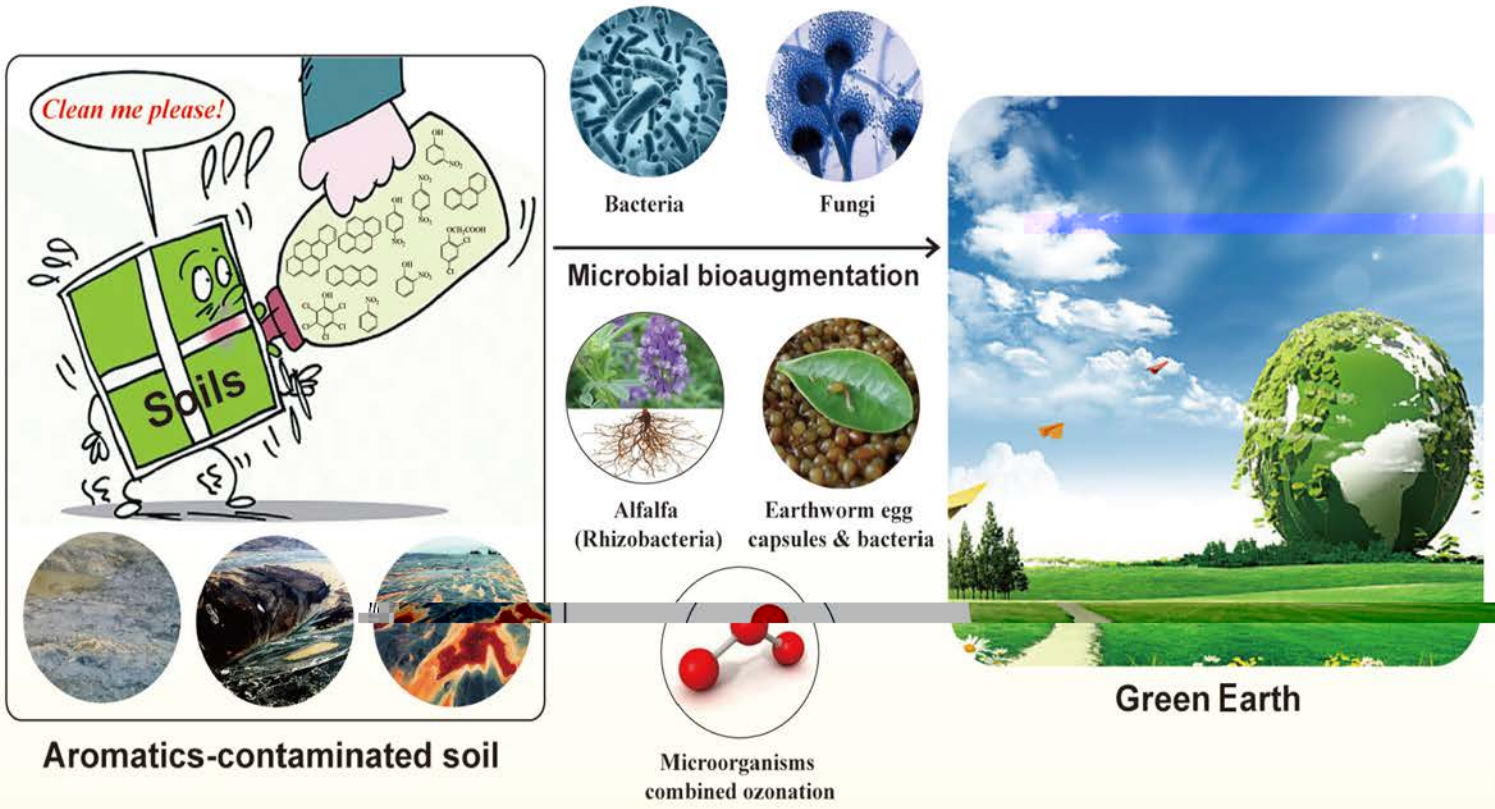
ISSN 2095-2201 Volume 11 · Number 2 · April 2017

Engineering
Transactions of CAE

Frontiers of Environmental Science & Engineering



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Higher Education Press
Springer

Front. Environ. Sci. Eng. 2017, 11(2): 1
DOI 10.1007/s11783-017-0894-x

FEATURE ARTICLE

Microbial remediation of aromatics-contaminated soil

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1 Introduction

During the past 100 years, the worldwide area of polluted soil has substantially increased because of the inappropriate use and accidental leakage of xenobiotics. The presence of xenobiotics in soil, including aromatic pollutants, has become a serious threat to the use of land resources and the safety of agricultural production. Most aromatic pollutants are recalcitrant, persistent and remain in the environment for long periods of time [1]. Xenobiotics in the environ-

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ment can cause serious damage to human and animal health because of their toxic, mutagenic and carcinogenetic properties as well as their ability to bioaccumulate [2]. There are many methods of removing these pollutants from soils, involving physical, chemical or biological approaches. Physico-chemical methods tend to be expensive, requiring high energy input and consumption of lots of different chemical reagents. During recent decades, many microbial strains have been shown to degrade organic pollutants owing to their metabolic machinery [3–11] and ability to adapt to inhospitable environments. It is generally understood that, in a non-polluted environment, bacteria, fungi, protists, and other microorganisms constantly metabolize inorganic and organic matter to provide energy for growth. What occurs in xenobiotics-contaminated environments? Some microorganisms die, while others with the ability to degrade or withstand the xenobiotics. Thus, biological approaches have attracted increasing attention in recent years.

