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Bioremediation of Quinoline-contaminated Soil Using Bioaugmentation in Slurry-phase Reactor[1]

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Objective To investigate the possibility of using bioaugmentation as a strategy for remediating quinoline-contaminated soil. **Methods** Microorganisms were introduced to the soil to assess the feasibility of enhancing the removal of quinoline from quinoline-contaminated soil. Slurry-phase reactor was used to investigate the bioremediation of quinoline-contaminated soil. HPLC (Hewlett-Packard model 5050 with an UV detector) was used for analysis of quinoline concentration. **Results** The biodegradation rate of quinoline was increased through the introduction of *Burkholderia pickettii*. Quinoline, at a concentration of 1 mg/g soil, could be removed completely within 6 and 8 hours with and without combined effect of indigenous microbes, respectively. Although the indigenous microbes alone had no quinoline-degrading ability, they cooperated with the introduced quinoline-degrader to remove quinoline more quickly than the introduced microbes alone. Bioaugmentaion process was accelerated by the increase of inoculum size and bio-stimulation. The ratio of water to soil in slurry had no significant impact on bioremediation results. **Conclusion** Bioaugmetation is an effective way for bioremediation of quinoline-contaminated soil.

Key words: Bioaugmentation; Biodegradation; Quinoline; Persistent organic pollutants; Slurry-phase bioreactor

INTRODUCTION

Quinoline belongs to a class of organic compounds called NHAs (N-heterocyclic aromatic compounds) which are ubiquitous and persistent environmental contaminants[1]. Quinoline and its derivatives are widely used in chemical processes, pharmaceutical industries and wood treatment[2]. The removal of quinoline from wastewater, groundwater and soil has received increasing attention worldwide due to its significant amount produced every year and resistance to microbial attack[3]. Some studies have shown that quinoline is a carcinogen in mice and rats and a mutagenic agent with Ames assay[4]. Discharge of quinoline-containing wastes affects human health and causes environmental damage. So far most of the research efforts on quinoline removal from the environment have been devoted to the isolation of

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different quinoline degraders and pathways of quinoline transformation by these strains[5-8]. However there are few reports concerning bioremediation of quinoline-contaminated soil.

Pollutant toxicity is often used as justification for bioaugmentation, because this toxicity could inhibit the degradative activity of indigenous microorganisms. Although few sites with obvious toxicity have been reported, the sites that have been described are of clear potential for bioaugmentation if the introduced microorganisms can also resist the toxicity. One site where exogenous microorganisms were employed required dilution by soil washing or bioslurry techniques to achieve pollutant degradation[9].

Bioaugmentation, the addition of microorganisms to enhance a specific biological activity, has been practiced intentionally for years in a number of areas, including agriculture and forestry[10] and wastewater treatment[11]. Bioaugmentation clearly provides certain advantages over bio-stimulation in cases where pollutant toxicity or lack of appropriate microorganisms (both quantity and quality) is important. Bioaugmentation as a soil bioremediation approach has received increasing attention in recent years[12].

Using bioaugmentation as a strategy for bioremediating polluted soil has proved to be a feasible and economic method compared with other treatment techniques[13]. Through the addition of specialized microbes, the biodegradation of contaminants in soil can be enhanced. Even the complete destruction could be achieved within a short time, if the right environmental conditions were provided[14,15].

Bioaugmentation with carefully selected consortia may improve the opportunity to create reproducible systems enhancing degradative ability. Bioaugmentation with pure cultures into multi-substrate polluted systems (such as wastewater, groundwater, soil or slurry) has had variable results[16]. However, bioaugmentation using strains enriched from sites containing the target contaminant, where the populations have acquired the catabolic ability, has achieved some success[17,18]. Bioaugmentation of activated sludge systems with specialized microorganisms could be a powerful tool to improve the wastewater treatment processes, for example, to improve the flocculation of activated sludge and to enhance the removal efficiency of recalcitrant compounds. The specialized microbes include indigenous or genetically modified organisms. Bioaugmentation has been reported to enhance removal of 3-chlorobenzoate, 4-methyl benzoate, toluene, phenol, 2,4,6-trinitrotoluene, carbon tetrachloride, pentachlorophenol [PCP] and chlorinated solvents[19-27].

In the previous studies, we have investigated the biodegradation of quinoline by free and immobilized microbial cells, as well as the enhancement of quinoline removal from wastewater through introducing isolated pure culture[3,6-8,26]. The aim of this study was to remediate quinoline-contaminated soil by indigenous and introduced microorganisms, to probe the effectiveness of bioaugmentation technique as a strategy for remediating contaminated soil.

