

## Slurry-phase Biological Treatment of Nitrophenol Using Bioaugmentation Technique<sup>1</sup>

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**Objective** To investigate the performance of soil-slurry bioreactor used for remediating contaminated soil with 4-nitrophenol (4-NP). **Methods** The slurry bioreactor was used to degrade different concentrations of 4-nitrophenol with or without inoculating the acclimated activated sludge. HPLC system (Hewlett-Packard model 5050 with a UV detector) was used for the quantification of 4-nitrophenol. **Results** The indigenous microorganisms exhibited a little activity for simulated soil with 50 mg 4-NP/kg soil. However, at the concentration of 10 mg 4-NP/kg soil, a considerable degradation occurred within two weeks. It appeared that high concentrations of 4-nitrophenol apparently produced an inhibitory effect on microbial activity. For system receiving 50 mg 4-NP/kg soil, the maximum rate of 4-NP degradation measured in the reactor inoculated with 25 g sludge/kg soil was approximately 10 times higher than the uninoculated reactor, suggesting that the degradation rate of 4-nitrophenol could be enhanced greatly by means of inoculating acclimated sludge. **Conclusion** The addition of sludge capable of degrading 4-nitrophenol can result in enhance the degradation rate of 4-nitrophenol.

**Key words:** Biodegradation; Bioremediation; Slurry bioreactor; 4-nitrophenol; Soil

### INTRODUCTION

Nitroaromatic compounds are common pollutants produced by the industrial manufacturing of dyes, explosives, pesticides, herbicides, and drugs<sup>[1-2]</sup>. They are also formed through photochemical<sup>[3]</sup> or biological processes<sup>[4-5]</sup> in the environment. Several nitroaromatics, including 4-nitrophenol, are listed as priority pollutants by China Environmental Monitoring Center, U. S. Environmental Protection Agency and other similar authorities. Nitrophenolic compounds exhibit high toxicity and/or mutagenicity for many living organisms either directly or through some of their catabolic metabolites.

Nitrophenols in surface waters and surface soils are readily degraded by photochemical oxidation. However, in deeper soils and groundwater, nitrophenol degradation depends primarily on biodegradation. The biodegradation of nitroaromatics can be initiated by either reductive or oxidative mechanisms<sup>[6-12]</sup>.

Numerous investigations into the microbial trans-

formations of nitroaromatic compounds are reported in the literature<sup>[13-17]</sup>. These studies often used microbial communities selected from engineered processes, e.g., activated sludge systems. Relatively little is known about the degradative capability of indigenous microbial communities.

In this paper the bioremediation of 4-nitrophenol in slurry bioreactor was investigated with soil samples inoculating or uninoculating acclimated sludge.

### MATERIALS AND METHODS

#### Soil Samples

Soil samples used in this study were taken from the top layer (10 cm-20 cm) of the grounds of Tsinghua garden and air-dried (40%) and sieved (2 mm mesh size). The samples were stored at 4°C until used, and had the similar characteristics as the soil studied earlier<sup>[18]</sup>.

<sup>1</sup>The work was supported by the National Natural Science Foundation of China (Grant No. 50325824, 29637010) and the Excellent Young Teacher Program of MOE, China.

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## Chemicals

4-nitrophenol (4NP) was obtained from Beijing Chemical Plant (purity exceeded 98%) and used as received. The inorganic reagents used were of analytical grade.

## Acclimation of Activated Sludge

Activated sludge obtained from wastewater treatment plant of coke-oven plant was used as seeding sludge. The acclimation of sludge was carried out in a fill-and-draw operation of one cycle per two days, in a reactor of 2.0 L at 25°C using the 4-nitrophenol-containing medium shown in Table 1. In each cycle, half of the supernatant, after settled for 30 min in the reactor, was drawn before the fresh medium of the same volume was added. Air was supplied through a sparger at the bottom of the reactor. During the experimental period, 4-nitrophenol and biomass concentrations were monitored.

