



Review

Synthetically engineered microbial scavengers for enhanced bioremediation

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ABSTRACT

Microbial bioremediation has gained attention as a cheap, efficient, and sustainable technology to manage the increasing environmental pollution. Since microorganisms in nature are not evolved to degrade pollutants, there is an increasing demand for developing safer and more efficient pollutant-scavengers for enhanced bioremediation. In this review, we introduce the strategies and technologies developed in the field of synthetic biology and their applications to the construction of microbial scavengers with improved efficiency of biodegradation while minimizing the impact of genetically engineered microbial scavengers on ecosystems. In addition, we discuss recent achievements in the biodegradation of fastidious pollutants, greenhouse gases, and microplastics using engineered microbial scavengers. Using synthetic microbial scavengers and multidisciplinary technologies, toxic pollutants could be more easily eliminated, and the environment could be more efficiently recovered.

1. Introduction: microbial degradation for bioremediation

There is a great concern about increasing environmental contamination generated due to human intervention. There are various toxic contaminants, such as aromatic compounds (Cvetnic et al., 2019; Li et al., 2020c), halogens (Benedetti et al., 2016), pesticides (Fang et al., 2018), dyes (Liu et al., 2020b), and heavy metals (Tay et al., 2017; Xu et al., 2018a; Zhang et al., 2020) from factories; microplastics from disposable products; and air pollutants such as CO₂ (Anenberg et al., 2019), CH₄ (Mayr et al., 2020), and SO₂ (Zhong et al., 2020) from industrial and agricultural activities. These toxic chemicals may cause respiratory and cardiovascular diseases and cancers (Simkovich et al., 2019; Zhang et al., 2020).

Bioremediation is of great interest today owing to its sustainability, safety, and economics (Horemans et al., 2017; Kobayashi and Rittmann, 1982; Ramos et al., 2002). Conventional remediation methods are operated via chemical and physical processes. Contaminants are transferred into another medium, such as air or water, and are collected in a concentrated form, following which they are destroyed or decomposed, e.g., via oxidation, in a smaller and benign form (Fox, 1996). However, chemical methods often display low efficiencies and are costly, and the chemicals used for decomposition reactions may produce toxic intermediates, which also need to be cleared (Mishra et al., 2019).

Bioremediation is carried out by living organisms such as bacteria that utilize pollutants and toxic chemicals as nutrient sources for growth. The contaminants are removed completely or transformed into non-hazardous products via the metabolism (a cascade of biochemical reactions) of living organisms (Horemans et al., 2017; Kobayashi and Rittmann, 1982; Ramos et al., 2002). Bioremediation offers many advantages over other cleanup methods. Since bioremediation relies only on natural metabolic processes in living systems, it produces few byproducts and thus the damage to the surrounding environment is minimized. In addition, since living organisms are small factories that include all the materials to decompose toxic chemicals, bioremediation does not require substantial equipment, that is powered by fossil fuels and emit pollutants. Therefore, bioremediation is cheaper and cleaner than other methods. In this regard, the global bioremediation market was valued at USD 106 billion in 2019 and is estimated to reach up to USD 335 billion by 2027 at a compound annual growth rate of 15.5% (Emergence Research, 2020).

Microbes for bioremediation can be isolated from natural bacterial communities, mostly from contaminated sites. For example, *Pseudomonas putida* is able to utilize toxic chemicals such as benzene, toluene, and xylene as carbon sources (Safari et al., 2019) and is also resistant to the toxic chemicals (Ibrahim et al., 2020). Thus, *P. putida* is a good material to remediate toxic organic compounds. A general strategy to

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isolate appropriate bacteria for bioremediation includes (1) sample collection, e.g., from sewage treatment plants and contaminated soils, (2) screening and isolation by using a specific medium containing toxic pollutants of interest, (3) identification of the bacterium, e.g., by 16S rRNA sequencing or genome sequencing, and (4) characterization of the physiology and bioremediation ability of the bacterium (Aldhafiri et al., 2018; Azaroff et al., 2021; Shabbir et al., 2018). In addition to single species, microbial communities that co-metabolize toxic chemicals have been recently identified and utilized for bioremediation; these include rhizosphere bacteria for petroleum (Viesser et al., 2020), *Bacillus* species for hydrocarbons (Oyetibo et al., 2017), and *Paraburkholderia* species for aromatic compounds (Lee et al., 2019). Natural bacterial species used for bioremediation are listed in Table 1.

Despite the impressive innate abilities of microbes, recently there has been an increasing demand for engineered synthetic microbial scavengers with enhanced efficiency of degradation and improved tolerance to toxic contaminants (Ishag et al., 2016; Viesser et al., 2020; Zhang et al., 2021). The isolated natural microbes, however, have not evolved to convert contaminants efficiently but have evolved to survive in the contaminated site. Therefore, they often display poor metabolic efficiency in breaking down toxic compounds due to low enzymatic activity or low substrate specificity, or they are susceptible to the toxic compounds (Dvořák et al., 2017; Joutey et al., 2013).

Synthetic microbial scavengers are microorganisms rationally designed using advanced genetic engineering techniques in the fields of synthetic biology and metabolic engineering and using computational methods in the field of systems biology (DeLorenzo and Moon, 2019; Ofaim et al., 2020; Young et al., 2021). The advanced techniques enabled us to develop synthetic microbial scavengers with superior metabolism and tolerance by creating new synthetic catabolic pathways or rewiring and optimizing gene regulatory and metabolic pathways (Gong et al., 2016; Ilic Durdic et al., 2020; Li et al., 2020c; Nikel and de Lorenzo, 2013).

Here, we review the methodologies used to develop synthetic microbial scavengers for efficient bioremediation, including chassis development and reconstruction, and optimization of degradation pathways. We also discuss the recent achievements in development of synthetic microbial scavengers, the major challenges in this field, and the biosafety issue of genetically engineered microorganisms (GEMs).

2. Recent strategies for the construction of microbial scavengers

Owing to the recent advancements in microbial biotechnology driven by synthetic biology and metabolic engineering, it has now become possible to rationally design and create synthetic microbial scavengers with superior metabolic efficiency and tolerance to natural microbes. Novel synthetic catabolic pathways can be constructed using heterologous enzymes from various organisms, which transform any bacterial species into pollutant-degrading microbial scavengers. Further metabolic flux optimization with tunable gene expression systems allows us to maximize the metabolic capacity and efficiency of bioremediation. In this section, strategies used to develop synthetic microbial scavengers are introduced: construction of novel degradation pathways, pathway optimization, and tolerance improvement.

2.1. Construction of metabolic degradation pathways

Recent advances in sequencing technology and bioinformatic algorithms have enabled the analysis of genomic sequences and the identification of innate metabolic enzymes and pathways for bioremediation (Hong et al., 2017). For example, a complete innate catabolic pathway for the detoxification of carcinogenic cyanotoxin microcystin-LR was identified in *Sphingopyxis* sp. YF1, via whole-genome and multi-omic analysis (Yang et al., 2020). To date, several identified degradation pathways have been implemented in other bacterial species to enhance bioremediation, which have allowed the degradation of phenol by

Table 1

Natural bacterial species used for bioremediation.

Contaminants	Bacterial species	Efficiency of bioremediation	References
2-nitrotoluene (2NT), 3-nitrotoluene (3NT), 4-nitrotoluene (4NT), nitrobenzene (NB) and 2-nitrobenzoic acid (2NBA)	<i>Cupriavidus</i> sp. strain a3	16–59% of nitroaromatics was degraded after 14 days.	(Tiwari et al., 2020)
Chlorinated pesticides (2,4-D and DDT)	<i>P. fluorescens</i>	Up to 500 mg/L of 2,4-D was removed with an efficiency of approximately 99% within 24 h. Approximately 55–99% of 150 mg/L DDT was degraded in 24 h.	(Santacruz et al., 2005)
Pentachlorophenol (PCP)	<i>S. chlorophenolicum</i>	Wild-type <i>S. chlorophenolicum</i> could degrade 300 µM PCP within 8 h but could not grow at >0.4 mM PCP.	(Dai and Copley, 2004)
Petroleum hydrocarbon	<i>Rhodococcus erythropolis</i> S67	S67 strain was capable of converting 2% n-hexadecane supplied in medium into 780 mg/L of glycolipids.	(Petrakov et al., 2013)
	<i>B. thuringiensis</i> , <i>B. pumilus</i> and <i>R. hoagii</i>	<i>R. hoagii</i> was the highest consumer capable of degrading 85% of total petroleum hydrocarbons in 24 h.	(Viesser et al., 2020)
Crude oil	<i>Halobacillus</i> sp. EG1HP4QL	This bacterium could consume up to 35.3% of crude oil as the sole carbon source within 12 days.	(Ibrahim et al., 2020)
	<i>Alcanivorax borkumensis</i>	<i>A. borkumensis</i> was able to degrade up to 90% of hexadecane within 5 days.	(Omarova et al., 2019)
Naphthalene, BTEX, and aliphatic hydrocarbons	<i>Paraburkholderia aromaticivorans</i> BN5	A mixture of BTEX mixture (30 mg/L) was degraded completely within 5 days.	(Lee et al., 2019)
3, 4-dichloroaniline (DCA)	<i>C. testosteroni</i> WDL7	0.1 mM 3,4-DCA was degraded completely within 24 h.	(Horemans et al., 2017)
Phenol	<i>R. opacus</i> PD630	Molecular tracking of 0.5 g/L U- ¹³ C phenol in amino acid showed that the strain could produce biomass in 40 h using 0.5 g/L phenol.	(Roell et al., 2019)
	<i>G. nicotianae</i> MSSRFPD35	Degradation efficiency of phenol was up to 1117 mg/L within 60 h.	(Duraismy et al., 2020)
Polyethylene terephthalate (PET)	<i>I. sakaiensis</i> sp. nov.	The isolated strain could use the PET thin film as the sole carbon source.	(Tanasupawat et al., 2016)

Escherichia coli (Wang et al., 2019), haloalkanes by *P. putida* (Benedetti et al., 2016), heavy metals (mercury) by *E. coli* MC4100 strain PQN4 (Tay et al., 2017), and azo dye in *Pichia pastoris* (Liu et al., 2020b) (Table 2). However, inherent metabolic degradation pathways are often insufficient and unreliable for industrial applications because the inherent pathways have been optimized through evolution for survival, not for detoxification. Thus, further genetic manipulation is required to engineer the pathways to further increase cleanup capacity to meet industrial demands.

The advancement in bioinformatic algorithms and DNA synthesis technology has enabled the design and realization of artificial metabolic pathways using individual enzymes (Safari et al., 2019). For such rational synthetic pathway construction, the approach of the design-build-test-learn cycle is generally used (Fig. 1) (Lawson et al., 2019). Briefly, feasible metabolic pathways are designed using heterologous enzymes, and an appropriate bacterial species, generally possessing high tolerance and metabolic capacity, are selected. Thereafter, their genetic sequences are chemically synthesized and assembled into plasmids (Wang et al., 2019). If *in vitro* evaluation is possible, the kinetic characteristic of the realized pathways could be evaluated by high-throughput techniques (Zeng et al., 2020). The plasmids harboring the pathway genes are introduced into other living microbes (French et al., 2020; Ghatge et al., 2021) or they are integrated into the genome of the microbes (Zhao et al., 2020). The bioremediation efficiency of the engineered microorganisms is then evaluated (Benedetti et al., 2016; Gong et al., 2016; Yang et al., 2016). If the bioremediation efficiency is insufficient, further omic analysis or other analyses would be required to assess the factors that limit efficiency. Once the limiting factors are identified, various synthetic biology tools could be applied to solve the restrictions and enhance the rate of degradation (Young et al., 2021). To date, various synthetic metabolic pathways have been designed and implemented using heterologous enzymes to decompose toxic xenobiotic into non-toxic or beneficial end products (Benedetti et al., 2016; Gong et al., 2016, 2017; Martinez et al., 2016; Mohamed et al., 2020b; Wang et al., 2019).

2.2. Enhancement of the catalytic activity of adopted enzymes and pathways

Since most contaminant-decomposing pathways are secondary metabolism in living organisms, the enzymes involved in these pathways are not suitable for industrial use. For efficient decontamination, enzymes need to be engineered for higher activity and higher or altered substrate specificity to detoxify a broad spectrum of pollutants (Fig. 2) (Ilic Durdic et al., 2020; Liu et al., 2020a; Wang et al., 2020a; Yan et al., 2020). Conventional protein engineering strategies can be applied to enzyme modification, which involves mutagenesis of an enzyme gene and selection of the fittest enzyme (Ilic Durdic et al., 2020; Li et al., 2020a; Maxel et al., 2020; Wang et al., 2020b). However, the conventional random mutagenesis and screening approach is laborious and time-consuming because the complexity and search space of amino acid mutations are extraordinarily large (Khan and Akhtar, 2011). With the aid of computational methodologies, enzymes can be computationally designed to improve their binding affinity and specificity to substrates through molecular docking simulations. Molecular docking is a computational simulation method used to estimate the binding affinity of a small molecule (substrate) and a macromolecule (enzyme) by calculating the forces among atoms (Meng et al., 2011; Shaker et al., 2020). Recently, Zhang et al. (2019b) converted an oxygen-transporting myoglobin into an artificial dye-decolorizing peroxidase by introducing amino acid mutations (F43Y/F138W/P88W) in the heme pocket of the myoglobin, which was rationally chosen by docking simulation (Zhang et al., 2019b). Although the artificial peroxidase showed poorer catalytic activity than known natural peroxidases, it demonstrated the possibility of rational design of enzymes with a desired substrate specificity and activity.

