholderia thailendensis* E264, *chromobacterium violaceum* SC11378 and *Pseudomonas syringae* DC3000. Based on growth characteristics and metabolite detection, researchers uncovered the molecular mechanisms of antagonism between *P. syringae* and *B. thailandensis*. Nevertheless, these two approaches have their own limitations. The top-down approach may miss the information on microbial metabolic networks and related molecular transformation processes (Rodríguez Amor and Dal Bello, 2019), whereas the bottom-up approach may neglect naturally occurring microbial interdependences via artificially simplified combination patterns (Che and Men, 2019). For the ecosystem with highly diverse microbial communities (e.g., soil, activated sludge and human gut), a judiciously balanced blend of the two above approaches could facilitate to successfully build synthetic microbial community. This pattern not only captures more microbial interdependences in the natural environments, and also focuses on the metabolic networks and the metabolite transfer among microbial individuals. For instance, Yu et al. (2019) enriched a bisphenol A (BPA)-degrading microbial community from the activated sludge through a top-down approach, constructed a community-level BPA metabolic pathway with targeted metabolites and multi-omics data, and identified the key BPA degraders (*Sphingonomas* and *Pseudomonas* sp.) in the community. At the same time, they used a bottom-up approach to reconstruct synthetic microbial communities to confirm the synergistic interaction of cross-feeding between BPA-degrading species (*Sphingonomas* sp.) and intermediate species (*Pseudomonas* sp.).

Based on the theories and methods of synthetic microbial ecology, we present a framework to construct and study synthetic PAE-degrading communities via the design-build-test-learn (DBTL) cycle, which is crucial for understanding the interaction mechanism of indigenous bacteria communities and improving the efficiency of *in situ* PAE biodegradation (Fig. 4). First, we choose environmental sites with long-term PAE pollution to collect samples (e.g., agricultural soil, municipal wastewater and activated sludge). Through metabolite detection and stoichiometric calculation, we try to uncover the PAE biodegradation processes and their kinetics in the contaminated environments. Such information was the prerequisite of whether we can get a targeted PAE-degrading consortium. Second, we need to screen out the optimal enrichment parameters, such as inoculum size, PAE loading rate, culturing temperature, pH and oxygen level, thereby obtaining a targeted PAE-degrading consortium after enrichment culture. Third, by analyzing microbial diversity and function in response to various concentrations of PAE using 16S rRNA gene amplicon sequencing, multi-omics technologies and SIP, we could identify key PAE degraders and reconstruct PAE metabolic pathways at the community level, and then use network approach to explore potential interactions among PAE