TABLE 1

Composition of Medium Used for Acclimating Activated Sludge

Component	Concentration (g/L)
Glucose	0.1-0.5
4-nitrophenol	0.01-0.1
KH <sub>2</sub> PO <sub>4</sub>	1.0
KNO <sub>3</sub>	0.5
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.1
CaCl <sub>2</sub>	0.1
FeCl <sub>3</sub>	0.01
NaCl	1.0

## Bioremediation Experiments

The degradation rate of 4-nitrophenol was examined in soil-slurry bioreactor for unamended soils as well as soils inoculated with previously acclimated sludge with 4-nitrophenol-degrading bacteria.

The bioremediation experiments were carried out in 500 mL flasks containing 50 g dry weight of soil and 100 mL distilled water. Each bioremediation experiment was performed in triplicate.

## Analytical Methods

Following completion of the bioremediation tests, the soil-water residue in each flask was extracted by agitating overnight with 100 mL of 1:1 hexane:acetone mixture. The contents in the flasks were then allowed to settle, the solvent mixture decanted into graduated conical tip tubes, and the solvent was allowed to be evaporated to a final volume of 10 mL.

For quantification of 4-nitrophenol, HPLC system (Hewlett-Packard model 5050 with an UV detector) was used. Twenty  $\mu$ L of samples was injected after centrifugation and filtration. Separation was carried out in a C<sup>18</sup> reverse-phase column, 250 mm×4.6 mm, 5  $\mu$ m (Hewlett-Packard Zorbax SB-C<sup>18</sup>, U.S.A). The elution solvent, which consisted of a mixture of methanol and water (60:40, v/v), was introduced to the column at a flow rate of 1 mL/min. 4-nitrophenol was detected at 280 nm.

## RESULTS

### Effect of 4-nitrophenol Concentration on Bioremediation Rate

Two different 4-nitrophenol concentrations were used in the bioremediation experiments: 10 mg and 50 mg 4-nitrophenol per kg soil. Biological degradation of 4-nitrophenol was monitored. 4-nitrophenol losses through volatilization were assessed through the use of sterilized soil as a control.

Fig. 1 showed the microbial degradation of 4-nitrophenol in soil-water systems receiving 10 mg/kg soil and 50 mg/kg soil, respectively.

From Figs. 1A and 1B, it was apparent that the increased concentration of 4-nitrophenol appeared to have an inhibitory effect on microbial degradation. For the system receiving 10 mg 4-NP/kg soil, sixty percent of 4-nitrophenol could be degraded within 30 days by indigenous microorganisms. However, when the 4-nitrophenol concentration increased to 50 mg/kg soil, the degradation extent was only about 25% after 30 days incubation, and about 10 days lag phase was observed, suggesting that microbial activity in these systems was inhibited due to the high concentration of 4-nitrophenol. 4-nitrophenol was not very hydrophobic, with an octanol/water partition constant

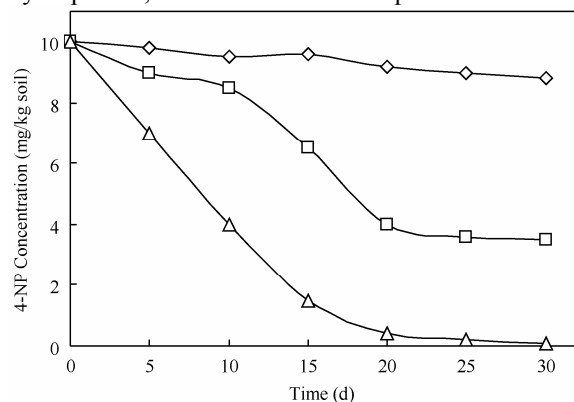


FIG. 1A. Degradation of 4-nitrophenol in slurry bioreactor with 10 mg 4-nitrophenol/kg soil (◇) sterilized soil as a control; (□) soil sample; (△) soil sample inoculated acclimated sludge.