In addition to enzyme engineering for improved catalytic activity, overall metabolic pathway activity can be also improved based on the idea that the enzymes belonging to the same pathway are physically in close proximity. Inherent enzymes belonging to the same pathway or reaction cascade generally stick together and thus a product of an enzyme can be immediately utilized as a substrate of the following enzyme without diffusion. This effect, known as the substrate tunneling effect, dramatically increases the overall metabolic reaction rates of a pathway. However, synthetic degradation pathways composed of heterologous enzymes do not show such high catalytic activity, because the heterologous enzymes do not link together and move freely in the cytoplasm. To resolve this issue, artificial scaffolds, targeting peptides, and immobilization methods have been developed to mimic the substrate tunneling effect of enzymes, and such scaffold strategies successfully enhanced the overall reaction rate of synthetic pathways (Kang et al., 2019; Parameswarappa et al., 2008). For example, a synthetic scaffold protein containing PDZ-SH3-GBD domains, which could recognize particular peptides, was constructed (Dueber et al., 2009). The domain-binding peptides were attached to each heterologous enzyme involved in a synthetic pathway. The scaffold protein recruited the enzymes and aligned them in close proximity and consequently mimicked the substrate tunneling effect. The use of the protein scaffold enhanced the efficiency of the pathway up to 77 folds. Similarly, a modular enzyme assembly approach was developed to enhance the metabolic efficiency of biocatalytic cascades using a pair of short peptide tags (RIAD and RIDD). These peptides interact with each other, and the proteins fused with the tags assemble (Kang et al., 2019). The peptides were applied to the catalytic optimization of two heterologous pathways in *E. coli*, carotenoid biosynthesis and mevalonate biosynthesis. The last enzyme of the mevalonate pathway and the first enzyme of the carotenoid pathway were fused with the short peptide tags to assemble. This assembly increased the overall carotenoid production by 5.7-fold (Kang et al., 2019). To date, diverse scaffolds and assembly methods were developed and utilized (Horn and Sticht, 2015).

2.3. Enhancing the performance of synthetic metabolic pathways through fine-tuning of gene expression

Enzyme levels should be optimized to balance metabolic flux and prevent the accumulation of metabolic intermediates in the host cell (Tsoi et al., 2018; Varman et al., 2018). For example, overexpression of an enzyme may deplete nutrients essential for enzyme production, which may reduce cell growth or limit its metabolic capacity. In addition, the overexpressed enzyme may accumulate its product metabolites inside the host cell, which may cause toxic effects. Therefore, in order to maximize metabolic efficiency, the expression levels of individual enzymes in the same pathway should be balanced. However, expression kinetics is determined by many different factors including promoter strength, ribosome strength, and mRNA secondary structure (DeLorenzo and Moon, 2019; Yoo et al., 2020). Of all the processes involved in gene expression, translation is affected by complex factors such as motifs in the 5' untranslated region, ribosome binding site strength, and secondary structure around the translation initiation region. Due to the complexity, it was difficult to predict translation efficiency, and thus several computational tools for prediction (Espah Borujeni et al., 2017; Na et al., 2010; Salis, 2011) and pre-constructed expression cassettes with a desired expression level were developed (Fig. 3) (Yoo et al., 2020). These tools were utilized for the metabolic engineering of various bacterial species and metabolites (Farasat et al., 2014; Jeschek et al., 2016; Sebesta and Peebles, 2020; Yoo et al., 2020).

2.4. Tolerance improvement of microbial scavengers

Scavenger host cells should survive under a working concentration of pollutants. Exposure of host cells to contaminants results in cellular damage and eventually cell death (Ramos et al., 2002; Sikkema et al.,

Table 2

Genetically engineered microbial scavengers for enhanced bioremediation.

Group	Pollutant	Engineered strain	Description	References
Petroleum and aromatic compounds	Phenol	<i>E. coli</i> BL21(DE3)	Synthetic metabolic modules were introduced to degrade phenol and produce biomass via the TCA cycle. The engineered strain was able to degrade 5 mM phenol completely within 7 h and 1 mM phenol completely within 3 h in raw coking waste water.	(Wang et al., 2019)
	Dibenzothiophene (DBT)	<i>P. putida</i> KT2440	A 4S pathway for the removal of recalcitrant sulfur of aromatic heterocycles in fuels was constructed by implementing the <i>dszABCD</i> genes in <i>P. putida</i> . The specific desulfurization activity was 23 $\mu\text{mol/g DCW/hour}$.	(Martinez et al., 2016)
	3-methyl diphenyl ether	<i>E. coli</i> BL21 (DE3)	The study involved introducing a cluster of <i>mdeAB</i> , <i>mdeD</i> , and <i>mdeC</i> genes of <i>Hydrogenophaga atypical</i> QY7–2 mediating methyl-oxidation reactions. The catalytic activity of recombinant enzymes was 113.8 ± 3.5 , 274.5 ± 6.2 , and 673.4 ± 8.7 nmol/min/mg, respectively.	(Yang et al., 2016)
	Haloalkanes	<i>P. putida</i> KT2440	The study involved genetic programming for catalytic biofilm formation using <i>yedQ</i> (diguanylate cyclase) or <i>yjhH</i> (c-di-GMP phosphodiesterase), which was integrated into <i>P. putida</i> KT2440 genome. The engineered strains were incubated in 1 mM cyclohexanone within 48 h. The catalytic activity of dehalogenase in the biofilm fraction was 0.75–1.85 units mg/protein.	(Benedetti et al., 2016)
	Phenanthrene, pyrene, and benzo(a) pyrene	A synthetically microbial consortium	A synthetic microbial consortium was constructed to tolerate and degrade phenanthrene, pyrene, benzo(a)pyrene. Ligninolytic enzymes were produced from engineered fungal strains, which improved the degradation rates of PAHs by 10%.	(Zafra et al., 2017)
	Acenaphthene	<i>E. coli</i> strain MX203	A mutant oxygenase of cytochrome P450-BM3 (D222N) was developed by directed evolution, which possessed an enhanced catalytic activity of converting acenaphthene to 1-acenaphenol up to 2.5-fold.	(Maxel et al., 2020)
Pesticides	Bensulfuron-methyl (BSM)	<i>Methylobionas</i> sp. strain LW13	A strain LW13 was engineered to harbor a BSM-degrading hydrolase gene (<i>sulE</i>) originated from <i>Hansschlegelia zhihuaiae</i> S11. The methanotrophic scavenger efficiently degraded 90% of BSM within 9 days.	(Liu et al., 2021c)
	Butralin (n-butan-2-yl-4-tert-butyl-2,6-dinitroaniline)	<i>E. coli</i>	A butralin degradation pathway was found by genome sequencing of isolated bacteria (<i>Sphingopyxis</i> sp. strain HMH). When the key enzyme, nitroreductase (NfnB), was expressed in <i>E. coli</i> , the cells could degrade butralin and pendimethalin at 294.3 ± 10.2 and 164.0 ± 5.2 $\mu\text{mol/min/mg}$, respectively.	(Ghatge et al., 2021)
	Organochloride pesticides, organophosphorus pesticides, carbamates, and pyrethroid	<i>E. coli</i> strain BL21	<i>E. coli</i> was engineered to express a carboxylesterase B1 gene (<i>carE</i> B1) capable of degrading pesticides and to possess an inducible suicide system. Over 50% of pyrethroid, 80% of fenpropathrin, and 80% of permethrin were removed by the engineered strain after several hours, while the rates of degradation of chlorpyrifos and plifenate were very low. The engineered cells could be eliminated by inducing the expression of a nuclease gene originated from <i>S. marcescens</i> .	(Li et al., 2020d)
	Methyl parathion	<i>P. putida</i> KT2440	An artificial degradation pathway was integrated into the chromosome of KT2440 for complete mineralization of methyl parathion and γ -hexachlorocyclohexane. The cells completely degraded 50 mg/L of MP and γ -HCH in soils in 6 and 11 days, respectively.	(Gong et al., 2016)
Heavy metals	γ -Hexachlorocyclohexane	<i>E. coli</i> MC4100 strain PQN4	The study involved construction of a synthetic circuit and redesign of the MerR operon to detect Hg^{2+} in the environment. The limit of detection of Hg^{2+} was above 400 ppb in the curli-expressing bacteria that showed a 4.5-fold increase in dry weight compared to that of the control when exposed to 1200 ppb Hg^{2+} .	(Tay et al., 2017)
	Mercury (Hg)	<i>E. coli</i>	About 92% of <i>E. coli</i> EcSSMO was resistant to 50 mg/L of Pb^{2+} and Cd^{2+} .	(Liu et al., 2021a)
	Lead (Pb) and Cadmium (Cd)	<i>S. oneidensis</i> MR-1	CRISPR-dAsCpf1 was used to inhibit the expression of the genes involved in an extracellular electron transfer system of <i>S. oneidensis</i> MR-1. The repression enhanced the removal of chromium and methyl orange up to 3 folds.	(Li et al., 2020c)
	Chromium (Cr)	<i>E. coli</i>	Copper-binding peptides with a high binding affinity were screened and identified. The peptides improved the copper tolerance of <i>E. coli</i> cells.	(Gahlot et al., 2020)
Greenhouse gases	Copper (Cu)	<i>E. coli</i>	Copper-binding peptides with a high binding affinity were screened and identified. The peptides improved the copper tolerance of <i>E. coli</i> cells.	(Gahlot et al., 2020)
	CO_2	<i>E. coli</i>	<i>E. coli</i> was engineered to use CO_2 using non-native Calvin cycle. The doubling of the engineered strains was still slow (approximately 18 h).	(Gleizer et al., 2019)
Microplastics	Methanol	<i>E. coli</i> SM1	Metabolic reprogramming, DNA-protein crosslinking, and metabolic flux analysis were used to develop a fast-growing methanol-consuming <i>E. coli</i> . The resulting methanotrophic <i>E. coli</i> strain showed a doubling time of 8 h.	(Chen et al., 2020)
	Poly (ethylene terephthalate) (PET)	<i>P. tricornutum</i>	A photosynthetic microalga, <i>P. tricornutum</i> , was engineered as a microbial cell factory to secrete PETase for biological PET degradation in the marine ecosystem.	(Moog et al., 2019)

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Table 2 (continued)

Group	Pollutant	Engineered strain	Description	References
Others	Polyvinyl chloride (PVC)	<i>P. aeruginosa</i>	A synthetic microplastic 'capture and release' system was developed by controlling a biofilm formation via c-di-GMP signal molecule.	(Liu et al., 2021b)
	Azo dyes	<i>E. coli</i>	Enzyme versatile peroxidase (VP) was engineered by saturation mutagenesis to obtain up to 16-fold higher catalytic efficiency than the wild type in <i>E. coli</i> XL10 Gold.	(Ilic Durdic et al., 2020)
		<i>P. pastoris</i> GS115	Laccase (Lac), manganese peroxidase (MnP), and lignin peroxidase (LiP) were cloned and expressed for secretion in <i>P. pastoris</i> . The decolorization rate of Lac on Congo red was 45.5% within the first 5 s	(Liu et al., 2020b)
	Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	<i>S. maltophilia</i> OK-5	Genome-shuffled strain completely degraded up to 75 μ M RDX after 50 days, with a 1.5-fold increase in cell number.	(Lee et al., 2017)
	Microcystin	<i>Novosphingobium</i> sp. THN1	Heterologous expression, bioinformatics, and site-directed mutagenesis were used to optimize the expression of Mlrc enzyme, and novel sites of Glu ³⁹ , His ¹³³ , and Asp ³³² were discovered for the enzyme activity enhancement.	(Wang et al., 2020a)

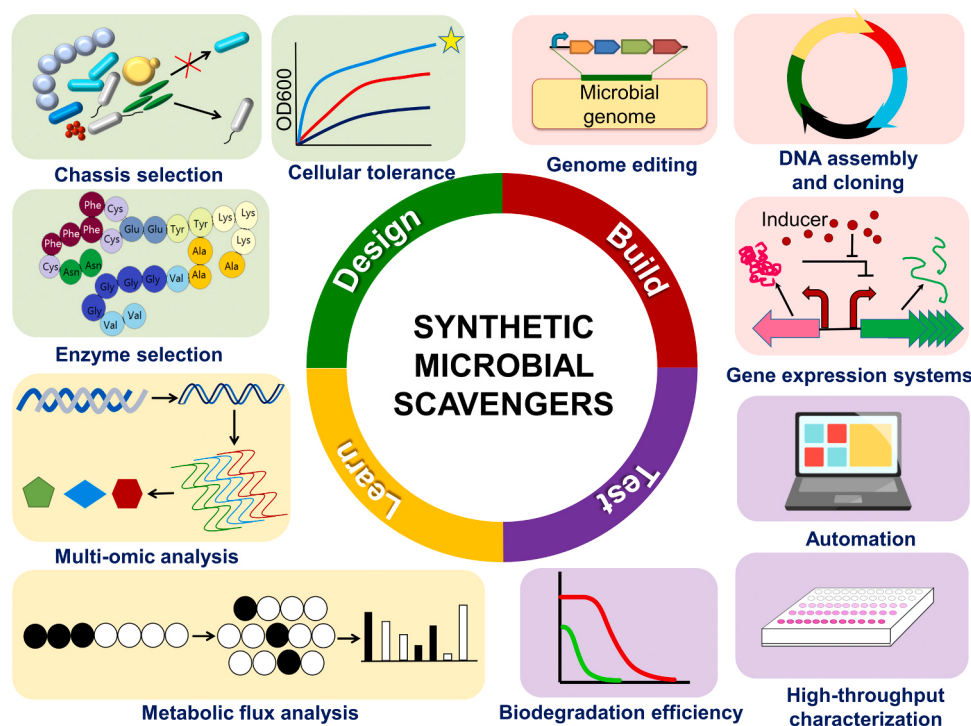


Fig. 1. The design–build–test–learn cycle for synthetic microbial scavenger construction. The cycle begins with the selection of an appropriate chassis and enzymes for pollutant degradation. The designed systems are then realized with genetic engineering techniques. The constructed microbial scavengers are evaluated and investigated for further optimization.

1995). Several strategies were used to improve cellular tolerance, including alteration in membrane lipid composition (Gialama et al., 2017), phenotypic screening through adaptive laboratory evolution (ALE) (Xu et al., 2018a), modification in global gene expression (Zhang et al., 2015), genome shuffling (Dai and Copley, 2004), directed evolution (Maxel et al., 2020), and other techniques (Mukhopadhyay, 2015) (Fig. 4).