Bioremediation, a biological approach, is a waste management technique that aims to remove or neutralize pollutants from a contaminated site using indigenous or introduced organisms [12], with the addition of fertilizer, oxygen, or other factors to encourage the rapid growth of pollutant degraders. These pollutant-degrading organisms would then be able to break down the organic pollutant, relying on their immense metabolic capacities for transformation of toxic pollutants into essentially harmless or, at least, less toxic compounds at a correspondingly faster rate [13]. Therefore, bioremediation provides a technique for cleaning up pollution by enhancing naturally-occurring biodegradation processes; bioremediation has many advantages including high efficiency, low expense and limited or no secondary pollution when compared with traditional soil remediation techniques, such as incineration or landfilling. A further advantage of bioremediation is that soil, sediment or water can be treated in situ without digging or pumping the contaminated matrix out of the ground. This is why microorganisms play increasingly important roles in site bioremediation. Bioaugmentation can be applied as part of in situ bioremediation treatments and is thought to be a promising approach to clean up pollutants in soil [13,14]; bioaugmentation introduces a specific strain or a consortium of microorganisms with the desired catalytic capabilities for accelerating or enhancing the removal efficiency of pollutants in situ [15,16]. Bioaugmentation has been intentionally performed for years in a number of areas, including agriculture and forestry [17] and wastewater treatment [18]. In recent decades, bioaugmentation has been practiced in the remediation of aromatic compound-contaminated soil [19–27]. Multivariate analysis, previously used in plant ecology, is now also being applied to microbial ecology during bioaugmentation treatments [21].

This review addresses the bioaugmentation of aromatic compound-contaminated soils using a specific strain, a consortium of microorganisms, or microorganisms combined with physical, chemical and other biological methods. However, it should be noted that the strategies described might be also effective for cleaning up other kinds of contaminated soils, such as heavy metal contaminated soils [28–33].

2 Bioaugmentation of aromatic-contaminated soil

2.1 Bioaugmentation of aromatic-contaminated soil with single bacterial strains

Many bacterial strains have the capacities of transforming or metabolizing aromatic pollutants into essentially harmless or, at least, less toxic compounds. Then some contaminated soils with one kind of aromatic were successfully remediated with adding single strains.

2.1.1 Bioaugmentation of nitroaromatic-contaminated soil

Nitrobenzene is highly toxic, water-insoluble, and readily absorbed through the skin. *Pseudomonas putida* ZWL73 is able to grow on nitrobenzene as the sole carbon and nitrogen source with the release of free ammonium [5,34]. At the laboratory scale, bioaugmentation of nitrobenzene-contaminated soil using strain ZWL73 was shown to be feasible [21]. The bioaugmentation of nitrobenzene-contaminated soil with strain ZWL73 effectively enhanced removal of nitrobenzene concurrently with the accumulation of ammonium. Changes in the community structure of ammonia-oxidizing bacteria in nitrobenzene-contaminated soil associated with changes in environmental factors (including concentrations of nitrobenzene, ammonium, nitrite and nitrate) were attenuated in the soil bioaugmented with strain ZWL73 [21].

Fenitrothion (*O*,*O*-dimethyl *O*-*p*-nitro-*m*-tolyl phosphorothioate) is a toxic nitrophenolic pesticide that can build up the concentration of nitrophenolic compounds in soils. *Burkholderia* sp. FDS-1, a fenitrothion-degrading strain [35], was used to study factors affecting its growth, and then evaluated for its capacity to eliminate fenitrothion in soil [24]. Varying concentrations (1–200 $mg \cdot kg^{-1}$ dry soil) of fenitrothion were added into soils with strain FDS-1; the results showed that fenitrothion in the range of 1–50 $mg \cdot kg^{-1}$ dry soil was effectively degraded by strain FDS-1. The addition of strain FDS-1 at 2×10^6 colony forming $units \cdot g^{-1}$ dry soil was found to be suitable for fenitrothion degradation over a temperature range of 20°C–40°C and at pH 7.5. These results indicated that strain FDS-1 had the potential to be used for bioaugmentation treatment of fenitrothion and its metabolite-contaminated soils at field scale [24].

***para*-Nitrophenol (PNP)**, a mononitrophenol isomer, is a priority environmental pollutant that enters the environ-

ment through industrial release and degradation of parathion-based pesticides. To date, several pure bacterial strains have been identified with the ability to metabolize PNP [4,36–38]. Among the pure PNP degraders, *Arthrobacter protophormiae* RKJ100 was inoculated into PNP-contaminated soil as a bioaugmentation treatment [39]. Results showed that, in the process of bioaugmentation, the stability of the introduced strain RKJ100 was enhanced with immobilization, and the rate of PNP consumption varied with increasing depth of soil. Under natural environmental conditions, small-scale field studies (in 1 m^2 plots) were conducted, in which strain RKJ100 was inoculated into PNP-contaminated soil from an agricultural field. In this experiment, PNP was completely removed in 5 days by immobilized cells of strain RKJ100, whereas free cells were only able to consume 75% of the total PNP within the same time period [39].

Organophosphate pesticides, a major class of pollutants, are essential to modern agriculture, particularly in developing countries. Methyl parathion (*O*, *O*-dimethyl *O*-*p*-nitrophenol phosphorothioate; MP) belongs to the group of highly toxic organophosphate pesticides, which are extensively used as agricultural and domestic pesticides, fungicides, and herbicides [40]. *Pseudomonas* sp. strain WBC-3 was isolated with the ability to utilize MP and PNP as the sole sources of carbon, nitrogen and energy for growth [4,40]. The efficacy of bioaugmentation with strain WBC-3 was tested in MP-contaminated soil at the laboratory scale. Compared with the control (uninoculated) treatment, MP was removed more quickly in the bioaugmented treatment. Within 15 days, MP (0.536 $mg \cdot g^{-1}$ dry soil) in the bioaugmented treatment was completely removed without accumulation of toxic intermediates. Strain WBC-3 existed stably during the entire bioaugmentation period, as determined by denaturing gradient gel electrophoresis (DGGE) and real-time PCR analyses. Based on redundancy analysis of the relationships between the environmental factors and microbial community structure, it was clearly shown that the inoculated strain WBC-3 significantly influenced the indigenous bacterial community structure ($P = 0.002$) [20].