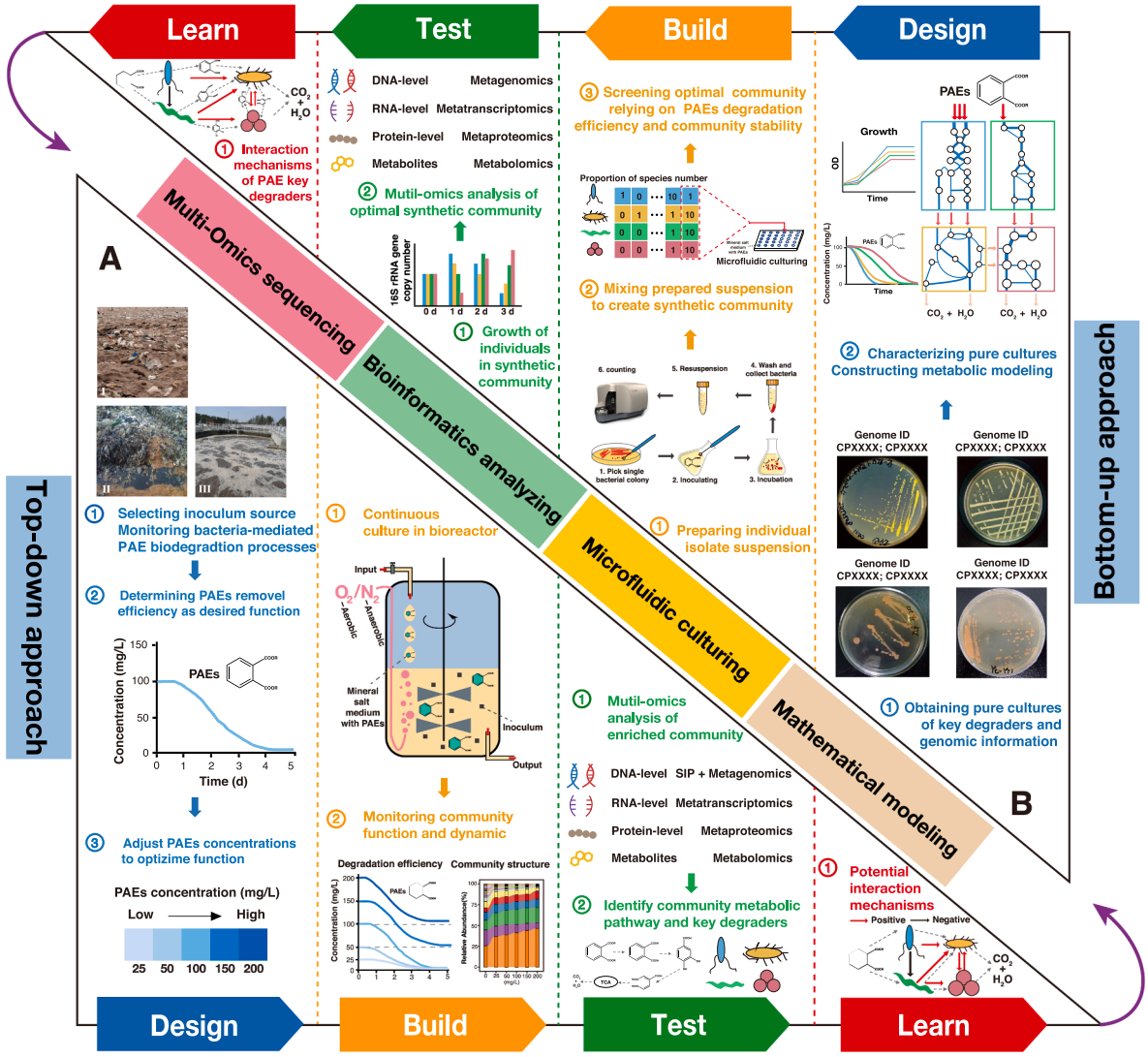



**Fig. 4.** Top-down and bottom-up approaches to design and construct synthetic bacterial community for PAE degradation. (A) Top-down approach for enriching and studying the PAE-degrading bacterial communities. **(B)** Bottom-up approach for building and studying the synthetic PAE-degrading bacterial communities. I: agricultural soil; II: landfill leachate; III: municipal wastewater.

degraders (Fig. 4A). Fourth, we endeavor to isolate the key PAE degraders by advanced culture techniques (e.g., culturomics) (Lagier et al., 2016) and examine their genetic, physiological, biochemical characterizations. With the help of mathematical modeling and microfluidics technique, we subsequently mix PAE-degrading isolates via bottom-up approach to create stable synthetic community. Last, we test PAE degradation rate of stable synthetic community across time and detect the variations of community members at abundance-, gene-, protein- and metabolite-levels, thereby deciphering the microbial interactions and underlying mechanisms within PAE-degrading bacterial community (Fig. 4B).