Since tolerance is an outcome of complex cellular interactions, multiple genetic modification techniques were developed. ALE is an artificial evolution method that is widely used to improve cellular tolerance to toxic chemicals and metabolites. In ALE, during cell culture, a selection pressure such as a high concentration of pollutants is continuously provided. Mutant cells that successfully adapted to the pollutants are able to grow faster than wild-type cells under the pressure. After several rounds of cell culture, the medium is populated by the adapted mutant cells that are tolerant to the high concentration of pollutants (Mohamed et al., 2020a; Wang et al., 2020b; Xu et al., 2018a). Through laboratory evolution of *P. putida*, mutants (mutations within

the *ttyB* gene, genes involved in flagellar movement, and genes encoding transcriptional regulators) tolerant to *p*-coumaric acid and ferulic acid were generated (Mohamed et al., 2020a). ALE was also successfully applied to other bacterial species to generate tolerant mutants: *Corynebacterium glutamicum* to methanol (Wang et al., 2020b), *Synechocystis* sp. PCC 6803 to cadmium (Xu et al., 2018a), yeast strains to copper (Adamo et al., 2012), and *P. putida* to acids to clean up pollutants dissolved in acids (Zhou et al., 2019).

Global transcription machinery engineering (gTME) was used to induce extensive cellular changes by mutating genes encoding global regulators such as sigma factors (Tan et al., 2016) or transcriptional regulators (Liu et al., 2008). For gTME, a gene encoding a target global regulator is selected, and mutations are introduced into the gene by error-prone PCR to construct a mutant library. After introducing the mutant genes into a bacterial host, cells are screened for the desired phenotype. gTME of σ^{70} factor encoded by *rpoD*, a sigma subunit of *E. coli* RNA polymerase, successfully increased tolerance to benzene by up to 69% (Zhang et al., 2015). gTME was also used to increase tolerance

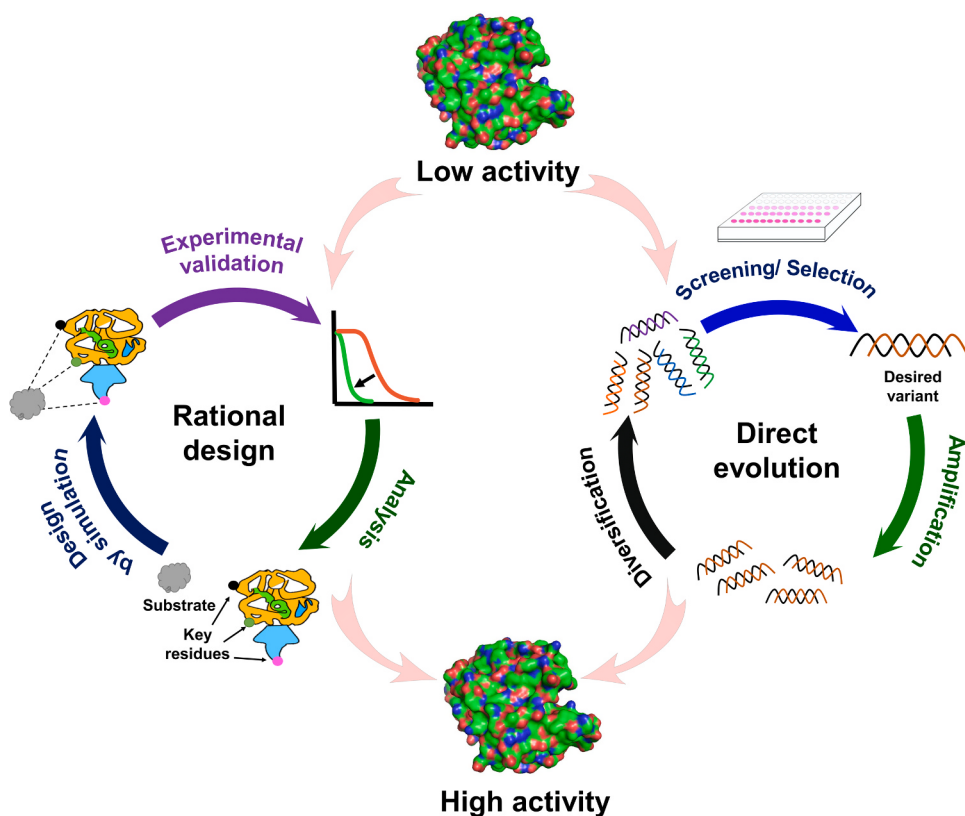


Fig. 2. Activity enhancement of the adopted heterologous enzymes in a synthetic degradation pathway. The catalytic activity of an enzyme can be improved by protein engineering technologies such as in silico rational design and direct evolution. Rational design is more efficient than randomness-based directed evolution, but it requires prior structural information of an enzyme-substrate complex. In the absence of such prior information, which is common in newly identified bacterial enzymes, directed evolution approach can be employed.

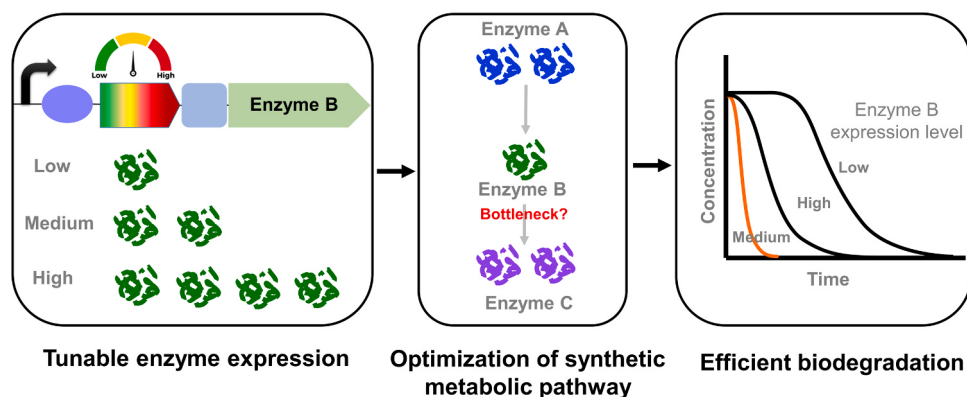


Fig. 3. Catabolic flux optimization for efficient biodegradation through fine-tuning of gene expression. Unbalanced expression level of the enzymes in a pathway may result in accumulation of intermediate metabolites, which reduces overall pathway activity. Optimization of enzyme expression by tunable gene expression systems increases the rate of biodegradation in microbial scavengers.

to valuable but toxic chemicals, such as ethanol (Liu et al., 2008).

Genome shuffling uses protoplast fusion to generate a library of mutants from multi-parent strains. Lee et al. (2017) successfully screened *Stenotrophomonas maltophilia* OK-5 genomes to shorten the decay time of hexahydro-1,3,5-trinitro-1,3,5-triazine, also known as RDX, developed to replace trinitrotoluene, but can cause seizure and death (Schneider et al., 1977). The mutant strain reached maximum growth 1.5-times faster than that of its ancestral strain. Moreover, the strain degraded RDX two times faster than the wild type (Lee et al., 2017). Genome shuffling is an efficient method to enhance catabolic enzyme activity and improve the robustness of microbial scavengers.

Aside from the above-mentioned techniques, other genome manipulation tools such as multiplex automated genome engineering (MAGE) (Wang et al., 2009), transcription activator-like effector nucleases (TALENs) (Bedell et al., 2012), CRISPR/Cas systems (Su et al., 2016),

and metagenomic methods are also efficient approaches to improve tolerance and degradation in microbial scavengers.

3. Synthetically engineered microbial scavengers for bioremediation

3.1. Aromatic compounds and petroleum

Simple aromatic compounds such as benzene, phenol, toluene, and xylene, and complex aromatic hydrocarbons are the most prevalent and persistent pollutants in the environment (Seo et al., 2009). Due to the increasing contamination caused by pollutants such as aromatic compounds, diverse microbial species with an inherent ability of biodegradation were isolated from contaminated sites (Duraismy et al., 2020; Haddadi and Shavandi, 2013; Nelson et al., 2002; Panigrahy et al., 2020;

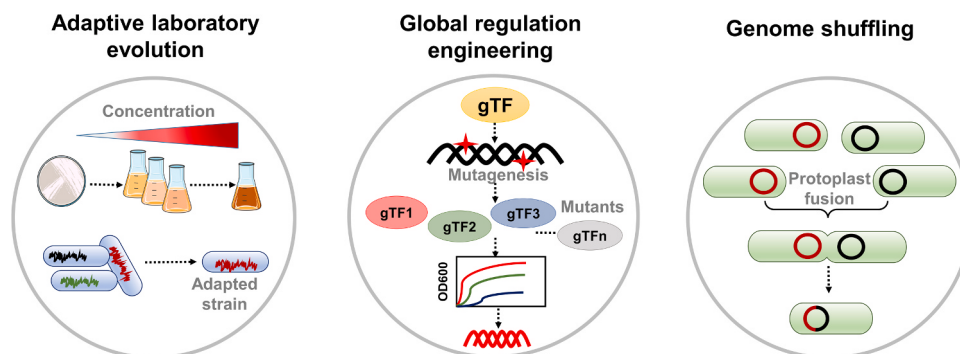


Fig. 4. Tolerance improvement of microbial scavengers. The tolerance of microbial scavengers can be improved by several strategies such as adaptive laboratory evolution, global regulation engineering, and genome shuffling. The mechanisms of each methodology are introduced discussed in Section 2.4.

Seo et al., 2009). However, the low metabolic efficiency of natural bacteria hindered their use as an agent to bioremediate aromatic compounds. Thus, synthetic microbial scavengers were developed to efficiently degrade aromatic compounds by reconstructing a synthetic aromatic-decomposing pathway in various microbes such as *E. coli* BL21 (DE3) for phenol degradation (Wang et al., 2019), *P. putida* KT2440 for oil sulfur compound decomposition (Martinez et al., 2016), and *P. putida* mt-2 for toluene biodegradation (Tsipa et al., 2017).

Of the many aromatic compounds, phenol is the most prevalent because of its wide use in industries. Various natural phenol-degrading bacteria were identified including *Halomonas* sp. strain PH2-2 and *Glutamicibacter nicotianae* MSSRFPD35 (Duraisamy et al., 2020). An artificial metabolic pathway for complete biodegradation of phenol was reconstructed in *E. coli* BL21 (DE3) (Wang et al., 2019), by which phenol was degraded into catechol by a phenol hydroxylase, which was then converted into muconic acid by an *ortho*-ring cleavage, and was finally consumed in the tricarboxylic acid (TCA) cycle. Nine foreign enzymes from *R. erythropolis*, *Rhodococcus* sp. AN-22, *R. jostii*, and *P. putida* were expressed under the control of a T7 promoter. The engineered strain was able to completely degrade 5 mM of phenol within 7 h and 1 mM of phenol in raw coking waste water within 3 h (Wang et al., 2019).

Polycyclic aromatic hydrocarbons (PAHs), composed of multiple aromatic rings, are widespread in the environment due to industrial activities. Since they are semi-volatile and bioaccumulative compounds, they are carcinogenic and teratogenic (Alegbeye et al., 2017). For the cleanup of PAHs, a microbial consortium, composed of six fungal and seven bacterial native strains, was constructed and the consortium showed high tolerance to phenanthrene, pyrene, and benzo(a)pyrene, and was able to utilize PAHs as a sole carbon source (Zafra et al., 2017). When two genetically engineered fungal strains, which produced ligninolytic enzymes highly effective for the initial oxidation of PAHs, were included in the consortium, degradation rates of PAHs increased by 10%. In another study, for the bioremediation of acenaphthene, one of the PAHs, an oxygenase of cytochrome P450-BM3 was directly evolved (Maxel et al., 2020). A mutant (D222N) showed 2.5-fold increased catalytic activity of converting acenaphthene to 1-acenaphenol, which was more soluble and biodegradable than acenaphthene.

The increasing use of petroleum hydrocarbons results in global environmental pollution that needs urgent remediation. For the bioremediation of petroleum-contaminated sites, natural microorganisms and microbial communities were isolated and utilized for petroleum degradation, which included *Rhodococcus ruber*, *Achromobacter* sp. HZ01, *Dietzia papillomatosis*, *Bacillus subtilis* A1, *Pseudomonas aeruginosa*, *R. erythropolis* HX-2, and others (Ali et al., 2020; Hong et al., 2017; Xu et al., 2018b). Recently, engineered microbial scavengers showed remarkable successes in remediating petroleum-contaminated sites without adverse effects (Lim et al., 2016; Ossai et al., 2020). *P. putida* KT2440 was engineered to decompose dibenzothiophene (DBT), an oil sulfur compound, into sulfur-free 2-hydroxybiphenyl (2HBP) via the 4S

pathway composed of nondestructive oxidative reactions (Martinez et al., 2016). For the implementation, three functional units from *R. erythropolis* IGTS8 were constructed, which included two monooxygenases (*dszA* and *dszC*), one desulfinase (*dszB*), and one flavin reductase (*dszD*). Moreover, a mutation was introduced into *dszB* to improve its stability and activity. The specific desulfurization activity was 23 $\mu\text{mol/g DCW/hour}$, which was higher than that previously reported (Martinez et al., 2016).

3.2. Pesticides

Pesticides protect crops from diseases and pests, but due to their persistence and toxicity, they are potential risks for living organisms including humans. Various microorganisms employ metabolic pathways to mineralize hazardous pesticides (Bajaj et al., 2017; Bhatt et al., 2020; Copley, 2009). Aldrin is a widely used and dangerous organochlorine insecticide. *Micrococcus* (phylum Actinobacteria), *Pseudomonas* (phylum Gammaproteobacteria), and *Bacillus* sp. (phylum Firmicutes) were isolated from aldrin-polluted soil and were identified to possess a P450 hydroxylase gene involved in pesticide degradation. Cultures of *B. polymyxa* and *P. fluorescens* efficiently removed about 40% of aldrin in 12 days (Doolotkeldieva et al., 2018). Though individual microorganisms can be utilized for bioremediation, a community of bacteria could be more efficient in bioremediation where each bacterial species plays a different role in removing contaminants (Martinez et al., 2016). Li et al. (2020b) developed a synthetic microbial community (PSC-1) to efficiently degrade seven persistent pesticides: chlorpyrifos, imidacloprid, carbendazim, chlorothalonil, lambda-cyhalothrin, beta-cypermethrin, and deltamethrin. The artificial community was composed of 10 bacterial strains and 24 fungal genera including several dominant species such as *Pseudomonas*, *Enterobacter*, *Aspergillus*, and *Rhodotorula* (Li et al., 2020b).