$\log K_{ow}$  value reported between 1.91 and 2.04<sup>[19]</sup>. This suggested that excessive 4-nitrophenol sorption onto soil organic carbon was unlikely.

*Effect of Inoculating Sludge on Bioremediation Rate*

The influence of inoculating acclimated activated

sludge on 4-nitrophenol degradation was studied. The inoculum size was 5 g sludge/kg soil, 10 g sludge/kg soil and 25 g sludge/kg soil, respectively. The results of degradation data for systems receiving 50 mg 4-NP/kg soil with addition of different amounts of acclimated sludge are depicted in Fig. 2.

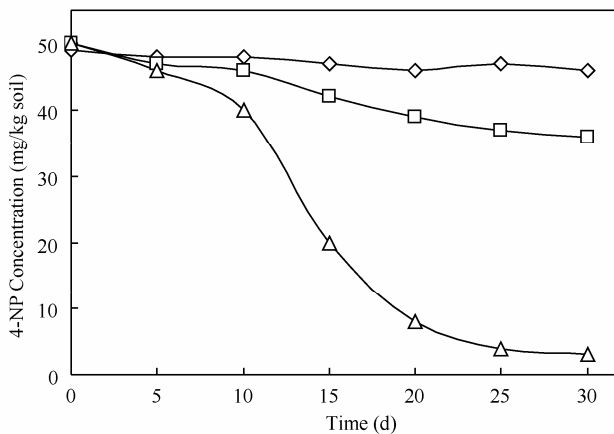


FIG. 1B. Degradation of 4-nitrophenol in slurry bioreactor with 50 mg 4-nitrophenol/kg soil, (◇) sterilized soil as a control; (□) soil sample; (△) soil sample inoculated acclimated sludge.

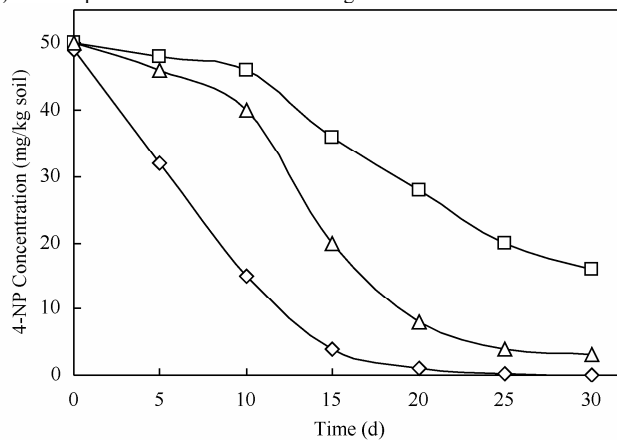


FIG. 2. Degradation of 4-nitrophenol in slurry bioreactor inoculated different amount of acclimated sludge, (◇) 5 g sludge/kg soil; (□) 10 g sludge/kg soil; (△) 25 g sludge/kg soil.

It was evident that the rate and extent of 4-NP degradation increased with increase of acclimated activated sludge added. 4-nitrophenol with the concentration of 50 mg/kg soil could be completely degraded within four weeks in a soil-slurry bioreactor when acclimated sludge at 25 g/kg soil was inoculated and there was no lag phase for 4-nitrophenol degradation. However, less than 60% 4-nitrophenol could be degraded when 5 g sludge/kg soil was inoculated and about 10 days lag phase was observed.

*Estimation of Maximum Degradation Rate*

In order to estimate the maximum rate and extent of biodegradation in each microcosm, the observed

biodegradation data were modeled as a saturation type curve<sup>[20]</sup>:

$$P = \frac{k_1 t}{1 + k_2 t} \tag{1}$$

Where: P is the degradation rate (%), t is the time since initiation of degradation (day),  $k_1$  and  $k_2$  are constants derived from curve fitting.