It has been well acknowledged that the mathematical modeling tools and computational techniques played an indispensable role in bottom-up designing of synthetic communities (Faust, 2019). Because it is hard to directly measure the extent and direction of microbial interaction via experimental technologies, researchers developed and used the mathematical modeling to study how microbes interact to accomplish complex tasks (Zomorrodi and Segrè, 2016). It has been widely accepted that Genome-scale metabolic models (GSMM) and Flux Balance Analysis (FBA) were the key model tools to predict how network-based metabolic fluxes generate community-level function (Orth et al., 2010; Thiele and Palsson, 2010). GSMM described the molecular mechanisms in chemical details by computing phenotypes from genotypes of microbes, which allowed us to trace the substrate transformation through the metabolic networks and compute the growth rates of microbes (Zengler and Palsson, 2012). FBA defined objective functions of the single species and community, and predicted the optimal metabolic model (Xu et al., 2018a,b). For the bottom-up designing of synthetic PAE-degrading bacterial community, we recommend to reconstruct the metabolic models of PAE-degrading isolates on the basis of their genomic information and then perform manual curation (Kanehisa et al., 2013; Nordberg et al., 2013; The UniProt Consortium, 2016). Under the different culture conditions, it is necessary to investigate whether there is biomass production of each isolate based on FBA in COBRA Toolbox v.3.0 (Heirendt et al., 2019), which will produce high-quality metabolic models of microbes. Importantly, such isolate-level models could help us construct community-level model via dynamic Flux Balance Analysis (dFBA) in the model frameworks (e.g., COMETS, OptCom and μbialSim) (Harcombe et al., 2014; Popp and Centler, 2020; Zomorrodi et al., 2014). In simulation with community-level modeling based on biomass production, we would screen out the best combination of those PAE-degrading isolates, which guides us to build synthetic PAE-degrading bacterial community (Fig. 4B). To overcome the logistical complexity of strain combinations, the construction of synthetic community

requires a high-throughput culturing platform that enables us to adequately sample combinatorial space in a single experiment (Nai and Meyer, 2018). Microfluidics culturing was one of the most promising techniques to achieve this goal. Kehe et al. (2019) developed the kChip, a droplets-based platform that performs rapid, massively parallel, bottom-up construction of synthetic microbial communities. Amazingly, it could parallelly construct ~$10^5$ synthetic microbial communities per day and required no robotic liquid handling. Taken together, the development of mathematical modeling (e.g., metabolic modeling) and automation technique (e.g., microfluidics culturing) provides a powerful guarantee for the construction of synthetic PAE-degrading bacterial communities.

### 4.2. *Tapping bioremediation potential of synthetic bacterial communities in PAE-contaminated sites*

Currently, the main bioremediation strategies included two categories: bioaugmentation (introduction of specific degrading strains or microbial consortia) and biostimulation (addition of nutrients or electron acceptors) (El Fantroussi and Agathos, 2005; Löffler and Edwards, 2006). Both bioaugmentation and biostimulation were successful to clear up pollutants in laboratory experiments, but they did not function well in the field (Tyagi et al., 2011). Fortunately, synthetic microbial communities hold great potentials to address this question and promote the developments of efficient bioremediation strategies. For instance, through the metabolic modeling and synthetic community construction, Xu et al. (2018a,b) deciphered the synergistic interactions between the atrazine degrader (*Arthrobacter aurescens* TC1) and non-degrader (*Halobacillus* sp. BAB-2008 or *Halomonas stevensii* S18214) in soil. Based on mechanistic understanding on atrazine biodegradation, they further developed the novel strategies of bioaugmentation (introducing *Halobacillus,* and/or *Halomonas* to the atrazine-contaminated soil) and biostimulation (adding additional carbon sources such as glucose), demonstrating that synthetic bacterial communities could facilitate tapping bioremediation potential to reduce atrazine contamination. In support of this notion, Zhuang et al. (2011) also found that synthetic bacterial community could serve as a useful model system to study microbial interactions between *Geobacter sulfurreducens* and *Rhodoferax ferrireducens* in the uranium-contaminated groundwater. Competition interaction between *Geobacter* and *Rhodoferax* provided an important clue to determine factors regulating uranium *in situ* bioremediation. Thus, it is anticipated that, with the aid of mathematical modeling, the constructions of synthetic PAE-degrading bacterial communities will spur new discoveries of *in situ* PAE biodegradation mediated by bacteria and lay a solid foundation for designing the effective bioremediation solutions (e.g., strain selection for bioaugmentation and selection of energy sources or electron accepts for biostimulation).