Bensulfuron-methyl (BSM) is one of the popular sulfonylurea herbicides. For BSM clearance, *Methylomonas* sp. strain LW13 was engineered to harbor a BSM-degrading hydrolase gene (*sulE*) originated from *Hansschlegelia zhihuaiae* S11 (Liu et al., 2021c). In addition, since the LW13 strain was capable of utilizing methane as a sole source of carbon and energy, the methanotrophic scavenger was able to degrade a persistent pesticide (BSM) as well as a greenhouse gas (methane) simultaneously. Efficient pesticide degradation was further achieved by synthetic biology approaches, including ribosome binding site optimization. The methanotrophic scavenger removed 90% of BSM within 9 days.

Dinitroaniline herbicides are widely used to inhibit the growth of weeds (Parka and Soper, 1977). Several bacterial species were isolated, which decomposed dinitroaniline herbicides such as trifluralin (Bellinaso et al., 2003) and pendimethalin (Ni et al., 2016). Butralin (n-butan-2-yl-4-tert-butyl-2,6-dinitroaniline) is a synthetic herbicide belonging to the dinitroaniline group and is highly toxic to human as

well as aquatic ecosystems. In a study, a bacterial species (*Sphingopyxis* sp. strain HMH) was isolated from contaminated soil and its genome was analyzed to identify a butralin catabolic pathway, since the butralin degradation pathway has not been identified before (Ghatge et al., 2021). A nitroreductase (NfnB) was identified from the genome, and biochemical assays proved that the enzyme was able to break down butralin and pendimethalin at 294.3 ± 10.2 and 164.0 ± 5.2 $\mu\text{mol}/\text{min}/\text{mg}$, respectively. The identified butralin degradation pathway could allow for the construction of new microbial scavengers possessing better growth and tolerance properties for industrial applications.

E. coli strain BL21 was engineered to detoxify organochloride pesticides, organophosphorus pesticides, carbamates, and pyrethroid insecticides (Li et al., 2020d). The strain harbored a carboxylesterase B1 gene (*carE* B1) capable of degrading the pesticides. Since one of the major concerns about the application of genetically engineered microbial scavengers is biosafety (discussed in Section 4), a conditional suicide plasmid containing a nuclease gene of *Serratia marcescens* was also introduced into the strain. Thus, the engineered scavenger could be eliminated by inducing the expression of the nuclease gene after completing the clean-up. Synthetic microbial scavengers with a suicide system could have diverse bioremediation applications and might address the concerns involved in the release of GEMs.

3.3. Organic halogen compounds

Organic halides are compounds where hydrogen atoms are replaced with halogen atoms including chlorine, bromine, fluorine, and iodine. The toxicity of halogen compounds has been studied during the past century (Hirade and Ninomiya, 1950) and the compounds were found to be neurotoxic, immunotoxic, and carcinogenic (Kodavanti and Loganathan, 2017).

For the construction of an efficient microbial scavenger for degrading halogen compounds, the genome of *P. putida* KT2440 was engineered to degrade 1,2,3-trichloropropane by implementing a degradation pathway of which the enzymes genes originated from *Sphingobium japonicum* UT26, *R. rhodochrous* NCIMB 13064, and *Agrobacterium radiobacter* AD1 were codon-optimized and chemically synthesized (Gong et al., 2017). In another study, for rapid and efficient integration of the genes into the genome of *P. putida* KT2440, a strategy consisting of DNA assembler-assisted pathway assembly and counter-selection system-based chromosomal integration was developed to degrade γ -hexachlorocyclohexane (γ -HCH) and 1,2,3-trichloropropane (TCP) (Zhao et al., 2020).

3.4. Heavy metals

Toxic heavy metals are released into marine and soil environments in large quantities every year due to human intervention. Heavy metals such as cadmium, chromium, mercury, and lead cause allergic reactions, bone diseases, metabolic disorders, ataxia, and various other diseases (Singh et al., 2011).

Since one of the bottlenecks in synthetic microbial scavenger construction is the lack of appropriate engineering tools, various genetic engineering techniques have been developed and advances in engineering techniques would facilitate the development of efficient microbial scavengers. For example, *Comamonas testosteroni* can reduce heavy metals and also decompose other contaminants such as aromatic compounds and steroids (Tang et al., 2018). Moreover, the bacterium can move toward target compounds by chemotaxis. *C. testosteroni* is a promising microbial scavenger chassis for bioremediation. However, the lack of well-developed genetic engineering techniques hindered its application to bioremediation. In a study, several genetic techniques were developed to engineer *C. testosteroni*, which included a shuttling plasmid system, IPTG-inducible promoters, and an orthogonal gene expression system using T7 RNA polymerase (Tang et al., 2018). Recent

advances in CRISPR systems to modulate gene expression also allowed the engineering of microorganisms, specifically *Shewanella oneidensis* MR-1, to remove heavy metals and dyes (Li et al., 2020c). The repression of genes associated with the extracellular electron transfer system via CRISPR-ddAsCpf1 increased the activity of the electron transfer system three folds and consequently promoted the degradation of methyl orange and chromium.

It has been reported that absorption capacity and resistance to heavy metals could be enhanced by genetic modification of bacteria. For example, Liu et al. (2021a) observed that in the presence of Pb^{2+} and Cd^{2+} , the expression of sulfur metabolism-related operons (*iscSAU* and *moaEDAB*) was up-regulated in heavy metal-tolerant *Cupriavidus taiwanensis*. When the sulfur metabolism operons were introduced into *E. coli*, the engineered *E. coli* showed higher tolerance and uptake than non-engineered cells (Liu et al., 2021a). This result shows that the knowledge of the physiology of native resistant bacteria could help to engineer well-studied bacterial cells. In another study, expression of metal-binding peptides could enhance the tolerance of host cells (Gahlot et al., 2020). Copper-binding peptides were semi-randomly screened and two peptides with a high copper-binding affinity were identified. Due to the high absorption capability of the peptides, *E. coli* cells expressing the peptides were more resistant to higher concentrations of copper than the cells not expressing the peptides.

Bacterial biofilm engineering is a promising approach in bioremediation owing to its ability to self-assemble. Biofilm is formed by bacteria and consists of extracellular polymeric substances, including polysaccharides, extracellular DNA, and polymeric proteins. Curli amyloid fibers encoded by the *curli* gene in *E. coli*, known as self-assembling amyloid proteins and the major component of the extracellular matrix, have been widely used in the bioremediation of several toxic pollutants, such as heavy metals, radioactive substances (uranium), persistent organic pollutants, and explosives (Manobala et al., 2019; Mohapatra et al., 2020). Tay et al. (2017) developed a synthetic circuit for Hg^{2+} detoxification using a mercury-sensing promoter (pMerR) activated by Hg^{2+} and the self-assembling functional amyloids from *E. coli* MC4100 (Tay et al., 2017). The system detected and sequestered Hg^{2+} using the self-assembled protein nanofibers that absorbed Hg^{2+} via a positive feedback mechanism when exposed to Hg^{2+} (up to 400 ppb). The study demonstrated the practical applicability of dynamic synthetic circuits to bioremediation.

3.5. Greenhouse gases (CO_2 and CH_4)

Microorganisms capable of assimilating one-carbon compounds can be used to decrease the concentration of greenhouse gases (CH_4 , CO_2 , and CO) (DeLisi et al., 2020). For example, methanotrophs convert methane to methanol using an enzyme, methane monooxygenase (MMO), and methanol is further oxidized to other metabolites for cellular metabolism (Nguyen et al., 2019). Autotrophic bacteria are able to assimilate CO_2 via Calvin–Benson–Bassham cycle to provide cellular metabolites (Gleizer et al., 2019).

One of the hurdles of bioremediation using the bacteria is their low growth rate and low cell density (Chen et al., 2020; Takeguchi and Okura, 2000; Yu et al., 2003). Thus, for efficient bioremediation, there have been attempts to implement carbon assimilation systems for CO_2 or CH_4 in fast-growing microorganisms. An *E. coli* strain was engineered to fix CO_2 molecules into sugars via metabolic engineering and directed evolution (Antonovsky et al., 2016). In another study, metabolic engineering of central carbon metabolism and directed evolution allowed *E. coli*, a heterotrophic bacterium, to become a synthetic autotrophic bacterium with the ability of CO_2 fixation while formate (HCOO^-) provided the reducing power and energy demands (Gleizer et al., 2019). Methanol anabolism has been implemented in *E. coli* strains (Murrell et al., 2000). However, the *E. coli* strains showed a low growth rate (doubling time of 55 h) and a low cell density with an optical density (OD_{600}) of 0.2 when cultured with methane. The cells also required

additional supplements for growth (Chen et al., 2018; Gonzalez et al., 2018; Kim et al., 2020; Meyer et al., 2018). Recently, it was found that formaldehyde, a toxic metabolite generated during methanol metabolism, induced DNA-protein crosslinking and aggregation (Chen et al., 2020), leading to cell death or an extraordinarily long lag phase. In addition to rational ribulose monophosphate (RuMP) cycle engineering and adaptive evolution, further metabolic engineering for balanced generation and consumption of formaldehyde increased cell growth. The resulting methanotrophic *E. coli* strain showed a doubling time of 8 h, which was notably shorter than that of previously engineered synthetic methanotrophic *E. coli* strains. However, since the newly engineered fast-growing *E. coli* strain could not grow with methane as a sole carbon source, there remains a need for the construction of efficient scavengers that utilize methane and have a high growth rate.

3.6. Microplastics

Recently, microplastics are gaining attention for their negative impact on ecosystems as well as human health, since they are found in soil and marine environments and even in foods (Alimba and Faggio, 2019; Zhang et al., 2019a). Microplastics exposure via respiration, digestion, and dermal absorption has been demonstrated to cause human health issues including carcinogenicity, neurotoxicity, and metabolic disturbances (Rahman et al., 2021). Thus, there is an urgent demand for the bioremediation of microplastics.

Poly(ethylene terephthalate) (PET) is a well-known thermoplastic polymer that is widely used for packaging beverages and foods because it was approved as safe (Crawford and Quinn, 2017). However, microplastics broken down from plastics, including PET, are toxic to animals (Holland et al., 2016; Song et al., 2019). Recently, a bacterium, *Ideonella sakaiensis* 201-F6, was isolated from outside a bottle-recycling facility, which contained PET-hydrolyzing enzymes (Yoshida et al., 2016) that were able to degrade PET into non-hazardous monomers, terephthalic acid, and ethylene glycol. The bacterial species could be utilized to break down PET microplastics or its hydrolyzing enzymes could be introduced into other bacteria to construct more efficient microbial scavengers. In this regard, a photosynthetic microalga, *Phaeodactylum tricornutum*, was engineered as a new chassis to produce and secrete PET-hydrolyzing enzymes to the surrounding environment (Moog et al., 2019).

In addition to microplastic degradation, a strategy to capture polyvinyl chloride (PVC) within bacterial biofilms was developed (Liu et al., 2021b). *P. aeruginosa* was engineered to capture and accumulate microparticles in its biofilm, specifically within its sticky exopolymeric substances. To enhance the capturing efficiency of *P. aeruginosa*, the *wspF* gene was deleted because WspF negatively regulates WspR that generated c-di-GMP, a secondary signaling molecule for biofilm formation. The mutant scavenger formed more biofilm and consequently showed >1.3-fold increased capturing efficiency. The microplastics captured within the biofilm could then be released for treatment. Thus, the *yhjH* gene was designed under the control of an arabinose-induced promoter and introduced into the bacterium. Since the function of *yhjH* was to decrease c-di-GMP levels, induced expression of the gene reduced the biofilm enough to release captured microplastics. The synthetic 'capture and release' system would enable the creation of efficient microplastic scavengers for bioremediation of aquatic ecosystems.

4. Biosafety issue of genetically engineered scavengers

With the recent achievements in synthetic biology and metabolic engineering, genetically engineered microbial scavengers have been successfully applied for the bioremediation of toxic pollutants (French et al., 2020; Gong et al., 2016; Mohamed et al., 2020b; Wang et al., 2019). However, due to regulatory hurdles prohibiting the release of GEMs the bioremediation with GEMs was validated only in laboratories, not in the environment.

Several genetic tools and systems were developed to eliminate the negative impacts of GEMs on the natural environment. These include antibiotic gene-free genetic engineering tools (Ji et al., 2019) and suicide genetic systems (Honjo et al., 2019; Marguet et al., 2010; Scott et al., 2017). The complete mineralization pathways for degrading methyl parathion and γ -hexachlorocyclohexane were integrated into the genome of *P. putida* KT2440 without any antibiotic resistance marker genes using the uracil phosphoribosyltransferase gene (*upp* gene) as a counter-selectable marker (Gong et al., 2016). Using synthetic biology, there have been attempts to develop programmed cell death circuits for biomedical applications of synthetically engineered microorganisms (Marguet et al., 2010; Sedlmayer et al., 2018). Such programmed cell death circuits can be used to self-eliminate the microbial scavengers after completing bioremediation.

5. Challenges and future perspectives

The hurdles that have limited the application of microbial scavengers for bioremediation are overcome by genetic engineering techniques developed in synthetic biology, system biology, and metabolic engineering not only for the efficient degradation of pollutants but also for the safe use of GEMs in nature. However, there remain several challenges in microbial scavenger construction.