This model is defined by a limiting or maximum extent of degradation,  $P_{max} = k_1/k_2$ , and an initial linear degradation rate given by  $(dP/dt)_{max} = k_1$ .

Equation (1) could be linearized by taking the reciprocal of both sides, and rearranged as:

$$\frac{t}{P} = \frac{k_2}{k_1} t + \frac{1}{k_1} \tag{2}$$

A plot of the ratio of time passed/percent degraded *versus* time passed yields a straight line, parameters  $k_1$  and  $k_2$  could be derived by linear regression.

Table 2 presents the maximum rates of 4-nitrophenol degradation observed in the soil-water microcosms, values for the squared correlation coefficient ( $R^2$ ) for linear regression are also listed in Table 2.

TABLE 2

Maximum Rate of 4-nitrophenol Degradation in Soil Slurry Bioreactor

System	4-nitrophenol (mg/kg soil)	Maximum Degradation Rate (%/day)	$R^2$
Unamended Soil	10	15.5	0.833
Inoculated Soil	50	0.98	0.821
Inoculated Soil	50	10.6	0.878

## DISCUSSION

The use of unamended soil samples resulted in maximum degradation rates of 15.5 and 0.98%/day for the systems receiving 10 mg 4-NP/kg soil and 50 mg 4-NP/kg soil, respectively. For the system receiving 50 mg 4-NP/kg soil and inoculated with 25 g sludge/kg soil, the maximum rate of degradation measured was approximately 10.6%/day, which was about 10 times higher than unamended soil, that is to say, the addition of acclimated sludge capable of degrading 4-nitrophenol could greatly increase the degradation rate, compared with uninoculated soil microcosms.

The results obtained in this study indicated that the indigenous microorganisms appeared to be able to biodegrade 4-nitrophenol without any observed lag period up to the concentration of 10 mg/kg soil, suggesting the potential for natural attenuation of the adverse effects of 4-nitrophenol in natural soil environments, which was also supported by other researchers. Zaidi *et al.*<sup>[21]</sup> carried out a study to evaluate the viability of bacterial inoculation in enhancing the biodegradation of organic pollutants, 4-nitrophenol was observed to be mineralized extensively in lake water inoculated with 4-nitrophenol degraders, whereas no degradation was measured in uninoculated lake water over one week incubation. Spain and van Veld<sup>[22]</sup> reported the ability of microbial communities in various sediment water samples collected from the Pensacola area, Florida, to degrade radiolabeled 4-nitrophenol. After a lag period of two to four days, the rates of  $^{14}\text{CO}_2$  release increased considerably in all freshwater communities.

Schmidt and Gier<sup>[23]</sup> measured almost 50% mineralization of 2,4-dinitrophenol (DNP) in soil-water systems receiving up to 200  $\mu\text{g/g}$  DNP, and approximately 10% mineralization in systems receiving 500  $\mu\text{g/g}$  DNP. Heitkamp *et al.*<sup>[15]</sup> observed 45% to 70% 4-nitrophenol was degraded within four days in liquid culture. The degradation rates measured in this study were somewhat less than these results. The reason may be due to the differences in microbial activity in indigenous soil and acclimated activated sludge.

## CONCLUSION

In this study, the microbial degradation of 4-NP was examined in soil-slurry systems. Experimental results indicate that at the contamination levels up to 10 mg 4-NP/kg soil, 60% 4-nitrophenol can be degraded in unamended soil-slurry systems within 30 days with no lag period detected prior to the onset of degradation. The degradation rate can be increased when a soil-slurry bioreactor is inoculated with acclimated sludge. The addition of sludge capable of degrading 4-nitrophenol can enhance the degradation rate of 4-nitrophenol. For soil microcosms receiving 50 mg 4-NP/kg soil, inoculation with acclimated sludge can result in a significant increase in degradation rate.

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(Received November 1, 2003 Accepted June 20, 2004)