MATERIALS AND METHODS

Chemicals and Media

Quinoline used in this study was purchased from Beijing Chemical Plant. It is chemical grade. All other chemicals were reagent grade or analytical grade. Liquid mineral salts medium (MSM) with 0.2 g/L quinoline was used for enrichment experiments. The composition of MSM was illustrated in Table 1. Solid quinoline-MSM (2% agar) was used for isolation and maintenance of microorganisms. Nutrient broth was used for massive growth of microorganisms.

TABLE1

Composition of MSM

Chemicals	Concentration (in g/L)
Na_2HPO_4	4260
KH_2PO_4	2650
$CaCl_2$	200
$MgSO_4 \cdot 7H_2O$	10
$FeSO_4 \cdot 7H_2O$	20
$MnSO_4 \cdot 7H_2O$	2

Soil Samples

Soil samples used in this study were taken from the top layer (10-20 cm) of the grounds of Tsinghua Garden and air-dried (40%) and sieved (2 mm mesh size)[28]. The characteristic features of the soil were analyzed and presented in Table 2.

TABLE 2

Physicochemical Characteristics of Soil Used in This Study

Parameter	Value	Unit
Total C	1.56	%
Inorganic C	0.42	%
Organic C	1.14	%
Total N	0.06	%
NH_4^+-N	5.82	mg/kg
NO_3^--N	22.15	mg/kg
Total P	9.0	mg/kg
K^+	30.2	mg/kg
Mg^{2+}	52.3	mg/kg
pH	7.2	-

Enrichment and Isolation of Microorganisms

Activated sludge from aeration tank of a coke-oven wastewater treatment in our lab was used for enrichment. After centrifugation, one gram of inoculum was introduced into 100 mL of MSM containing 0.2 g/L quinoline. Cultivation was performed at 28℃ on a rotary shaker (180 rpm). After every five days of incubation, 5 mL of the enrichment culture was transferred to 100 mL of fresh MSM containing 0.2 g/L quinoline. Three to four transfers were made before pure culture of microorganisms was recovered from the liquid enrichment medium by streak plating onto solid quinoline-containing MSM. The microorganisms were identified using Biolog Microstation System (Biolog Inc, U.S.A) in the Institute of Microbiology, the Chinese Academy of Sciences.

Bioaugmentation Procedures

Bioaugmetation experiments were carried out in Erlenmeyer flasks containing 5 g of

quinoline-contaminated soil and 20 mL of sterilized water on a rotary shaker (28℃, 180 rpm). Quinoline-contaminated soil was prepared by thorough mixing of soil samples and quinoline to reach a final concentration of 1 mg quinoline per gram of soil. The isolated quinoline-degrader was introduced as inoculum to enhance quinoline biodegradation in slurry. The normal inoculum size was 1.0×10^9 cells in one flask, but another inoculum size (3.0×10^9 cells) was also used to determine its effect on bioaugmentation result. Different ratio of water to soil in the slurry (40 mL of sterilized water and 5 g of quinoline-contaminated soil in one flask) was used to see if the extra water in the slurry had any effect on the removal of quinoline. Bio-stimulation effect was studied by the addition of NH_4Cl (1 g/L) and KH_2PO_4 (1 g/L) to the slurry. A small part of the slurry was withdrawn at different time intervals for the analysis of quinoline concentrations.

All experiments were performed in triplicate. The data were mean values of three experiments.

Analytical Method

For quinoline quantification, HPLC system (Hewlett-Packard model 5050 with an UV detector) was used. 20 μL of samples was injected after centrifugation and filtration. Separation was carried out in a C^{18} reverse-phase column, 250×4.6 mm, 5 μm (Hewlett-Packard Zorbax SB-C^{18}, U.S.A). The elution solvent consisting of a mixture of methanol and water (60:40, v/v), was introduced to the column at a flow rate of 1 mL/min. Quinoline was detected at 275 nm.

RESULTS

Isolation and Identification of Quinoline-degrader

A quinoline degrader, which can use quinoline as the sole source of carbon, nitrogen and energy, was isolated from the enrichment culture after four transfers. The degrader was identified as *Burkholderia pickettii* according to the report of Biolog Microstation System (ID=0.733). It was a gram-negative rod-shaped aerobe (6 μm long and 2 μm wide). Colonies were mucoid and grey when grown on solid quinoline-MSM. This isolate was used then in the following studies as a bioaugmentation agent for remediating the quinoline-contaminated soil.