There are numerous toxic contaminants, but the number of contaminants that can be degraded by living organisms is very small due to the lack of knowledge on catabolic pathways and appropriate enzymes. Identification of novel bacterial species could enable the discovery of new enzymes and pathways, but the cultivation and isolation of natural bacteria remain challenging (Bodor et al., 2020; Pham and Kim, 2012). The recent advances in metagenomic analysis and engineering of uncultivated microbial communities, particularly those sampled from contaminated sites, should help develop the process of bioremediation of the contaminants (Schloss and Handelsman, 2005). Briefly, as shown in Fig. 5A, total microbial DNA extracted from environmental samples are sub-cloned into an expression vector and used to construct metagenomic libraries in laboratory bacteria such as *E. coli*. By screening the sub-clones with a specific medium containing target pollutant, functional enzymes are identified from metagenomic libraries. Eventually, DNA sequencing and genome assembly enable the discovery of *de novo* enzymes and new biodegradation pathways from the bacterial communities (Colatrisano et al., 2018; Fang et al., 2018; Hemme et al., 2010; Sousa et al., 2020; Yang et al., 2020). Thus, culture-independent isolation of microbes opens a new avenue to explore unknown metabolic pathways of bioremediation in nature.

In nature, living organisms in the same environment continuously interact with each other and sometimes co-evolve to survive by taking advantage of their physiological characteristics. Similarly, the indigenous bacteria in communities often co-metabolize chemical compounds for efficient utilization or detoxification (Borchert et al., 2021; Honjo et al., 2019; McCarty and Ledesma-Amaro, 2019; Wanapaisan et al., 2018). In previous studies, bioremediation by a synthetic microbial consortium was often more efficient than that by a single species, in which each bacterium played a unique catalytic function. Thus, a synthetically constructed bacterial consortium could help decrease the metabolic burden on a single scavenger in degrading a broad range of pollutants into non-toxic end products (Fig. 5B) (Alexandrino et al., 2020; Martinez et al., 2016; Wanapaisan et al., 2018). Consequently, the overall efficiency of bioremediation could be enhanced, thus accelerating the sustainable development of microbial scavengers for industrial use.

In conclusion, advancements in multidisciplinary science, particularly in biotechnology, make it possible to design and reconstruct smart synthetic microbial scavengers or their consortia to efficiently degrade hazardous pollutants.

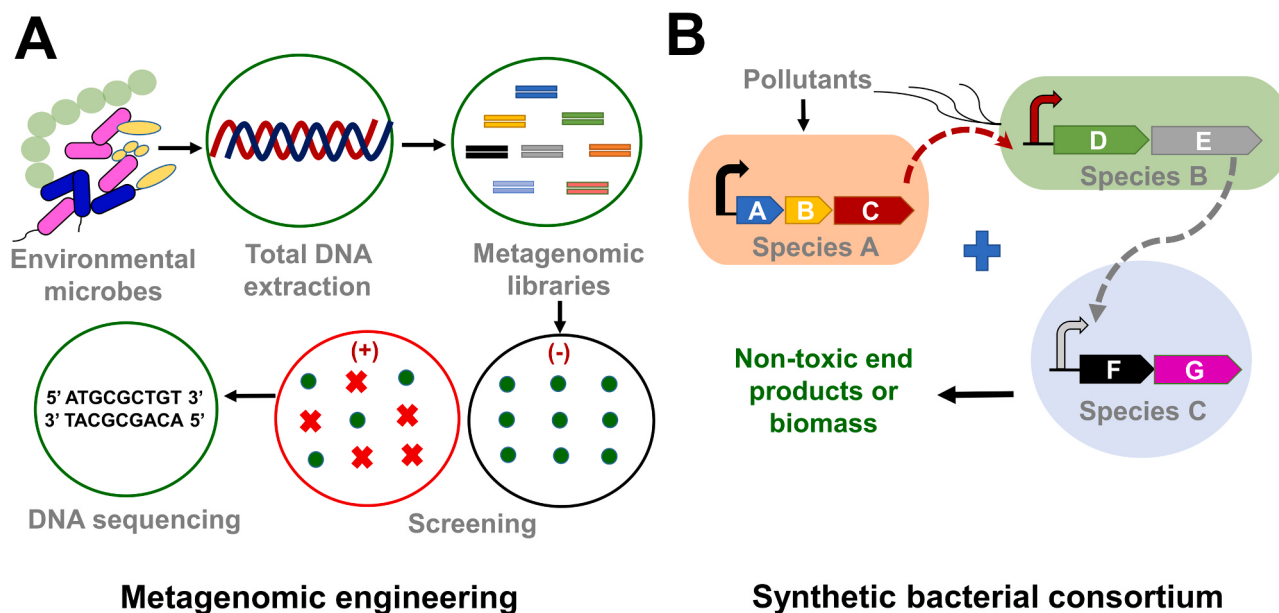


Fig. 5. Synthetic biology approaches for microbial bioremediation of hazardous pollutants. A. From contaminated sites, bacteria capable of pollutant detoxification are isolated and their catabolic genes are identified by metagenomic analysis. The total DNA of the bacterial community is extracted for metagenomic library construction. Screening is performed to select functional catabolic genes. B. A synthetic bacterial consortium is used to convert pollutants into non-toxic end products by cooperative metabolism.

CRedit authorship contribution statement

Kha Mong Tran: Conceptualization, Investigation, Writing - original draft. **Hyang-Mi Lee:** Conceptualization, Investigation, Writing - original draft. **Thi Duc Thai:** Investigation, Writing - original draft. **Junhao Shen:** Investigation, Writing - original draft. **Seong-il Eyun:** Writing - review & editing. **Dokyun Na:** Writing - review & editing, Supervision.

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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References

- Adamo, G.M., Brocca, S., Passolunghi, S., Salvato, B., Lotti, M., 2012. Laboratory evolution of copper tolerant yeast strains. *Microb. Cell Fact.* 11 (1), 1. <https://doi.org/10.1186/1475-2859-11-1>.
- Aldhafiri, S., Mahmoud, H., Al-Sarawi, M., Ismail, W.A., 2018. Natural attenuation potential of polychlorinated biphenyl-polluted marine sediments. *Pol. J. Microbiol.* 67 (1), 37–48. <https://doi.org/10.5604/01.3001.0011.6140>.
- Alegbeleye, O.O., Opeolu, B.O., Jackson, V., 2017. Bioremediation of polycyclic aromatic hydrocarbon (PAH) compounds: (acenaphthene and fluorene) in water using indigenous bacterial species isolated from the Diep and Plankenburg rivers, Western Cape, South Africa. *Braz. J. Microbiol.* 48 (2), 314–325. <https://doi.org/10.1016/j.bjm.2016.07.027>.
- Alexandrino, D.A.M., Mucha, A.P., Almeida, C.M.R., Carvalho, M.F., 2020. Microbial degradation of two highly persistent fluorinated fungicides – epoxiconazole and fludioxonil. *J. Hazard. Mater.* 394, 122545 <https://doi.org/10.1016/j.jhazmat.2020.122545>.
- Ali, N., Dashti, N., Khanafer, M., Al-Awadhi, H., Radwan, S., 2020. Bioremediation of soils saturated with spilled crude oil. *Sci. Rep.* 10 (1), 1–9. <https://doi.org/10.1038/s41598-019-57224-x>.

- Alimba, C.G., Faggio, C., 2019. Microplastics in the marine environment: current trends in environmental pollution and mechanisms of toxicological profile. *Environ. Toxicol. Pharmacol.* 68, 61–74. <https://doi.org/10.1016/j.etap.2019.03.001>.
- Anenberg, S.C., Achakulwisut, P., Brauer, M., Moran, D., Apte, J.S., Henze, D.K., 2019. Particulate matter-attributable mortality and relationships with carbon dioxide in 250 urban areas worldwide. *Sci. Rep.* 9 (1), 1–6. <https://doi.org/10.1038/s41598-019-48057-9>.
- Antonovsky, N., Gleizer, S., Noor, E., Zohar, Y., Herz, E., Barenholz, U., Tepper, N., 2016. Sugar synthesis from CO₂ in *Escherichia coli*. *Cell* 166 (1), 115–125. <https://doi.org/10.1016/j.cell.2016.05.064>.
- Azaroff, A., Monperrus, M., Miossec, C., Gassie, C., Guyoneaud, R., 2021. Microbial degradation of hydrophobic emerging contaminants from marine sediment slurries (Capbreton Canyon) to pure bacterial strain. *J. Hazard. Mater.* 402, 123477 <https://doi.org/10.1016/j.jhazmat.2020.123477>.
- Bajaj, S., Sagar, S., Khare, S., Singh, D.K., 2017. Biodegradation of γ-hexachlorocyclohexane (lindane) by halophilic bacterium *Chromohalobacter* sp. LD2 isolated from HCH dumpsite. *Int. Biodeterior. Biodegrad.* 122, 23–28. <https://doi.org/10.1016/j.ibiod.2017.04.014>.
- Bedell, V.M., Wang, Y., Campbell, J.M., Poshusta, T.L., Starker, C.G., Krug II, R.G., Leung, A.Y., 2012. *In vivo* genome editing using a high-efficiency TALEN system. *Nature* 491 (7422), 114–118. <https://doi.org/10.1038/nature11537>.
- Bellinaso, M.D.L., Greer, C.W., Peralba, M.d.C., Henriques, J.A.P., Gaylarde, C.C., 2003. Biodegradation of the herbicide trifluralin by bacteria isolated from soil. *FEMS Microbiol. Ecol.* 43 (2), 191–194. [https://doi.org/10.1016/S0168-6496\(02\)00388-4](https://doi.org/10.1016/S0168-6496(02)00388-4).
- Benedetti, I., de Lorenzo, V., Nikel, P.I., 2016. Genetic programming of catalytic *Pseudomonas putida* biofilms for boosting biodegradation of haloalkanes. *Metab. Eng.* 33, 109–118. <https://doi.org/10.1016/j.ymben.2015.11.004>.
- Bhatt, P., Huang, Y., Rene, E.R., Kumar, A.J., Chen, S., 2020. Mechanism of allethrin biodegradation by a newly isolated *Sphingomonas trueperi* strain CW3 from wastewater sludge. *Bioresour. Technol.* 305, 123074 <https://doi.org/10.1016/j.biortech.2020.123074>.
- Bodor, A., Bounedjoum, N., Vincze, G.E., Erdeiné Kis, Á., Laczi, K., Bende, G., Rákhegyi, G., 2020. Challenges of unculturable bacteria: environmental perspectives. *Rev. Environ. Sci. Biotechnol.* 19 (1), 1–22. <https://doi.org/10.1007/s11157-020-09522-4>.
- Borchert, E., Hammerschmidt, K., Hentschel, U., Deines, P., 2021. Enhancing microbial pollutant degradation by integrating eco-evolutionary principles with environmental biotechnology. *Trends Microbiol.* <https://doi.org/10.1016/j.tim.2021.03.002>.
- Chen, C.T., Chen, F.Y.H., Bogorad, I.W., Wu, T.Y., Zhang, R., Lee, A.S., Liao, J.C., 2018. Synthetic methanol auxotrophy of *Escherichia coli* for methanol-dependent growth and production. *Metab. Eng.* 49, 257–266. <https://doi.org/10.1016/j.ymben.2018.08.010>.
- Chen, F.Y., Jung, H.W., Tsuei, C.Y., Liao, J.C., 2020. Converting *Escherichia coli* to a synthetic methylotroph growing solely on methanol. *Cell* 182 (4), 933–946. <https://doi.org/10.1016/j.cell.2020.07.010>.
- Colatruano, D., Tran, P.Q., Guéguen, C., Williams, W.J., Lovejoy, C., Walsh, D.A., 2018. Genomic evidence for the degradation of terrestrial organic matter by pelagic Arctic Ocean Chloroflexi bacteria. *Commun. Biol.* 1 (1), 90. <https://doi.org/10.1038/s42003-018-0086-7>.