2.1.2 Bioaugmentation of chloroaromatic-contaminated soil

Pentachlorophenol (PCP) is extensively used as a wood preservative as well as in a wide variety of agricultural and industrial applications, which has led to serious environment pollution with this chemical [41]. *Sphingobium chlorophenolicum* ATCC 39723, a well-known PCP degrader [42], when inoculated into PCP-contaminated soil with plants showed faster PCP degradation than the uninoculated treatment [25]. In the bioaugmented soil system, plants acted as a vector for strain ATCC 39723 to PCP, the target compound. Monitoring of the plant growth showed a protective role of strain ATCC 39723 against the toxicity of PCP to the plant. In a bioaugmented hydroponic system, strain ATCC 39723 degraded 16 $mg \cdot L^{-1}$ PCP after 7 days at both concentrations tested (20 and 30 $mg \cdot L^{-1}$ PCP). The bioassay confirmed that bioaugmentation treatment of PCP was successful and initial toxicity was reduced with degradation progression at both concentrations tested [25].

Bioaugmentation with ATCC 39723 was also tested in an artificially PCP-contaminated loamy sandy soil [43]. The toxic effect of PCP on plants was assessed through monitoring plant weight and root length. Compared with the uninoculated soil, bioaugmentation with strain ATCC 39723 in planted soil led to faster degradation of PCP. This showed that the presence of strain ATCC 39723 not only enhanced PCP removal but also provided protection for plants from PCP toxicity. The combined use of bioaugmentation and plants suggests that the plant rhizosphere environment might facilitate microbial degradation of pollutants in soil.

4-Chlorophenol (4-CP), a monochlorophenol isomer, is toxic by skin absorption, inhalation, or ingestion. *Arthrobacter chlorophenolicus* A6L, a 4-CP degrader, was inoculated into 4-CP-contaminated soil (180 µg 4-CP $\cdot g^{-1}$ dry soil) to assess the impact of inoculated strain A6L on the indigenous microbiota [44]. After 8 days, less than 10

was enhanced compared with the uninoculated control, based on the gradual accumulation of ammonium and chloride. In 4CNB-contaminated soil, the quantity of 4CNB-resistant bacteria increased, while the diversity and quantity of cultivable heterotrophic bacteria decreased. DGGE analysis showed that the indigenous microbial community structure changed significantly with bioaugmentation [22]. Similarly, 4CNB-degrader *Comamonas* sp. strain CNB-1, isolated from activated sludge [45,46], was inoculated in combination with alfalfa for rhizoremediation of 4CNB-contaminated soil at the laboratory scale [23]. Confocal laser scanning microscopy proved that strain CNB-1 successfully colonized alfalfa roots. The abundance of strain CNB-1 was determined by cultivation and by quantitative competitive PCR targeting the chloronitrobenzene nitroreductase gene. The results showed that the abundance of strain CNB-1 in the rhizosphere was about 10–100 times higher than that in the bulk soil. Sterile and outdoor experiments both showed that 4CNB was completely removed within 1 or 2 days, and its phytotoxicity to alfalfa was also eliminated by strain CNB-1 [23].

2.2 Bioaugmentation of aromatic-contaminated soil with microbial consortia

Aromatic-contaminated soil often contains multiple pollutants. Therefore, microbial consortia are increasingly being used in the bioaugmentation of multi-aromatics-contaminated soils.

2.2.1 Bioaugmentation of multi-aromatics-contaminated soil with bacterial consortia

Nitrophenols are phenolic compounds that have a nitro group at different positions of the hydroxyl group on the benzene ring and they are toxic to many living organisms. In addition to PNP, there are two other isomeric mononitrophenols, *ortho*-nitrophenol (ONP) and *meta*-nitrophenol (MNP). A consortium consisting of PNP utilizer *Pseudomonas* sp. strain WBC-3 [4], MNP utilizer *Cupriavidus necator* JMP134 [6] and ONP utilizer *Alcaligenes* sp. strain NyZ215 [3,47] was inoculated into soil contaminated with three nitrophenol isomers to investigate the efficacy of bioaugmentation at the laboratory scale [19]. Compared with uninoculated soils, inoculated soils showed rapid and complete removal of the three isomeric mononitrophenols within between 2 to 16 days. Real-time PCR targeting functional catabolic genes of the three isomeric mononitrophenols indicated that the three strains survived and were stable during the bioaugmentation process. The abundance of total indigenous bacteria, measured by 16S rRNA genes using real-time PCR, was slightly negatively impacted by contamination with the three isomeric mononitrophenols. DGGE profiles of total and group-specific indigenous communities suggested that species richness of indigenous microorganisms dynamically changed during the process of bioaugmentation. Furthermore, Pareto-Lorenz curves and community organization parameters indicated that the bioaugmentation process had little impact on species evenness within the microbial community [19].

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants widely distributed in the ecosphere [48], which are composed of two or more fused benzene rings and pose serious risks to human and animal health owing to their carcinogenic potential [49]. PAHs in soil tend to interact with the non-aqueous phase and organic matter because of their high hydrophobicity; consequently, these persistent organic pollutants become less available for further microbial degradation [50].