Biodegradation of Quinoline by Indigenous Microbes

The soil was sterilized in order to investigate the role of indigenous microbes in removing quinoline in slurry. The abiotic and biotic loss of quinoline in contaminated soil, or alternatively, the quinoline biodegradation with and without indigenous microbes, is shown in Fig. 1. It can be seen that there was virtually no difference between biotic and abiotic treatment of soil. The decrease of quinoline concentration in slurry may be the result of its adsorption to soil. The indigenous microbes could not degrade quinoline readily, thus contributing little to the removal of quinoline.

Biodegradation of Quinoline by Introduced and Indigenous Microbes

The above results revealed that there were no appropriate microorganisms capable of degrading quinoline in the soil. Therefore, bioaugmentation was a powerful strategy for remediating recalcitrant pollutants in such a circumstance. The role of bioaugmentation in the remediation of quinoline-contaminated soil was investigated by introducing quinoline-

degrading microbes to contaminated soil. The effect of introduced microbes on quinoline degradation is depicted in Fig. 2.

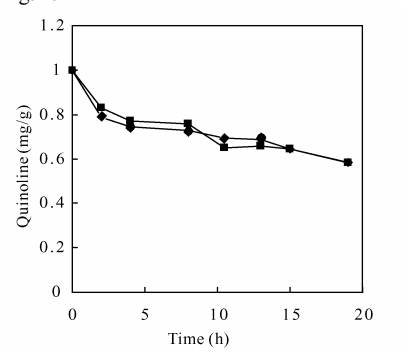

FIG .1. Quinoline degradation by indigenous microbes. Symbol (♦) indicates that the soil was sterilized to kill all the microbes in the soil, that is to say, toexclude the biotic loss of quinoline in the soil; (■) indicates that the soil was not sterilized, so the indigenous microbes were alive in the soil, the loss of quinoline was combination of biotic and abiotic effect.

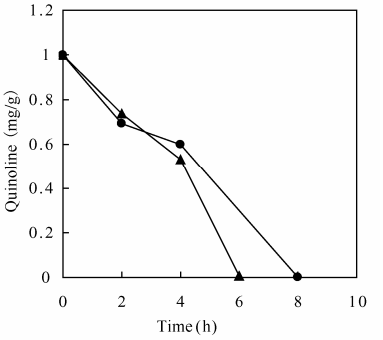

FIG. 2. Quinoline degradation by introduced and indigenous microbes. Symbol (▲) indicates that the soil was not sterilized, the biodegradation off quinoline was caused by the indigenous and introduced microbes; (●) indicates that the soil was sterilized, the biodegradation of quinoline was only related to the introduced microbes.

Fig. 2 shows that, after the addition of quinoline-degrader, the degradation of quinoline was enhanced greatly. Quinoline could be removed within a few hours. In a bioaugmented slurry reactor with and without indigenous microbes, quinoline at a concentration of 1.0 mg/g soil was degraded completely in 6 h and 8 h, respectively.

Effect of Inoculum Size on Quinoline Degradation

The effect of inoculum size on quinoline biodegradation was investigated and the result is shown in Fig. 3.

It was evident that with the increase in inoculum size, quinoline biodegradation could be accelerated, but there was no proportional relationship between the increase in inoculum size and the enhancement of biodegradation rate. After the addition of three times of

quinoline-degrading bacteria, the degradation rate was enhanced approximately by 25%.

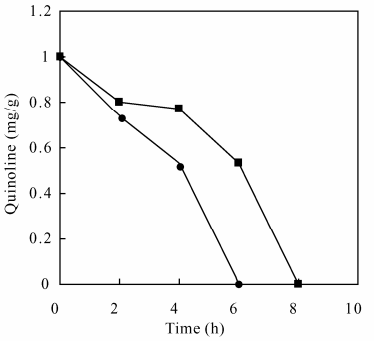

FIG.3. Effect of inoculum size on quinoline degradation. Symbol(●) indicates that the introduced microbes were 3.0×10^9 cells; (■) indicates that the introduced microbes were 1.0×10^9 cells.

Effect of Bio-stimulation on Quinoline Degradation

The effect of nutrients addition to the slurry reactor on the quinoline degradation was investigated by adding NH_4Cl (1 g/L) and KH_2PO_4 (1 g/L) to the slurry. The result is shown in Fig. 4.