- Copley, S.D., 2009. Evolution of efficient pathways for degradation of anthropogenic chemicals. *Nat. Chem. Biol.* 5 (8), 559–566. <https://doi.org/10.1038/nchembio.197>.
- Crawford, C.B., Quinn, B., 2017. Physicochemical properties and degradation. In: Crawford, C.B., Quinn, B. (Eds.), *Microplastic Pollutants*. Elsevier, Amsterdam, pp. 57–100. <https://doi.org/10.1016/B978-0-12-809406-8.00004-9>.
- Cvetnic, M., Perisic, D.J., Kovacic, M., Ukic, S., Bolanca, T., Rasulev, B., Bozic, A.L., 2019. Toxicity of aromatic pollutants and photooxidative intermediates in water: a QSAR study. *Ecotoxicol. Environ. Saf.* 169, 918–927. <https://doi.org/10.1016/j.ecoenv.2018.10.100>.
- Dai, M., Copley, S.D., 2004. Genome shuffling improves degradation of the anthropogenic pesticide pentachlorophenol by *Sphingobium chlorophenolicum* ATCC 39723. *Appl. Environ. Microbiol.* 70 (4), 2391–2397. <https://doi.org/10.1128/AEM.70.4.2391-2397.2004>.
- DeLisi, C., Patrinos, A., MacCracken, M., Drell, D., Annas, G., Arkin, A., Lidstrom, M., 2020. The role of synthetic biology in atmospheric greenhouse gas reduction: prospects and challenges. *BioDesign Res.* 2020, 1–8. <https://doi.org/10.34133/2020/1016207>.
- DeLorenzo, D.M., Moon, T.S., 2019. Construction of genetic logic gates based on the T7 RNA polymerase expression system in *Rhodococcus opacus* PD630. *ACS Synth. Biol.* 8 (8), 1921–1930. <https://doi.org/10.1021/acssynbio.9b00213>.
- Doolotkeldieva, T., Konurbayeva, M., Bobusheva, S., 2018. Microbial communities in pesticide-contaminated soils in Kyrgyzstan and bioremediation possibilities. *Environ. Sci. Pollut. Res.* 25 (32), 31848–31862. <https://doi.org/10.1155/2014/157974>.
- Dueber, J.E., Wu, G.C., Malmirchegini, G.R., Moon, T.S., Petzold, C.J., Ullal, A.V., Keasling, J.D., 2009. Synthetic protein scaffolds provide modular control over metabolic flux. *Nat. Biotechnol.* 27 (8), 753–759. <https://doi.org/10.1038/nbt.1557>.
- Duraissamy, P., Sekar, J., Arunkumar, A.D., Ramalingam, P.V., 2020. Kinetics of phenol biodegradation by heavy metal tolerant rhizobacteria *Glutamicibacter nicotianae* MSSRFPD35 from distillery effluent contaminated soils. *Front. Microbiol.* 11, 1573. <https://doi.org/10.3389/fmicb.2020.01573>.
- Dvořák, P., Nikel, P.I., Damborský, J., de Lorenzo, V., 2017. Bioremediation 3.0: engineering pollutant-removing bacteria in the times of systemic biology. *Biotech. Adv.* 35 (7), 845–866. <https://doi.org/10.1016/j.biotechadv.2017.08.001>.
- Emergence Research, 2020. Bioremediation market by services (soil, wastewater and oilfield remediation, others), by technology (phytoremediation, biostimulation, bioaugmentation, bioreactors, fungal remediation, land-based treatments) and by regions forecasts to 2027. ID: ER_00215. Retrieved from <https://www.emergenceresearch.com/industry-report/bioremediation-market>.
- Espah Borujeni, A., Cetnar, D., Farasat, I., Smith, A., Lundgren, N., Salis, H.M., 2017. Precise quantification of translation inhibition by mRNA structures that overlap with the ribosomal footprint in N-terminal coding sequences. *Nucleic Acids Res.* 45 (9), 5437–5448. <https://doi.org/10.1093/nar/gkx061>.
- Fang, H., Zhang, H., Han, L., Mei, J., Ge, Q., Long, Z., Yu, Y., 2018. Exploring bacterial communities and biodegradation genes in activated sludge from pesticide wastewater treatment plants via metagenomic analysis. *Environ. Pollut.* 243 (Pt B), 1206–1216. <https://doi.org/10.1016/j.envpol.2018.09.080>.
- Farasat, I., Kushwaha, M., Collens, J., Easterbrook, M., Guido, M., Salis, H.M., 2014. Efficient search, mapping, and optimization of multi-protein genetic systems in diverse bacteria. *Mol. Syst. Biol.* 10 (6), 731. <https://doi.org/10.15252/msb.20134955>.
- Fox, R.D., 1996. Physical/chemical treatment of organically contaminated soils and sediments. *J. Air Waste Manag. Assoc.* 46 (5), 391–413. <https://doi.org/10.1080/10473289.1996.10467473>.
- French, K.E., Zhou, Z., Terry, N., 2020. Horizontal 'gene drives' harness indigenous bacteria for bioremediation. *Sci. Rep.* 10 (1), 15091. <https://doi.org/10.1038/s41598-020-72138-9>.
- Gahlot, D.K., Taheri, N., Mahato, D.R., Francis, M.S., 2020. Bioengineering of non-pathogenic *Escherichia coli* to enrich for accumulation of environmental copper. *Sci. Rep.* 10 (1), 20327. <https://doi.org/10.1038/s41598-020-76178-z>.
- Ghatge, S., Yang, Y., Moon, S., Song, W.-Y., Kim, T.-Y., Liu, K.-H., Hur, H.-G., 2021. A novel pathway for initial biotransformation of dinitroaniline herbicide butralin from a newly isolated bacterium *Sphingopyxis* sp. strain HMH. *J. Hazard. Mater.* 402, 123510. <https://doi.org/10.1016/j.jhazmat.2020.123510>.
- Gialama, D., Kostelidou, K., Michou, M., Delivoria, D.C., Kolisis, F.N., Skretas, G., 2017. Development of *Escherichia coli* strains that withstand membrane protein-induced toxicity and achieve high-level recombinant membrane protein production. *ACS Synth. Biol.* 6 (2), 284–300. <https://doi.org/10.1021/acssynbio.6b00174>.
- Gleizer, S., Ben-Nissan, R., Bar-On, Y.M., Antonovsky, N., Noor, E., Zohar, Y., Milo, R., 2019. Conversion of *Escherichia coli* to generate all biomass carbon from CO₂. *Cell* 179 (6), 1255–1263. <https://doi.org/10.1016/j.cell.2019.11.009>.
- Gong, T., Liu, R., Zuo, Z., Che, Y., Yu, H., Song, C., Yang, C., 2016. Metabolic engineering of *Pseudomonas putida* KT2440 for complete mineralization of methyl parathion and gamma-hexachlorocyclohexane. *ACS Synth. Biol.* 5 (5), 434–442. <https://doi.org/10.1021/acssynbio.6b00025>.
- Gong, T., Xu, X., Che, Y., Liu, R., Gao, W., Zhao, F., Yang, C., 2017. Combinatorial metabolic engineering of *Pseudomonas putida* KT2440 for efficient mineralization of 1,2,3-trichloropropane. *Sci. Rep.* 7 (1), 7064. <https://doi.org/10.1038/s41598-017-07435-x>.
- Gonzalez, J.E., Bennett, R.K., Papoutsakis, E.T., Antoniewicz, M.R., 2018. Methanol assimilation in *Escherichia coli* is improved by co-utilization of threonine and deletion of leucine-responsive regulatory protein. *Metab. Eng.* 45, 67–74. <https://doi.org/10.1016/j.ymben.2017.11.015>.
- Haddadi, A., Shavandi, M., 2013. Biodegradation of phenol in hypersaline conditions by *Halomonas* sp. strain PH2-2 isolated from saline soil. *Int. Biodeterior. Biodegrad.* 85, 29–34. <https://doi.org/10.1016/j.ibiod.2013.06.005>.
- Hemme, C.L., Deng, Y., Gentry, T.J., Fields, M.W., Wu, L., Barua, S., Zhou, J., 2010. Metagenomic insights into evolution of a heavy metal-contaminated groundwater microbial community. *ISME J.* 4 (5), 660–672. <https://doi.org/10.1038/ismej.2009.154>.
- Hirade, J., Ninomiya, A., 1950. Studies on the mechanism of the toxic action of organic halogen compounds. *J. Biochem.* 37 (1), 19–34. <https://doi.org/10.1093/oxfordjournals.jbchem.a126178>.
- Holland, E.R., Mallory, M.L., Shutler, D., 2016. Plastics and other anthropogenic debris in freshwater birds from Canada. *Sci. Total Environ.* 571, 251–258. <https://doi.org/10.1016/j.scitotenv.2016.07.158>.
- Hong, Y.H., Ye, C.C., Zhou, Q.Z., Wu, X.Y., Yuan, J.P., Peng, J., Wang, J.H., 2017. Genome sequencing reveals the potential of *Achromobacter* sp. HZ01 for bioremediation. *Front. Microbiol.* 8, 1507. <https://doi.org/10.3389/fmicb.2017.01507>.
- Honjo, H., Iwasaki, K., Soma, Y., Tsuruno, K., Hamada, H., Hanai, T., 2019. Synthetic microbial consortium with specific roles designated by genetic circuits for cooperative chemical production. *Metab. Eng.* 55, 268–275. <https://doi.org/10.1016/j.ymben.2019.08.007>.
- Horemans, B., Bruegelmans, P., Hofkens, J., Springael, D., 2017. Carbon catabolite repression and cell dispersal affect degradation of the xenobiotic compound 3,4-dichloroaniline in *Comamonas testosteroni* WDL7 biofilms. *FEMS Microbiol. Ecol.* 93 (3). <https://doi.org/10.1093/femsec/fix004>.
- Horn, A.H.C., Sticht, H., 2015. Synthetic protein scaffolds based on peptide motifs and cognate adaptor domains for improving metabolic productivity. *Front. Bioeng. Biotechnol.* 3, 191. <https://doi.org/10.3389/fbioe.2015.00191>.
- Ibrahim, I.M., Konnova, S.A., Sigida, E.N., Lyubun, E.V., Muratova, A.Y., Fedonenko, Y. P., Elbanna, K., 2020. Bioremediation potential of a halophilic *Halobacillus* sp. strain, EG1HP4QL: exopolysaccharide production, crude oil degradation, and heavy metal tolerance. *Extremophiles* 24 (1), 157–166. <https://doi.org/10.1007/s00792-019-01143-2>.
- Ilic Durdic, K., Ostafe, R., Durdevic Delmas, A., Popovic, N., Schillberg, S., Fischer, R., Prodanovic, R., 2020. Saturation mutagenesis to improve the degradation of azo dyes by versatile peroxidase and application in form of VP-coated yeast cell walls. *Enzym. Microb. Technol.* 136, 109509. <https://doi.org/10.1016/j.enzmictec.2020.109509>.
- Ishag, A.E., Abdelbagi, A.O., Hammad, A.M., Elsheikh, E.A., Elsaid, O.E., Hur, J.H., Laing, M.D., 2016. Biodegradation of chlorpyrifos, malathion, and dimethoate by three strains of bacteria isolated from pesticide-polluted soils in Sudan. *J. Agric. Food Chem.* 64 (45), 8491–8498. <https://doi.org/10.1021/acs.jafc.6b03334>.
- Jeschek, M., Gerngross, D., Panke, S., 2016. Rationally reduced libraries for combinatorial pathway optimization minimizing experimental effort. *Nat. Commun.* 7 (1), 11163. <https://doi.org/10.1038/ncomms11163>.
- Ji, X., Lu, P., van der Veen, S., 2019. Development of a dual-antimicrobial counterselection method for markerless genetic engineering of bacterial genomes. *Appl. Microbiol. Biotechnol.* 103 (3), 1465–1474. <https://doi.org/10.1007/s00253-018-9565-5>.
- Joutey, N.T., Bahafid, W., Sayel, H., El Ghachtouli, N., 2013. Biodegradation: involved microorganisms and genetically engineered microorganisms. *Biodegrad. Life Sci.* 289–320. <https://doi.org/10.5772/56194>.
- Kang, W., Ma, T., Liu, M., Qu, J., Liu, Z., Zhang, H., Liu, T., 2019. Modular enzyme assembly for enhanced cascade biocatalysis and metabolic flux. *Nat. Commun.* 10 (1), 4248. <https://doi.org/10.1038/s41467-019-12247-w>.
- Khan, I., Akhtar, M.W., 2011. Different approaches for protein engineering in industrial biotechnology. *Nat. Preced.* 1197, 1–11. <https://doi.org/10.1038/npre.2011.5601.1>.
- Kim, S., Lindner, S.N., Aslan, S., Yishai, O., Wenk, S., Schann, K., Bar-Even, A., 2020. Growth of *E. coli* on formate and methanol via the reductive glycine pathway. *Nat. Chem. Biol.* 16 (5), 538–545. <https://doi.org/10.1038/s41589-020-0473-5>.
- Kobayashi, H., Rittmann, B.E., 1982. Microbial removal of hazardous organic compounds. *Environ. Sci. Technol.* 16 (3), 170A–183A. <https://doi.org/10.1021/es00097a002>.
- Kodavanti, P.R.S., Loganathan, B.G., 2017. Organomet. Pollut. Hum. Health. <https://doi.org/10.1016/B978-0-12-803678-5.00318-0>.
- Lawson, C.E., Harcombe, W.R., Hatzenpichler, R., Lindemann, S.R., Löffler, F.E., O'Malley, M.A., McMahon, K.D., 2019. Common principles and best practices for engineering microbiomes. *Nat. Rev. Microbiol.* 17 (12), 725–741. <https://doi.org/10.1038/s41579-019-0255-9>.
- Lee, B.U., Choi, M.S., Kim, D.M., Oh, K.H., 2017. Genome shuffling of *Stenotrophomonas maltophilia* OK-5 for improving the degradation of explosive RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine). *Curr. Microbiol.* 74 (2), 268–276. <https://doi.org/10.1007/s00284-016-1179-5>.
- Lee, Y., Lee, Y., Jeon, C.O., 2019. Biodegradation of naphthalene, BTEX, and aliphatic hydrocarbons by *Paraburkholderia aromaticivorans* BN5 isolated from petroleum-contaminated soil. *Sci. Rep.* 9 (1). <https://doi.org/10.1038/s41598-018-36165-x>.
- Li, C., Zhang, R., Wang, J., Wilson, L.M., Yan, Y., 2020a. Protein engineering for improving and diversifying natural product biosynthesis. *Trends Biotechnol.* 38 (7), 729–744. <https://doi.org/10.1016/j.tibtech.2019.12.008>.
- Li, H., Qiu, Y., Yao, T., Ma, Y., Zhang, H., Yang, X., Li, C., 2020b. Evaluation of seven chemical pesticides by mixed microbial culture (PCS-1): degradation ability, microbial community, and *Medicago sativa* phytotoxicity. *J. Hazard. Mater.* 389, 121834. <https://doi.org/10.1016/j.jhazmat.2019.121834>.
- Li, J., Tang, Q., Li, Y., Fan, Y.Y., Li, F.H., Wu, J.H., Yu, H.Q., 2020c. Redirecting electron flux with an engineered CRISPR-dAdCasP system to enhance the pollutant