The bioaugmentation treatment of a PAH mixture, consisting of fluorene, phenanthrene and pyrene, was performed by a bacterial consortium (containing three bacterial strains, *Rhodococcus* sp., *Acinetobacter* sp. and *Pseudomonas* sp.) enriched from mangrove sediments under sediment-free and sediment slurry conditions [51]. The bacterial consortium had high PAH degradation capability with 100% degradation of fluorene and phenanthrene in sediment-free liquid medium after four weeks. After one week, the highest removal percentages of fluorene and phenanthrene were found in the non-sterile sediment slurry inoculated with the bacterial consortium, followed by the inoculated sterile slurry, whereas the non-sterile sediment slurry without any inoculation had the lowest removal percentages of all treatments. After 2 weeks, 99% of fluorene and 97% of phenanthrene were eliminated in all treatments, but very little pyrene was removed. Nevertheless, after 4 weeks, all three PAHs were completely degraded. The results indicated that the bacterial consortium accelerated removal of the three PAHs, but autochthonous microorganisms in sediments also had the ability to degrade PAHs. Complete biodegradation of pyrene took longer than that of fluorene and phenanthrene, perhaps indicating that the bacterial consortium preferentially utilized low-molecular-weight PAHs [51].

2.2.2 Bioaugmentation of PAH-contaminated soil using fungal consortia

Fungi, such as saprophytic and plant root-parasitic ground fungi, and ectomycorrhizal and mitosporic fungi, as well as wood decaying fungi, have been identified with the ability to degrade PAHs [52–55]. Based on their degradation abilities, fungi could potentially be applied for bioaugmentation of PAH-contaminated soil.

Low-molecular-weight PAHs (2–3 rings) were observed to be degraded most extensively by *Aspergillus* sp., *Trichocladium canadense*, and *Fusarium oxysporum*

[55]. *Trichocladium canadense*, *Aspergillus* sp., *Verticillium* sp., and *Achremonium* sp. all showed efficient ability to degrade high-molecular-weight PAHs (4–7 rings) [55]. These fungi revealed a great capability to degrade a broad range of PAHs under low-oxygen conditions. Ligninolytic enzyme activities were observed during the growth of fungi on these compounds. These results suggest that fungi are suitable for inoculation into PAH-contaminated soils because of their utilization of PAHs for growth [55].

Fluorene and its derivatives are commonly found in environmental soil, sediment and water samples. Bioaugmentation treatments were performed on an artificially fluorene-contaminated non-sterile soil with 47 fungal strains, including *Absidia cylindrospora*, that were isolated from a PAH-contaminated site. In the presence of fungal inoculants, more than 90% of the fluorene was degraded within 288 h, while in the absence of fungi, the same level of fluorene removal took up to 576 h. The results indicated that *A. cylindrospora* could be used successfully in the bioaugmentation treatment of fluorene-contaminated soil [56].

Bioaugmentation of a historically PAH-contaminated soil from a gasworks site was performed with 21 filamentous fungi isolated from the gasworks site soil. Bioaugmentation was performed separately for each fungal isolate with spore and mycelial inocula. When compared with the spore-based inocula, the extent of total PAH degradation was increased with mycelial inocula. The highest PAH degradation was obtained with *Coniothyrium* sp. (26.5%) and *Fusarium* sp. (27.5%) inoculants, especially for high molecular-weight PAHs. These results suggested that filamentous fungi such as those described above could be used for bioaugmentation of long-term PAH-contaminated soils [57], with the potential to eliminate or reduce the harm of PAHs to the environment.

2.2.3 Bioaugmentation using a consortia containing both bacterial and fungal strains

Bioaugmentation treatment of anthracene, phenanthrene and pyrene contaminated soil of different concentrations (0, 250, 500 and 1000 $mg \cdot kg^{-1}$ dry soil) was performed [58] by a defined microbial consortium (containing five bacteria: *Mycobacterium fortuitum*, *Bacillus cereus*, *Microbacterium* sp., *Gordonia polyisoprenivorans* and *Microbacteriaceae* sp., and a fungus: *Fusarium oxysporum*) isolated from a PAH-contaminated land farm site [59]. PAH degradation was detected by gas chromatography and respirometry. In the inoculated soil, on average, 99%, 99% and 96% of the different concentrations of anthracene, phenanthrene and pyrene, respectively, in the soil were removed by the microbial consortium over a 70-day period, whereas the autochthonous microbial community of the soil showed limited ability to mineralize the PAHs. When bacterial or fungal isolates from the consortium were respectively used for bioaugmentation, the mineralization of anthracene was found to be less efficient than when the whole consortium was present. The results indicated that the microbial mixtures tended to show synergistic activity compared with the monoculture isolates during the process of bioaugmentation treatment of PAH-contaminated soil [58].

A mixed consortium of five bacteria (*Bacillus* sp. B1F, *Bacillus* sp. B5A, *Bacillus* sp. B3G, *Chromobacterium* sp. 4015, and *Enterobacter agglomerans*

or without strain JMP222N (pJP4). Results showed that cocoons containing the 2,4-D-degrader were able to tolerate higher levels of 2,4-DCP. These results suggest that the presence of microbiota containing catabolic abilities might improve the survival rate of developing earthworms when they are exposed to toxic chemicals. In addition, cocoons can be used as carriers of beneficial bacteria, such as pollutant-degraders, for their introduction into the environment [63].

2.3.2 Bioaugmentation using combined ozonation and biodegradation

Bioaugmentation was performed in a prairie soil containing seven PAHs (naphthalene, fluorene, phenanthrene, anthracene, pyrene, chrysene, and benzo(a)pyrene) using a combination of ozonation and a consortium capable of degrading various PAHs [64]. The effect of ozonation on the removal of PAHs was detected with gaseous ozone at the concentration of 12 $mg \cdot day^{-1}$. The results showed that ozonation was quite efficient in the removal of low-molecular-weight PAHs (naphthalene, fluorene, phenanthrene, and anthracene) but not for the removal of high-molecular-weight PAHs (pyrene, chrysene, and benzo(a) pyrene) from the soil. However, elimination of PAHs increased appreciably with ozone treatment in sand containing 0.03% organic carbon [64]. These results suggest that the combined treatment with ozonation and biodegradation might be a promising bioremediation technology for PAH-contaminated soil. The combined ozonation/bioaugmentation approach might be suitable for historically contaminated sites such as the gasworks site mentioned above [57].