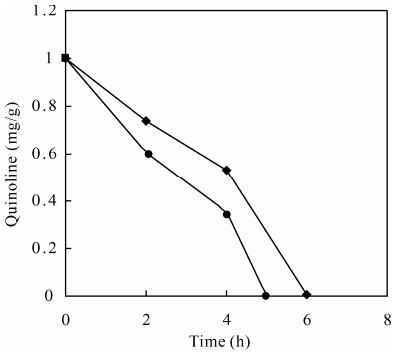

FIG .4. Effect of bio-stimulation on quinoline degradation. Symbol (■) indicates that nutrients (N and P) were added into the soil to investigate the effect of bio-stimulation on quinoline biodegradation; (●) indicates that no additional nutrients were supplemented to the soil.

Fig. 4 revealed that biodegradation of quinoline could be enhanced by addition of nutrients to the slurry reactor. The reason may be that the added nutrients could increase both the amount and activity of quinoline-degrader, therefore the process of quinoline degradation was stimulated.

DISCUSSION

Bioaugmentation of a wastewater treatment system with specialized microorganisms

could be a powerful tool to improve the treatment processes, for example, to enhance the removal efficiency of recalcitrant compounds[26,29]. The specialized microbes include naturally or genetically modified organisms. Bioaugmentation has been reported to enhance removal of 3-chlorobenzoate, 4-methyl benzoate, toluene, phenol, and chlorinated solvents. Yu and Mohn[29] investigated bioaugmentation with resin-acid-degrading bacteria to enhance resin acid removal from pulp mill effluents using 500-mL flasks as sequencing batch reactors. They monitored the indigenous microbial community composition by ribosomal intergenic spacer analysis (RISA) and the results indicated that the introduced bacteria did not substantially change structure of indigenous microbial community. Their results suggest that it is feasible and potentially useful to enhance resin acid removal by bioaugmentation with addition of resin-acid-degrading bacteria.

Comparing Figs. 1 and 2, it was clear that the biodegradation by quinoline-degrader contributed to 75% of the total quinoline removal, and the adsorption accounted for the other 25%. Although the indigenous microbes alone showed no quinoline-degrading ability, they cooperated with the introduced quinoline-degrader to remove quinoline more quickly than the quinoline-degrader added alone. The possible reasons for this phenomenon may be that even though indigenous microbes could not degrade quinoline, they could degrade the inter-metabolites formed during the quinoline degradation by introduced microbes, that is to say, some metabolites of quinoline biodegradtion by the introduced degrader could be further used by indigenous microbes, and therefore, the biodegradation rate of quinoline was increased.

Fig. 4 indicated that quinoline was completely removed in 5 hours with bio-stimulation, compared with 6 hours without bio-stimulation. Margesin *et al.*[30] investigated effect of different N-sources (such as NO_3^--N and NH_4^+-N) and P-sources on hydrocarbon decontamination in soil. The results indicated that hydrocarbon degradation was significantly lower in unfertilized soils than in fertilized ones. Besides nitrogen, phosphorus also enhanced bioremediation significantly and was independent of the nitrogen source. There was no significant difference between decontamination in soils containing both a N- and a P-source.

When we use bioaugmentation technique to remediate quinoline-contaminated soil, it is important to choose a suitable inoculum size and an appropriate amount of nutrients added for achieving a good bioremediation result in an economic way.

The effect of the ratio of water to soil was also investigated. It was suggested that the existence of extra water in slurry did not influence the degradation of quinoline (data not shown). Cho *et al.*[31] investigated the effect of soil moisture on bioremediation of chlorophenol-contaminated soil using 250-mL Erlenmeyer flask containing 100 g soil. The results indicated that soil moisture had a significant effect with the slowest degradation rate of chlorophenols at 25% in the range of 10%-40% moisture content. At 25%-40%, the rate of chlorophenol degradation was directly related to the soil moisture content, whereas at 10%-25%, it was inversely related.

CONCLUSIONS

The experimental results reveals that after the addition of quinoline-degrader as inoculum, complete degradation of quinoline in slurry can be achieved within a few hours, showing that bioaugmetation is an effective way for bioremediation of quinoline-contaminated soil. The indigenous microbes have no quinoline-degrading ability, but there might be a synergetic relationship between the quinoline-degrader and indigenous microbes. The experimental results indicate that combined effect of indigenous and introduced microbes can enhance the

removal of quinoline. Bioaugmentaion process can be accelerated by the increase of inoculum size and bio-stimulation. The ratio of water to soil in slurry has no significant impact on bioremediation results.

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