- degradation capacity of *Shewanella oneidensis*. *Environ. Sci. Technol.* 54 (6), 3599–3608 <https://pubs.acs.org/doi/10.1021/acs.est.9b06378>.
- Li, Q., Li, J., Kang, K.-L., Wu, Y.-J., 2020d. A safety type of genetically engineered bacterium that degrades chemical pesticides. *AMB Express* 10 (1), 33. <https://doi.org/10.1186/s13568-020-00967-y>.
- Lim, M.W., Von Lau, E., Poh, P.E., 2016. A comprehensive guide of remediation technologies for oil contaminated soil—present works and future directions. *Mar. Pollut. Bull.* 109 (1), 14–45. <https://doi.org/10.1016/j.marpolbul.2016.04.023>.
- Liu, H., Xu, L., Yan, M., Lai, C., Ouyang, P., 2008. gTME for construction of recombinant yeast co-fermenting xylose and glucose. *Chin. J. Biotechnol.* 24 (6), 1010–1015. [https://doi.org/10.1016/S1872-2075\(08\)60048-5](https://doi.org/10.1016/S1872-2075(08)60048-5).
- Liu, J., Wei, Y., Lin, L., Teng, L., Yin, J., Lu, Q., Zhang, Y., 2020a. Two radical-dependent mechanisms for anaerobic degradation of the globally abundant organosulfur compound dihydroxypropanesulfonate. *Proc. Natl. Acad. Sci. USA* 117 (27), 15599–15608. <https://doi.org/10.1073/pnas.2003434117>.
- Liu, J., Zhu, N., Zhang, Y., Ren, T., Shao, C., Shi, R., Yu, Q., 2021a. Transcription profiling-guided remodeling of sulfur metabolism in synthetic bacteria for efficiently capturing heavy metals. *J. Hazard. Mater.* 403, 123638 <https://doi.org/10.1016/j.jhazmat.2020.123638>.
- Liu, S., Xu, X., Kang, Y., Xiao, Y., Liu, H., 2020b. Degradation and detoxification of azo dyes with recombinant ligninolytic enzymes from *Aspergillus* sp. with secretory overexpression in *Pichia pastoris*. *R. Soc. Open Sci.* 7 (9), 200688 <https://doi.org/10.1098/rsos.200688>.
- Liu, S.Y., Leung, M.M.-L., Fang, J.K.-H., Chua, S.L., 2021b. Engineering a microbial 'trap and release' mechanism for microplastics removal. *Chem. Eng. J.* 404, 127079 <https://doi.org/10.1016/j.cej.2020.127079>.
- Liu, Y., Zhang, H., He, X., Liu, J., 2021c. Genetically engineered methanotroph as a platform for bioaugmentation of chemical pesticide contaminated soil. *ACS Synth. Biol.* 10 (3), 487–494. <https://doi.org/10.1021/acssynbio.0c00532>.
- Manobala, T., Shukla, S.K., Rao, T.S., Kumar, M.D., 2019. A new uranium bioremediation approach using radio-tolerant *Deinococcus radiodurans* biofilm. *J. Biosci.* 44 (5), 1–9. <https://doi.org/10.1007/s12038-019-9942-y>.
- Marguet, P., Tanouchi, Y., Spitz, E., Smith, C., You, L., 2010. Oscillations by minimal bacterial suicide circuits reveal hidden facets of host-circuit physiology. *PLOS One* 5 (7), e11909. <https://doi.org/10.1371/journal.pone.0011909>.
- Martinez, I., Mohamed, M.E., Rozas, D., Garcia, J.L., Diaz, E., 2016. Engineering synthetic bacterial consortia for enhanced desulfurization and revalorization of oil sulfur compounds. *Metab. Eng.* 35, 46–54. <https://doi.org/10.1016/j.ymben.2016.01.005>.
- Maxel, S., King, E., Zhang, Y., Luo, R., Li, H., 2020. Leveraging oxidative stress to regulate redox balance-based, *in vivo* growth selections for oxygenase engineering. *ACS Synth. Biol.* 9, 3124–3133. <https://doi.org/10.1021/acssynbio.0c00380>.
- Mayr, M.J., Zimmermann, M., Dey, J., Brand, A., Wehrli, B., Burghmann, H., 2020. Growth and rapid succession of methanotrophs effectively limit methane release during lake overturn. *Commun. Biol.* 3 (1), 108. <https://doi.org/10.1038/s42003-020-0838-z>.
- McCarty, N.S., Ledesma-Amaro, R., 2019. Synthetic biology tools to engineer microbial communities for biotechnology. *Trends Biotechnol.* 37 (2), 181–197. <https://doi.org/10.1016/j.tibtech.2018.11.002>.
- Meng, X.-Y., Zhang, H.-X., Mezei, M., Cui, M., 2011. Molecular docking: a powerful approach for structure-based drug discovery. *Curr. Comput. Aided Drug Des.* 7 (2), 146–157. <https://doi.org/10.2174/157340911795677602>.
- Meyer, F., Keller, P., Hartl, J., Gröninger, O.G., Kiefer, P., Vorholt, J.A., 2018. Methanol-essential growth of *Escherichia coli*. *Nat. Commun.* 9 (1), 1–10. <https://doi.org/10.1038/s41467-018-03937-y>.
- Mishra, S., Chowdhary, P., Bharagava, R.N., 2019. Conventional methods for the removal of industrial pollutants, their merits and demerits. *Emerging and Eco-friendly Approaches for Waste Management*. Springer, New York, pp. 1–31. https://doi.org/10.1007/978-981-10-8669-4_1.
- Mohamed, E.T., Werner, A.Z., Salvachua, D., Singer, C.A., Szostkiewicz, K., Rafael Jimenez-Diaz, M., Feist, A.M., 2020a. Adaptive laboratory evolution of *Pseudomonas putida* KT2440 improves p-coumaric and ferulic acid catabolism and tolerance. *Metab. Eng. Commun.* 11, e00143 <https://doi.org/10.1016/j.mec.2020.e00143>.
- Mohamed, M.S.M., El-Arabi, N.I., El-Hussein, A., El-Maaty, S.A., Abdelhadi, A.A., 2020b. Reduction of chromium-VI by chromium-resistant *Escherichia coli* FACU: a prospective bacterium for bioremediation. *Folia Microbiol.* 65 (4), 687–696. <https://doi.org/10.1007/s12223-020-00771-y>.
- Mohapatra, R.K., Behera, S.S., Patra, J.K., Thatoi, H., Parhi, P.K., 2020. Potential application of bacterial biofilm for bioremediation of toxic heavy metals and dye-contaminated environments. *New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Biotechnology*. Elsevier, Amsterdam, pp. 267–281. <https://doi.org/10.1016/B978-0-444-64279-0.00017-7>.
- Moog, D., Schmitt, J., Senger, J., Zarzycki, J., Rexer, K.H., Linne, U., Maier, U.G., 2019. Using a marine microalga as a chassis for polyethylene terephthalate (PET) degradation. *Microb. Cell Fact.* 18 (1), 171. <https://doi.org/10.1186/s12934-019-1220-z>.
- Mukhopadhyay, A., 2015. Tolerance engineering in bacteria for the production of advanced biofuels and chemicals. *Trends Microbiol.* 23 (8), 498–508. <https://doi.org/10.1016/j.tim.2015.04.008>.
- Murrell, J.C., Gilbert, B., McDonald, I.R., 2000. Molecular biology and regulation of methane monooxygenase. *Arch. Microbiol.* 173 (5), 325–332. <https://doi.org/10.1007/s002030000158>.
- Na, D., Lee, S., Lee, D., 2010. Mathematical modeling of translation initiation for the estimation of its efficiency to computationally design mRNA sequences with desired expression levels in prokaryotes. *BMC Syst. Biol.* 4 (1), 71. <https://doi.org/10.1186/1752-0509-4-71>.
- Nelson, K., Weinell, C., Paulsen, I., Dodson, R., Hilbert, H., Martins dos Santos, V., Holmes, M., 2002. Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. *Environ. Microbiol.* 4 (12), 799–808. <https://doi.org/10.1046/j.1462-2920.2002.00366.x>.
- Nguyen, T.T., Hwang, I.Y., Na, J.G., Lee, E.Y., 2019. Biological conversion of propane to 2-propanol using group I and II methanotrophs as biocatalysts. *J. Ind. Microbiol.* 46 (5), 675–685. <https://doi.org/10.1007/s10295-019-02141-1>.
- Ni, H., Yao, L., Li, N., Cao, Q., Dai, C., Zhang, J., He, J., 2016. Biodegradation of pendimethalin by *Bacillus subtilis* Y3. *J. Environ. Sci.* 41, 121–127. <https://doi.org/10.1016/j.jes.2015.04.035>.
- Nikel, P.I., de Lorenzo, V., 2013. Engineering an anaerobic metabolic regime in *Pseudomonas putida* KT2440 for the anoxic biodegradation of 1,3-dichloroprop-1-ene. *Metab. Eng.* 15, 98–112. <https://doi.org/10.1016/j.ymben.2012.09.006>.
- Ofaim, S., Zarecki, R., Porob, S., Gat, D., Lahav, T., Kashi, Y., Freilich, S., 2020. Genome-scale reconstruction of *Paenarthrobacter aureus* TC1 metabolic model towards the study of atrazine bioremediation. *Sci. Rep.* 10 (1), 13019. <https://doi.org/10.1038/s41598-020-69509-7>.
- Omarova, M., Swientoniewski, L.T., Mkam Tsengam, I.K., Blake, D.A., John, V., McCormick, A., Bose, A., 2019. Biofilm formation by hydrocarbon-degrading marine bacteria and its effects on oil dispersion. *ACS Sustain. Chem. Eng.* 7 (17), 14490–14499. <https://doi.org/10.1021/acssuschemeng.9b01923>.
- Ossai, I.C., Ahmed, A., Hassan, A., Hamid, F.S., 2020. Remediation of soil and water contaminated with petroleum hydrocarbon: a review. *Environ. Technol. Innov.* 17, 100526 <https://doi.org/10.1016/j.eti.2019.100526>.
- Oyetibo, G.O., Chien, M.-F., Ikeda-Ohtsubo, W., Suzuki, H., Obayori, O.S., Adebisoye, S. A., Endo, G., 2017. Biodegradation of crude oil and phenanthrene by heavy metal resistant *Bacillus subtilis* isolated from a multi-polluted industrial wastewater creek. *Int. Biodeterior. Biodegradation* 120, 143–151. <https://doi.org/10.1016/j.ibiod.2017.02.021>.
- Panigrahy, N., Barik, M., Sahoo, R.K., Sahoo, N.K., 2020. Metabolic profile analysis and kinetics of p-cresol biodegradation by an indigenous *Pseudomonas citronellolis* NS1 isolated from coke oven wastewater. *Int. Biodeterior. Biodegradation* 147, 104837. <https://doi.org/10.1016/j.ibiod.2019.104837>.
- Parameswarappa, S., Karigar, C., Nagenahalli, M., 2008. Degradation of ethylbenzene by free and immobilized *Pseudomonas fluorescens*-CS2. *Biodegradation* 19 (1), 137–144. <https://doi.org/10.1007/s10532-007-9121-y>.
- Parka, S.J., Soper, O.F., 1977. The physiology and mode of action of the dinitroaniline herbicides. *Weed Sci.* 25 (1), 79–87. <https://www.jstor.org/stable/4042744>.
- Petrikov, K., Deegan, Y., Surin, A., Ponamereva, O., Puntus, I., Filonov, A., Boronin, A., 2013. Glycolipids of *Pseudomonas* and *Rhodococcus* oil-degrading bacteria used in bioremediation preparations: formation and structure. *Process Biochem.* 48 (5–6), 931–935. <https://doi.org/10.1016/j.procbio.2013.04.008>.
- Pham, V.H.T., Kim, J., 2012. Cultivation of unculturable soil bacteria. *Trends Biotechnol.* 30 (9), 475–484. <https://doi.org/10.1016/j.tibtech.2012.05.007>.
- Rahman, A., Sarkar, A., Yadav, O.P., Achari, G., Slobodnik, J., 2021. Potential human health risks due to environmental exposure to nano- and microplastics and knowledge gaps: a scoping review. *Sci. Total Environ.* 757, 143872 <https://doi.org/10.1016/j.scitotenv.2020.143872>.
- Ramos, J.L., Duque, E., Gallegos, M.T., Godoy, P., Ramos-Gonzalez, M.I., Rojas, A., Segura, A., 2002. Mechanisms of solvent tolerance in gram-negative bacteria. *Annu. Rev. Microbiol.* 56, 743–768. <https://doi.org/10.1146/annurev.micro.56.012302.161038>.
- Roell, G.W., Carr, R.R., Campbell, T., Shang, Z., Henson, W.R., Czajka, J.J., Tang, Y.J., 2019. A concerted systems biology analysis of phenol metabolism in *Rhodococcus opacus* PD630. *Metab. Eng.* 55, 120–130. <https://doi.org/10.1016/j.ymben.2019.06.013>.
- Safari, M., Yakhchali, B., Shariati, J.V., 2019. Comprehensive genomic analysis of an indigenous *Pseudomonas pseudoalcaligenes* degrading phenolic compounds. *Sci. Rep.* 9 (1), 12736. <https://doi.org/10.1038/s41598-019-49048-6>.
- Salis, H.M., 2011. The ribosome binding site calculator. In: Voigt, C. (Ed.), *Methods in Enzymology*, 498. Academic Press, pp. 19–42. <https://doi.org/10.1016/B978-0-12-385120-8.00002-4>.
- Santacruz, G., Bandala, E.R., Torres, L.G., 2005. Chlorinated pesticides (2, 4-D and DDT) biodegradation at high concentrations using immobilized *Pseudomonas fluorescens*. *J. Environ. Sci. Health B* 40 (4), 571–583. <https://doi.org/10.1081/PFC-200061545>.
- Schloss, P.D., Handelsman, J., 2005. Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. *Genome Biol.* 6 (8), 229. <https://doi.org/10.1186/gb-2005-6-8-229>.
- Schneider, N.R., Bradley, S.L., Andersen, M.E., 1977. Toxicology of cyclotrimethylenetrinitramine: distribution and metabolism in the rat and the miniature swine. *Toxicol. Appl. Pharmacol.* 39 (3), 531–541. [https://doi.org/10.1016/0041-008X\(77\)90144-2](https://doi.org/10.1016/0041-008X(77)90144-2).
- Scott, S.R., Din, M.O., Bittihn, P., Xiong, L., Tsimring, L.S., Hasty, J., 2017. A stabilized microbial ecosystem of self-limiting bacteria using synthetic quorum-regulated lysis. *Nat. Microbiol.* 2 (8), 1–9. <https://doi.org/10.1038/nmicrobiol.2017.83>.
- Sebesta, J., Peebles, C.A.M., 2020. Improving heterologous protein expression in *Synechocystis* sp. PCC 6803 for alpha-bisabolene production. *Metab. Eng. Commun.* 10, 00117 <https://doi.org/10.1016/j.mec.2019.e00117>.
- Sedlmayer, F., Aubel, D., Fussenegger, M., 2018. Synthetic gene circuits for the detection, elimination and prevention of disease. *Nat. Biomed. Eng.* 2 (6), 399–415. <https://doi.org/10.1038/s41551-018-0215-0>.
- Seo, J.-S., Keum, Y.-S., Li, Q.X., 2009. Bacterial degradation of aromatic compounds. *Int. J. Environ. Res. Public Health* 6 (1), 278–309. <https://doi.org/10.3390/ijerph6010278>.