2.4 Environmental factors affecting bioaugmentation in situ

Successful bioaugmentation in situ depends not only on the catabolic capability of the inocula but also on their survival in contaminated soils [65–67]. Environmental factors are the major factors impacting the survival and catabolic activity of the inoculated strains. Well-known environmental factors, such as oxygen, pH, temperature, water content, phosphorus, nitrogen and soil texture, can have impacts on the survival and abundance of the inoculated and indigenous microorganisms. During the bioaugmentation of aromatic-contaminated soil, case-dependent specific environmental factors can also play important roles. For example, in the bioaugmentation of nitrobenzene-contaminated soil by strain ZWL73 [21] or PNP-contaminated soil by strain WBC-3 [68], multivariate analysis showed that the concentrations of pollutants (nitrobenzene or PNP), ammonium, nitrite and nitrate were correlated with the decrease in pollutant concentrations, the activity of degraders and the community structure of ammonia-oxidizing bacteria and archaea [21,68]. The persistence and survival of inoculated strains depended on their ability to utilize local resources and contaminants in soil [21,22,67]. Furthermore, indigenous communities have been demonstrated to resist ecological invasion [69]; the success or failure of bioaugmentation will depend on whether a peaceful coexistence is present between the inoculated strains and indigenous communities.

3 Perspective

Bioaugmentation provides certain advantages in cases with pollutant toxicity or an absence of appropriate microorganisms (both quantity and quality) [16]. Successful bioaugmentation requires a considerable bioavailability of the pollutants, the survival and activity of the inoculated microorganisms, and the appropriate environmental conditions [16]. As previously stated, bioaugmentation appears to have great potential for the remediation of aromatics-contaminated soil, and microorganisms play major roles in the process because of their immense metabolic capacities for transformation of aromatic pollutants into essentially harmless or less dangerous compounds. Selection of appropriate microbial strains is the most important step in ensuring successful bioaugmentation treatment for bioremediation.

Many strains used in bioaugmentation have been studied to identify their degradation mechanism of the corresponding aromatic pollutants [3–11]. Previous successful cases of bioaugmentation of aromatics-contaminated soil have shown that not only single strains but also mixed consortia played important roles in bioaugmentation treatments [19–22]. Furthermore, physical, chemical or biological methods, such as plants, earthworm egg capsules and ozone, could be introduced in combination with bioaugmentation to increase the remediation efficiency [23,44,64]. Biostatistical methods have been used in the analysis of the microbial ecology of bioaugmentation [19,21]. It is likely that the most effective elimination of contaminants will be achieved using microorganisms isolated from sites with long-term historical contamination [57]. To ensure a successful bioaugmentation treatment, it is valuable to know the composition, spatial and temporal distribution and population dynamics of indigenous microbial communities in the target habitat to predict the potential effects of soil inoculation [70]. To date, increasing amounts of microbial genomic data has been sequenced and is available in public databases. There is potential to analyze these databases to identify novel pollutant degradation genes or to develop engineered strains for the bioaugmentation of pollutant-contaminated soils. It is necessary to determine the biosafety and survival of inoculated degraders to optimize their efficacy. There are interactions between the inoculated strains or consortia and the indigenous microbial communities; the inoculated strains compete with the indigenous communities, which might decrease the ecological diversity of the soil. In particular,

in the case of bioaugmentation using genetically-engineered degraders, it is necessary to ensure that the engineered degraders do not survive beyond the complete removal of pollutants. Nevertheless, enhancing the survival and catabolic activity of inoculated degraders, and maintaining the original microbial ecological diversity of the soil at the same time, remain research priorities in the field of microbial remediation.

Acknowledgements This work is supported by the National Key Research and Development Program of China (No. 2016YFA0601102) and the National Natural Science Foundation of China (Grant Nos. 31370094 and 31570100).