## 5. Conclusion and future perspectives

Bacteria-driven PAE biodegradation had always been considered as the center of PAE bioremediation work (Benjamin et al., 2015; Ren et al., 2018). However, due to the inherent complexity of indigenous microbial community, we had little knowledges about the mechanism of *in situ* PAE biodegradation, which made us difficult to harness bacteria-driven biodegradation potential to clear up PAEs from the environments. With the advances in synthetic biology and metabolic modeling, synthetic microbial ecology allowed us to construct artificial PAE-degrading microbial communities by an iterative design-build-test-learn (DBTL) approach, opening up a new avenue to systematically decipher the fundamental principles of *in situ* PAE biodegradation (Fig. 4). A clear understanding of how key degraders interact to complete PAE biodegradation could help us rationally inoculate the specific microorganisms or change environmental conditions to accelerate the PAE degradation at the contaminated sites. Nevertheless, the study on synthetic PAE-degrading microbial community is still in early infancy, and we need to take more efforts in the following aspects: (1) collecting various samples from the PAE-contaminated environments as the inoculum of top-down enrichment culturing (Barrett et al., 2020); (2) incorporating chemical or biochemical analytical tools (e.g., Ion mobility mass spectrometry) to detect PAE metabolites and reconstruct PAE metabolic pathways in the polluted environments (Paglia et al., 2021); (3) improving molecular network analysis methods to accurately identify keystone taxa in PAE-degrading communities (Benedetti et al., 2020; Lee et al., 2020); (4) isolating and characterizing more diverse PAE-degrading bacteria at the genetic, physiological, biochemical and ecological levels, because the culture collection of PAE-degrading bacteria is one of the prerequisites for building synthetic model communities (Jiang et al., 2016); (5) developing new algorithms of mathematical modelling to guide the construction of synthetic PAE-degrading microbial community (Medema et al., 2012; Zomorrodi and Segrè, 2016); (6) engineering ecological niches to prompt PAE-degrading isolates stable coexistence (Shahab et al., 2020); (7) applying gene editing tools (e.g., CRISPR/Cas9) to modify intracellularly genetic circuits responsible for PAE degradation in the existing isolates, thereby improving metabolic activity of PAE-degrading isolate and optimizing functionality of synthetic microbial community (Wang et al., 2016a,b); and (8) expanding the diversity of synthetic community members covering fungi, protist and virus, and extending synthetic PAE-degrading bacterial community to synthetic PAE-degrading microbiome (Deveau et al., 2018; Lawson et al., 2019). The road ahead for applications of synthetic bacterial community in PAE bioremediation seems long, given our nascent understanding of synthetic microbial ecology. However, it is expected that the integration of synthetic microbial ecology with high-throughput sequencing, molecular biology, biochemistry and engineering could revolutionize current studies on bacteria-driven PAE biodegradation and facilitate the development of innovative bioremediation solutions.

## CRediT authorship contribution statement

**Ruiwen Hu:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Haiming Zhao:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Xihui Xu:** Conceptualization, Data curation, Formal analysis. **Zhigang Wang:** Data curation, Formal analysis, Funding acquisition, Investigation. **Ke Yu:** Data curation, Formal analysis, Funding acquisition. **Longfei Shu:** Investigation, Project administration, Writing - original draft. **Qingyun Yan:** Investigation, Project administration, Writing - original draft. **Bo Wu:** Project administration, Resources, Software, Supervision, Validation. **Cehui Mo:** Investigation, Methodology, Project administration. **Zhili He:** Project administration, Writing - original draft, Writing - review & editing. **Cheng Wang:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing - original draft, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106560.

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