- Shabbir, M., Singh, M., Maiti, S., Kumar, S., Saha, S.K., 2018. Removal enactment of organo-phosphorous pesticide using bacteria isolated from domestic sewage. *Bioresour. Technol.* 263, 280–288. <https://doi.org/10.1016/j.biortech.2018.04.122>.
- Shaker, B., Yu, M.-S., Lee, J., Lee, Y., Jung, C., Na, D., 2020. User guide for the discovery of potential drugs via protein structure prediction and ligand docking simulation. *J. Microbiol.* 58 (3), 235–244. <https://doi.org/10.1007/s12275-020-9563-z>.
- Sikkema, J., de Bont, J.A., Poolman, B., 1995. Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev.* 59 (2), 201–222. <https://doi.org/10.1128/mmbr.59.2.201-222.1995>.
- Simkovich, S.M., Goodman, D., Roa, C., Crocker, M.E., Gianella, G.E., Kirenga, B.J., Checkley, W., 2019. The health and social implications of household air pollution and respiratory diseases. *npj Prim. Care Respir. Med.* 29 (1), 1–17. <https://doi.org/10.1038/s41533-019-0126-x>.
- Singh, R., Gautam, N., Mishra, A., Gupta, R., 2011. Heavy metals and living systems: an overview. *Indian J. Pharmacol.* 43 (3), 246–253. <https://doi.org/10.4103/0253-7613.81505>.
- Song, Y., Cao, C., Qiu, R., Hu, J., Liu, M., Lu, S., He, D., 2019. Uptake and adverse effects of polyethylene terephthalate microplastics fibers on terrestrial snails (*Achatina fulica*) after soil exposure. *Environ. Pollut.* 250, 447–455. <https://doi.org/10.1016/j.envpol.2019.04.066>.
- Sousa, S.T.P.d., Cabral, L., Lacerda-Júnior, G.V., Noronha, M.F., Ottoni, J.R., Sartoratto, A., Oliveira, V.M.d., 2020. Exploring the genetic potential of a fosmid metagenomic library from an oil-impacted mangrove sediment for metabolism of aromatic compounds. *Ecotoxicol. Environ. Saf.* 189, 109974. <https://doi.org/10.1016/j.ecoenv.2019.109974>.
- Su, T., Liu, F., Gu, P., Jin, H., Chang, Y., Wang, Q., Qi, Q., 2016. A CRISPR-Cas9 assisted non-homologous end-joining strategy for one-step engineering of bacterial genome. *Sci. Rep.* 6 (1), 1–11. <https://doi.org/10.1038/srep37895>.
- Takeguchi, M., Okura, I., 2000. Role of iron and copper in particulate methane monooxygenase of *Methylosinus trichosporium* OB3b. *Catal. Surv. Jpn.* 4 (1), 51–63. <https://doi.org/10.1023/A:1019036105038>.
- Tan, F., Wu, B., Dai, L., Qin, H., Shui, Z., Wang, J., He, M., 2016. Using global transcription machinery engineering (gTME) to improve ethanol tolerance of *Zymomonas mobilis*. *Microb. Cell Fact.* 15, 4. <https://doi.org/10.1186/s12934-015-0398-y>.
- Tanasupawat, S., Takehana, T., Yoshida, S., Hiraga, K., Oda, K., 2016. *Ideonella sakaiensis* sp. nov., isolated from a microbial consortium that degrades poly (ethylene terephthalate). *Int. J. Syst. Evol. Microbiol.* 66 (8), 2813–2818. <https://doi.org/10.1099/ijsem.0.001058>.
- Tang, Q., Lu, T., Liu, S.J., 2018. Developing a synthetic biology toolkit for *Comamonas testosteroni*, an emerging cellular chassis for bioremediation. *ACS Synth. Biol.* 7 (7), 1753–1762. <https://doi.org/10.1021/acssynbio.7b00430>.
- Tay, P.K.R., Nguyen, P.Q., Joshi, N.S., 2017. A synthetic circuit for mercury bioremediation using self-assembling functional amyloids. *ACS Synth. Biol.* 6 (10), 1841–1850. <https://doi.org/10.1021/acssynbio.7b00137>.
- Tiwari, J., Gandhi, D., Sivanesan, S., Naoghare, P., Bafana, A., 2020. Remediation of different nitroaromatic pollutants by a promising agent of *Cupriavidus* sp. strain a3. *Ecotoxicol. Environ. Saf.* 205, 111138. <https://doi.org/10.1016/j.ecoenv.2020.111138>.
- Tsipia, A., Koutinas, M., Vernardis, S.I., Mantalaris, A., 2017. The impact of succinate trace on pWWO and ortho-cleavage pathway transcription in *Pseudomonas putida* mt-2 during toluene biodegradation. *Bioresour. Technol.* 234, 397–405. <https://doi.org/10.1016/j.biortech.2017.03.082>.
- Tsoi, R., Wu, F., Zhang, C., Bewick, S., Karig, D., You, L., 2018. Metabolic division of labor in microbial systems. *Proc. Natl. Acad. Sci. USA* 115 (10), 2526–2531. <https://doi.org/10.1073/pnas.1716888115>.
- Varman, A.M., Follenfant, R., Liu, F., Davis, R.W., Lin, Y.K., Singh, S., 2018. Hybrid phenolic-inducible promoters towards construction of self-inducible systems for microbial lignin valorization. *Biotechnol. Biofuels* 11 (1), 182. <https://doi.org/10.1186/s13068-018-1179-8>.
- Viesser, J.A., Sugai-Guerios, M.H., Malucelli, L.C., Pincerati, M.R., Karp, S.G., Maranhão, L.T., 2020. Petroleum-tolerant rhizospheric bacteria: isolation, characterization and bioremediation potential. *Sci. Rep.* 10 (1), 2060. <https://doi.org/10.1038/s41598-020-59029-9>.
- Wanapaisan, P., Laothamteep, N., Vejarano, F., Chakraborty, J., Shintani, M., Muangchinda, C., Pinyakong, O., 2018. Synergistic degradation of pyrene by five culturable bacteria in a mangrove sediment-derived bacterial consortium. *J. Hazard. Mater.* 342, 561–570. <https://doi.org/10.1016/j.jhazmat.2017.08.062>.
- Wang, B., Xu, J., Gao, J., Fu, X., Han, H., Li, Z., Yao, Q., 2019. Construction of an *Escherichia coli* strain to degrade phenol completely with two modified metabolic modules. *J. Hazard. Mater.* 373, 29–38. <https://doi.org/10.1016/j.jhazmat.2019.03.055>.
- Wang, H.H., Isaacs, F.J., Carr, P.A., Sun, Z.Z., Xu, G., Forest, C.R., Church, G.M., 2009. Programming cells by multiplex genome engineering and accelerated evolution. *Nature* 460 (7257), 894–898. <https://doi.org/10.1038/nature08187>.
- Wang, R., Li, J., Li, J., 2020a. Functional and structural analyses for MlrC enzyme of *Novosphingobium* sp. THN1 in microcystin-biodegradation: involving optimized heterologous expression, bioinformatics and site-directed mutagenesis. *Chemosphere* 255, 126906. <https://doi.org/10.1016/j.chemosphere.2020.126906>.
- Wang, Y., Fan, L., Tuyishime, P., Liu, J., Zhang, K., Gao, N., Ma, Y., 2020b. Adaptive laboratory evolution enhances methanol tolerance and conversion in engineered *Corynebacterium glutamicum*. *Commun. Biol.* 3 (1), 217. <https://doi.org/10.1038/s42003-020-0954-9>.
- Xu, C., Sun, T., Li, S., Chen, L., Zhang, W., 2018a. Adaptive laboratory evolution of cadmium tolerance in *Synechocystis* sp. PCC 6803. *Biotechnol. Biofuels* 11, 205. <https://doi.org/10.1186/s13068-018-1205-x>.
- Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Yu, H., 2018b. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. *Front. Microbiol.* 9, 2885. <https://doi.org/10.3389/fmicb.2018.02885>.
- Yan, F., Wei, R., Cui, Q., Bornscheuer, U.T., Liu, Y.J., 2020. Thermophilic whole-cell degradation of polyethylene terephthalate using engineered *Clostridium thermocellum*. *Microb. Biotechnol.* 14 (2), 374–385. <https://doi.org/10.1111/1751-7915.13580>.
- Yang, F., Huang, F., Feng, H., Wei, J., Massey, I.Y., Liang, G., Zhang, X., 2020. A complete route for biodegradation of potentially carcinogenic cyanotoxin microcystin-LR in a novel indigenous bacterium. *Water Res.* 174, 115638. <https://doi.org/10.1016/j.watres.2020.115638>.
- Yang, Q., Cai, S., Dong, S., Chen, L., Chen, J., Cai, T., 2016. Biodegradation of 3-methyldiphenylether (MDE) by *Hydrogenophaga atypical* strain QY7-2 and cloning of the methyloxydation gene *mdeABCD*. *Sci. Rep.* 6, 39270. <https://doi.org/10.1038/srep39270>.
- Yoo, S.M., Jung, S.W., Yeom, J., Lee, S.Y., Na, D., 2020. Tunable gene expression system independent of downstream coding sequence. *ACS Synth. Biol.* 9 (11), 2998–3007. <https://doi.org/10.1021/acssynbio.0c00029>.
- Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Oda, K., 2016. A bacterium that degrades and assimilates poly (ethylene terephthalate). *Science* 351 (6278), 1196–1199. <https://doi.org/10.1126/science.1246359>.
- Young, R., Haines, M., Storch, M., Freemont, P.S., 2021. Combinatorial metabolic pathway assembly approaches and toolkits for modular assembly. *Metab. Eng.* 63, 81–101. <https://doi.org/10.1016/j.ymben.2020.12.001>.
- Yu, S.S.-F., Chen, K.H.-C., Tseng, M.Y.-H., Wang, Y.-S., Tseng, C.-F., Chen, Y.-J., Chan, S. I., 2003. Production of high-quality particulate methane monooxygenase in high yields from *Methylococcus capsulatus* (Bath) with a hollow-fiber membrane bioreactor. *J. Bacteriol.* 185 (20), 5915–5924. <https://doi.org/10.1128/jb.185.20.5915-5924.2003>.
- Zafra, G., Absalón, Á.E., Anducho-Reyes, M.Á., Fernandez, F.J., Cortés-Espinoza, D.V., 2017. Construction of PAH-degrading mixed microbial consortia by induced selection in soil. *Chemosphere* 172, 120–126. <https://doi.org/10.1016/j.chemosphere.2016.12.038>.
- Zeng, W., Guo, L., Xu, S., Chen, J., Zhou, J., 2020. High-throughput screening technology in industrial biotechnology. *Trends Biotechnol.* 38 (8), 888–906. <https://doi.org/10.1016/j.tibtech.2020.01.001>.
- Zhang, B., Sun, L., Song, X., Huang, D., Li, M., Peng, C., Wang, W., 2021. Genetically engineered thermotolerant facultative anaerobes for high-efficient degradation of multiple hazardous nitroalkanes. *J. Hazard. Mater.* 405, 124253. <https://doi.org/10.1016/j.jhazmat.2020.124253>.
- Zhang, F., Qian, X., Si, H., Xu, G., Han, R., Ni, Y., 2015. Significantly improved solvent tolerance of *Escherichia coli* by global transcription machinery engineering. *Microb. Cell Fact.* 14, 175. <https://doi.org/10.1186/s12934-015-0368-4>.
- Zhang, J., Wang, L., Kannan, K., 2019a. Polyethylene terephthalate and polycarbonate microplastics in pet food and feces from the United States. *Environ. Sci. Technol.* 53 (20), 12035–12042. <https://doi.org/10.1021/acs.est.9b03912>.
- Zhang, L., Gao, Y., Wu, S., Zhang, S., Smith, K.R., Yao, X., Gao, H., 2020. Global impact of atmospheric arsenic on health risk: 2005 to 2015. *Proc. Natl. Acad. Sci. USA* 117 (25), 13975–13982. <https://doi.org/10.1073/pnas.2002580117>.
- Zhang, P., Xu, J., Wang, X.-J., He, B., Gao, S.-Q., Lin, Y.-W., 2019b. The third generation of artificial dye-decolorizing peroxidase rationally designed in myoglobin. *ACS Catal.* 9 (9), 7888–7893. <https://doi.org/10.1021/acscatal.9b02226>.
- Zhao, Y., Che, Y., Zhang, F., Wang, J., Gao, W., Zhang, T., Yang, C., 2021. Development of an efficient pathway construction strategy for rapid evolution of the biodegradation capacity of *Pseudomonas putida* KT2440 and its application in bioremediation. *Sci. Total Environ.* 761, 143239. <https://doi.org/10.1016/j.scitotenv.2020.143239>.
- Zhong, Q., Shen, H., Yun, X., Chen, Y., Ren, Y. a, Xu, H., Li, W., 2020. Global sulfur dioxide emissions and the driving forces. *Environ. Sci. Technol.* 54 (11), 6508–6517. <https://doi.org/10.1021/acs.est.9b07696>.
- Zhou, Z., Liu, Y., Zhanoli, G., Wang, Z., Xu, P., Tang, H., 2019. Enhancing bioremediation potential of *Pseudomonas putida* by developing its acid stress tolerance with glutamate decarboxylase dependent system and global regulator of extreme radiation resistance. *Front. Microbiol.* 10, 2033. <https://doi.org/10.3389/fmicb.2019.02033>.