References

1. O'Neil M J, Heckelman P E, Dobbelaar P H, Roman K J, Kenny C M, Karaffa L S. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 15th ed. London: The Royal Society of Chemistry, 2013
2. Toyoshima E, Mayer R F, Max S R, Eccles C. 2,4-Dichlorophenoxyacetic acid (2,4-D) does not cause polyneuropathy in the rat. Journal of the Neurological Sciences, 1985, 70(2): 225–229
3. Xiao Y, Zhang J J, Liu H, Zhou N Y. Molecular characterization of a novel *ortho*-nitrophenol catabolic gene cluster in *Alcaligenes* sp. strain NyZ215. Journal of Bacteriology, 2007, 189(18): 6587–6593
4. Zhang J J, Liu H, Xiao Y, Zhang X E, Zhou N Y. Identification and characterization of catabolic *para*-nitrophenol 4-monooxygenase and *para*-benzoquinone reductase from *Pseudomonas* sp. strain WBC-3. Journal of Bacteriology, 2009, 191(8): 2703–2710
5. Zhen D, Liu H, Wang S J, Zhang J J, Zhao F, Zhou N Y. Plasmid-mediated degradation of 4-chloronitrobenzene by newly isolated *Pseudomonas putida* strain ZWL73. Applied Microbiology and Biotechnology, 2006, 72(4): 797–803
6. Yin Y, Xiao Y, Liu H Z, Hao F, Rayner S, Tang H, Zhou N Y. Characterization of catabolic *meta*-nitrophenol nitroreductase from *Cupriavidus necator* JMP134. Applied Microbiology and Biotechnology, 2010, 87(6): 2077–2085
7. Pérez-Pantoja D, De la Iglesia R, Pieper D H, González B. Metabolic reconstruction of aromatic compounds degradation from the genome of the amazing pollutant-degrading bacterium *Cupriavidus necator* JMP134. FEMS Microbiology Reviews, 2008, 32(5): 736–794
8. Shen X H, Zhou N Y, Liu S J. Degradation and assimilation of aromatic compounds by *Corynebacterium glutamicum*: another potential for applications for this bacterium? Applied Microbiology and Biotechnology, 2012, 95(1): 77–89
9. Liu H, Wang S J, Zhang J J, Dai H, Tang H, Zhou N Y. Patchwork assembly of *nag*-like nitroarene dioxygenase genes and the 3-chlorocatechol degradation cluster for evolution of the 2-chloronitrobenzene catabolism pathway in *Pseudomonas stutzeri* ZWLR2-1. Applied and Environmental Microbiology, 2011, 77(13): 4547–4552
10. Min J, Zhang J J, Zhou N Y. A two-component *para*-nitrophenol monooxygenase initiates a novel 2-chloro-4-nitrophenol catabolism pathway in *Rhodococcus imtechensis* RKJ300. Applied and Environmental Microbiology, 2016, 82(2): 714–723
11. Chao H J, Chen Y F, Fang T, Xu Y, Huang W E, Zhou N Y. HipH catalyzes the hydroxylation of 4-hydroxyisophthalate to protocatechuate in 2,4-xylenol catabolism by *Pseudomonas putida* NCIMB 9866. Applied and Environmental Microbiology, 2016, 82(2): 724–731
12. Azubuike C C, Chikere C B, Okpokwasili G C. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World Journal of Microbiology and Biotechnology, 2016, 32(11): 180
13. El Fantroussi S, Agathos S N. Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Current Opinion in Microbiology, 2005, 8(3): 268–275
14. Dechesne A, Pallud C, Bertolla F, Grundmann G L. Impact of the microscale distribution of a *Pseudomonas* strain introduced into soil on potential contacts with indigenous bacteria. Applied and Environmental Microbiology, 2005, 71(12): 8123–8131
15. Martín-Hernández M, Suárez-Ojeda M E, Carrera J. Bioaugmentation for treating transient or continuous *p*-nitrophenol shock loads in an aerobic sequencing batch reactor. Bioresource Technology, 2012, 123: 150–156
16. Vogel T M. Bioaugmentation as a soil bioremediation approach. Current Opinion in Biotechnology, 1996, 7(3): 311–316
17. Jasper D A. Bioremediation of agricultural and forestry soils with symbiotic micro-organisms. Soil Research, 1994, 32(6): 1301–1319
18. Menashe O, Kurzbaum E. A novel bioaugmentation treatment approach using a confined microbial environment: a case study in a Membrane Bioreactor wastewater treatment plant. Environmental Technology, 37(12): 1–23
19. Chi X Q, Zhang J J, Zhao S, Zhou N Y. Bioaugmentation with a consortium of bacterial nitrophenol-degraders for remediation of soil contaminated with three nitrophenol isomers. Environmental Pollution, 2013, 172: 33–41
20. Wang L M, Chi X Q, Zhang J J, Sun D L, Zhou N Y. Bioaugmentation of a methyl parathion contaminated soil with *Pseudomonas* sp strain WBC-3. International Biodeterioration & Biodegradation, 2014, 87(1): 116–121
21. Zhao S, Ramette A, Niu G L, Liu H, Zhou N Y. Effects of nitrobenzene contamination and of bioaugmentation on nitrification and ammonia-oxidizing bacteria in soil. FEMS Microbiology Ecology, 2009, 70(2): 315–323
22. Niu G L, Zhang J J, Zhao S, Liu H, Boon N, Zhou N Y. Bioaugmentation of a 4-chloronitrobenzene contaminated soil with *Pseudomonas putida* ZWL73. Environmental Pollution, 2009, 157 (3): 763–771
23. Liu L, Jiang C Y, Liu X Y, Wu J F, Han J G, Liu S J. Plant-microbe association for rhizoremediation of chloronitroaromatic pollutants with *Comamonas* sp. strain CNB-1. Environmental Microbiology, 2007, 9(2): 465–473
24. Hong Q, Zhang Z H, Hong Y F, Li S. A microcosm study on bioremediation of fenitrothion-contaminated soil using *Burkholderia* sp. FDS-1. International Biodeterioration & Biodegradation, 2007, 59(1): 55–61
25. Dams R I, Paton G, Killham K. Bioaugmentation of pentachlorophenol in soil and hydroponic systems. International Biodeterioration & Biodegradation, 2007, 60(3): 171–177

26. 26. Qiao J, Zhang C D, Luo S M, Chen W. Bioremediation of highly contaminated oilfield soil: bioaugmentation for enhancing aromatic compounds removal. Frontiers of Environmental Science & Engineering, 2014, 8(2): 293–304
27. Xu W D, Guo S H, Li G, Li F M, Wu B, Gan X H. Combination of the direct electro-Fenton process and bioremediation for the treatment of pyrene-contaminated soil in a slurry reactor. Frontiers of Environmental Science & Engineering, 2015, 9(6): 1096–1107
28. Jézéquel K, Lebeau T. Soil bioaugmentation by free and immobilized bacteria to reduce potentially phytoavailable cadmium. Bioresource Technology, 2008, 99(4): 690–698
29. Beolchini F, Dell'Anno A, De Propris L, Ubaldini S, Cerrone F, Danovaro R. Auto- and heterotrophic acidophilic bacteria enhance the bioremediation efficiency of sediments contaminated by heavy metals. Chemosphere, 2009, 74(10): 1321–1326
30. Lebeau T, Braud A, Jézéquel K. Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environmental Pollution, 2008, 153(3): 497–522
31. Gavrilescu M, Pavel L V, Cretescu I. Characterization and remediation of soils contaminated with uranium. Journal of Hazardous Materials, 2009, 163(2–3): 475–510
32. Kumar R, Singh S, Singh O V. Bioremediation of radionuclides: emerging technologies. OMICS: A Journal of Integrative Biology, 2007, 11(3): 295–304
33. Sun Y B, Zhao D, Xu Y M, Wang L, Liang X F, Shen Y. Effects of sepiolite on stabilization remediation of heavy metal-contaminated soil and its ecological evaluation. Frontiers of Environmental Science & Engineering, 2016, 10(1): 85–92
34. Xiao Y, Wu J F, Liu H, Wang S J, Liu S J, Zhou N Y. Characterization of genes involved in the initial reactions of 4-chloronitrobenzene degradation in *Pseudomonas putida* ZWL73. Applied Microbiology and Biotechnology, 2006, 73(1): 166–171
35. Zhang Z H, Hong Q, Xu J H, Zhang X, Li S. Isolation of fenitrothion-degrading strain Burkholderia sp. FDS-1 and cloning of mpd gene. Biodegradation, 2006, 17(3): 275–283
36. Jain R K, Dreisbach J H, Spain J C. Biodegradation of *p*-nitrophenol via 1,2,4-benzenetriol by an *Arthrobacter* sp. Applied and Environmental Microbiology, 1994, 60(8): 3030–3032
37. Kadiyala V, Spain J C. A two-component monooxygenase catalyzes both the hydroxylation of p-nitrophenol and the oxidative release of nitrite from 4-nitrocatechol in *Bacillus sphaericus* JS905. Applied and Environmental Microbiology, 1998, 64(7): 2479–2484
38. Spain J C, Gibson D T. Pathway for Biodegradation of *p*-Nitrophenol in a *Moraxella* sp. Applied and Environmental Microbiology, 1991, 57(3): 812–819
39. Labana S, Pandey G, Paul D, Sharma N K, Basu A, Jain R K. Pot and field studies on bioremediation of *p*-nitrophenol contaminated soil using *Arthrobacter protophormiae* RKJ100. Environmental Science & Technology, 2005, 39(9): 3330–3337
40. Liu H, Zhang J J, Wang S J, Zhang X E, Zhou N Y. Plasmid-borne catabolism of methyl parathion and *p*-nitrophenol in *Pseudomonas* sp. strain WBC-3. Biochemical and Biophysical Research Communications, 2005, 334(4): 1107–1114
41. Middaugh D P, Thomas R L, Lantz S E, Heard C S, Mueller J G. Field scale testing of a hyperfiltration unit for removal of creosote and pentachlorophenol from ground water: chemical and biological assessment. Archives of Environmental Contamination and Toxicology, 1994, 26(3): 309–319
42. Cai M, Xun L. Organization and regulation of pentachlorophenol-degrading genes in *Sphingobium chlorophenolicum* ATCC 39723. Journal of Bacteriology, 2002, 184(17): 4672–4680
43. Dams R I, Paton G I, Killham K. Rhizoremediation of pentachlorophenol by *Sphingobium chlorophenolicum* ATCC 39723. Chemosphere, 2007, 68(5): 864–870
44. Jernberg C, Jansson J K. Impact of 4-chlorophenol contamination and/or inoculation with the 4-chlorophenol-degrading strain, *Arthrobacter chlorophenolicus* A6L, on soil bacterial community structure. FEMS Microbiology Ecology, 2002, 42(3): 387–397
45. Wu J F, Jiang C Y, Wang B J, Ma Y F, Liu Z P, Liu S J. Novel partial reductive pathway for 4-chloronitrobenzene and nitrobenzene degradation in *Comamonas* sp. strain CNB-1. Applied and Environmental Microbiology, 2006, 72(3): 1759–1765
46. Ma Y F, Wu J F, Wang S Y, Jiang C Y, Zhang Y, Qi S W, Liu L, Zhao G P, Liu S J. Nucleotide sequence of plasmid pCNB1 from *Comamonas* strain CNB-1 reveals novel genetic organization and evolution for 4-chloronitrobenzene degradation. Applied and Environmental Microbiology, 2007, 73(14): 4477–4483
47. Xiao Y, Liu T T, Dai H, Zhang J J, Liu H, Tang H, Leak D J, Zhou N Y. OnpA, an unusual flavin-dependent monooxygenase containing a cytochrome b_5 domain. Journal of Bacteriology, 2012, 194(6): 1342–1349
48. Kästner M, Breuer-Jammali M, Mahro B. Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil. Applied and Environmental Microbiology, 1998, 64(1): 359–362
49. Choi H, Harrison R, Komulainen H, Delgado-Saborit J M. Polycyclic aromatic hydrocarbons. In: World Health Organization, Regional Office for Europe, eds. Selected Pollutants: WHO Guidelines for Indoor Air Quality. Geneva: World Health Organization, 2010, 289–325
50. Johnsen A R, Wick L Y, Harms H. Principles of microbial PAH-degradation in soil. Environmental Pollution, 2005, 133(1): 71–84
51. Yu S H, Ke L, Wong Y S, Tam N F Y. Degradation of polycyclic aromatic hydrocarbons by a bacterial consortium enriched from mangrove sediments. Environment International, 2005, 31(2): 149–154
52. Gramss G, Voigt K D, Kirsche B. Degradation of polycyclic aromatic hydrocarbons with three to seven aromatic rings by higher fungi in sterile and unsterile soils. Biodegradation, 1999, 10(1): 51–62
53. Bamforth S M, Singleton I. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. Journal of Chemical Technology and Biotechnology (Oxford, Oxfordshire), 2005, 80(7): 723–736
54. Eibes G, Cajthaml T, Moreira M T, Feijoo G, Lema J M. Enzymatic degradation of anthracene, dibenzothiophene and pyrene by manganese peroxidase in media containing acetone. Chemosphere, 2006, 64(3): 408–414
55. Silva I S, Grossman M, Durranta L R. Degradation of polycyclic aromatic hydrocarbons (2–7 rings) under microaerobic and very-low-oxygen conditions by soil fungi. International Biodeterioration

& Biodegradation, 2009, 63(2): 224–229
56. Garon D, Sage L, Wouessidjewe D, Seigle-Murandi F. Enhanced degradation of fluorene in soil slurry by *Absidia cylindrospora* and maltosyl-cyclodextrin. Chemosphere, 2004, 56(2): 159–166
57. Potin O, Rafin C, Veignie E. Bioremediation of an aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil by flamentous fungi isolated from the soil. International Biodeterioration & Biodegradation, 2004, 54(1): 45–52
58. Jacques R J S, Okeke B C, Bento F M, Teixeira A S, Peralba M C R, Camargo F A O. Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. Bioresource Technology, 2008, 99(7): 2637–2643
59. Jacques R J S, Okeke B C, Bento F M, Peralba M C R, Camargo F A O. Characterization of a polycyclic aromatic hydrocarbon degrading microbial consortium from a petrochemical sludge landfarming site. Bioremediation Journal, 2007, 11(1): 1–11
60. Silva I S, Santos E C, Menezes C R, Faria A F, Franciscon E, Grossman M, Durrant L R. Bioremediation of a polyaromatic hydrocarbon contaminated soil by native soil microbiota and bioaugmentation with isolated microbial consortia. Bioresource Technology, 2009, 100(20): 4669–4675
61. Pemberton J M, Corney B, Don R H. Evolution and spread of pesticide degrading ability among soil micro-organisms, In: Timmis KN, Puhler A, eds. Plasmids of Medical, Environmental and Commercial Importance. Amsterdam, Netherlands: Elsevier/North-Holland Biomedical, 1979, 287–299
62. Don R H, Pemberton J M. Genetic and physical map of the 2,4-dichlorophenoxyacetic acid-degradative plasmid pJP4. Journal of Bacteriology, 1985, 161(1): 466–468
63. Daane L L, Häggblom M M. Earthworm egg capsules as vectors for the environmental introduction of biodegradative bacteria. Applied and Environmental Microbiology, 1999, 65(6): 2376–2381
64. Nam K, Kukor J J. Combined ozonation and biodegradation for remediation of mixtures of polycyclic aromatic hydrocarbons in soil. Biodegradation, 2000, 11(1): 1–9
65. Bouchez T, Patureau D, Dabert P, Juretschko S, Doré J, Delgenès P, Moletta R, Wagner M. Ecological study of a bioaugmentation failure. Environmental Microbiology, 2000, 2(2): 179–190
66. Singer A C, van der Gast C J, Thompson I P. Perspectives and vision for strain selection in bioaugmentation. Trends in Biotechnology, 2005, 23(2): 74–77
67. Cunliffe M, Kertesz M A. Effect of *Sphingobium yanoikuyae* B1 inoculation on bacterial community dynamics and polycyclic aromatic hydrocarbon degradation in aged and freshly PAH-contaminated soils. Environmental Pollution, 2006, 144(1): 228–237
68. Chi X Q, Liu K, Zhou N Y. Effects of bioaugmentation in *para*-nitrophenol-contaminated soil on the abundance and community structure of ammonia-oxidizing bacteria and archaea. Applied Microbiology and Biotechnology, 2015, 99(14): 6069–6082
69. Kennedy T A, Naeem S, Howe K M, Knops J M H, Tilman D, Reich P. Biodiversity as a barrier to ecological invasion. Nature, 2002, 417 (6889): 636–638
70. Mrozik A, Piotrowska-Seget Z. Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. Microbiological Research, 2010, 165(5): 363–375

Short biography — Ning-Yi Zhou

Professor Ning-Yi Zhou received his Bachelor's degree in Microbiology from Wuhan University and PhD in Microbiology from Imperial College London. Following postdoctoral training at Imperial College and University of Wales, Bangor, Prof. Zhou established his own laboratory in Wuhan Institute of Virology of Chinese Academy of Sciences. He has recently joined Shanghai Jiao Tong University as a distinguished professor. Over the last two decades, his research has been focusing on the genetics and biochemistry of microbial aromatic catabolism in various bacteria as well as the bioaugmentation of contaminated soil with microbial degraders, publishing more than 70 papers in this field. Currently, he serves an editor for "Applied and Environmental Microbiology" and an associate editor for "Frontiers in Microbiology". He was among the most cited Chinese researchers listed by Elsevier for 2014 and 2015 (Microbiology & Immunology Section).

Project for Enhancing International Impact of China STM Journals

Volume 11
Number 2
2017

Cover story (see: Ying Xu & Ning-Yi Zhou, 2017, 11(2): 1)

Aromatics-contaminated soil is of particular environmental concern as it exhibits carcinogenic and mutagenic properties. Bioremediation, a biological approach for the removal of soil contaminants, has several advantages over traditional soil remediation methodologies. Bioaugmentation is a widely applied bioremediation technology for soil remediation, defined as the introduction of specific competent strains or consortia of microorganisms. A number of reports have been published on the metabolic machinery of aromatics degradation by microorganisms and their capacity to adapt to aromatics-contaminated environments. Thus, microorganisms are major players in site remediation. This review addresses the bioaugmentation of aromatic compound-contaminated soils using a specific strain, a consortium of microorganisms, or microorganisms combined with physical, chemical and other biological methods. The bioremediation bioaugmentation process relies on the immense metabolic capacities of microbes for transformation of aromatic pollutants into essentially harmless or, at least, less toxic compounds. Aromatics-contaminated soils are successfully remediated with adding not only single strains but also bacterial or fungal consortia. Furthermore several novel approaches, which microbes combined with physical, chemical or biological factors, increase remediation efficiency of aromatics-contaminated soil. Meanwhile, the environmental factors also have appreciable impacts on the bioaugmentation process. The biostatistics method is recommended for analysis of the effects of bioaugmentation treatments.

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环境科学与工程前沿
CN 10-1013/X
邮发代号: **80-973**

ISSN 2095-2201