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Studies on Removal of Nitrobenzene from Contaminated Water and Sediment by Bioaugmentation

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Abstract: Nitrobenzene is highly toxic liquid aromatic compound which is readily absorbed through the skin. Although nitrobenzene is not currently known to be a carcinogen, prolonged exposure may cause serious damage to the central nervous system, impair vision, cause liver or kidney damage, anaemia and lung irritation. Natural attenuation, biostimulation and bioaugmentation of nitrobenzene contaminated sediments were investigated and compared. The removal rate of nitrobenzene from contaminated environments with bioaugmentation was much faster than with biostimulation and natural attenuation. Within 10 days, 8 mg/kg and 56 mg/L nitrobenzene in sediment and water, respectively, were degraded with the augmentation of *Pseudomonas putida* isolated from the contaminated sediment. There was no distinct performance difference between natural attenuation and biostimulation, demonstrating that addition of nutrients had no effect on the bioremediation process. The information on the current phase is a crucial step in making policy decisions for the application of bioremediation.

Key words: Biostimulation · Nitrobenzene-polluted River · Contaminated Sediments · Pseudomonas putida

INTRODUCTION

Nitrobenzene (NB) $C_6H_5NO_2$, very poisonous, flammable, pale yellow, liquid aromatic compound with an odor like that of bitter almonds. It is sometimes called oil of mirbane or nitrobenzol. Organic chemicals form the second largest segment of the Indian chemical industry. This segment has the largest number of products classified under it, most of which are knowledge driven. As a result, R and D forms a considerable part of the manufacturer's costs. This segment accounts for 20% of total chemical production in the country.Nitrobenzene is one of the top 40 industrial chemicals produced in India and has been on the United States Environmental Protection Agency (EPA) priority pollutant list [1]. About 19 million pounds of NB is released into the environment annually due to its usage, leakage or industrial accidents [2]. Due to its recalcitrant and hydrophobic nature, NB accumulates in the sediment of a water body, posing a concern for ecosystem health. Conventional treatment of NB has largely been through chemical or physical methods. These processes, however, have led to secondary contamination. Biological methods for the bulk removal of these pollutants are therefore generally preferred. Major advantages of bioremediation are the lower capital costs and the ability to perform the task on site. As an emerging alternative technology for restoration of contaminated environments, bioremediation normally includes bioaugmentation (addition of microorganisms) and biostimulation (addition of nutrients) [3].

In a previous study, a nitrobenzene-degrading bacteria was isolated. Seven strains that can degrade nitrobenzene at low temperature were isolated from the sediments of a nitrobenzene-polluted river. One of the strains, NB^{-1}, could mineralize 20 mg/L nitrobenzene completely from 3 to 30°C with an optimum temperature of 25°C. NB^{-1} was identified as *Pseudomonas putida* according to its morphology, biochemical properties and 16S rDNA sequence analysis [4]. The main objective of this study was to assess the bioremediation potential of nitrobenzene contaminated sediment using *P. putida* [5]. The effectiveness of different remediation strategies, such as natural attenuation, biostimulation and bioaugmentation, was investigated and compared.

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MATERIALS AND METHODS

Pseudomonas putida isolated from the river sediment was inoculated in river water collected from Paleru, a tributary of river Krishna, it flows through Warangal district and mingles into river Krishna near Jaggayyapeta, in a plastic container with 25 mg/L nitrobenzene as the sole carbon and energy source. After the bacterial grow to their exponential growth phase (115 cells/ mL), they are ready to be used for bioremediation. Bioremediation experiments were conducted in glass cylinder containers (diameter: 0.6 m, height: 0.8 m) with water and sediment (Table 1) collected from the Krishna River. Water and sediment samples were collected from the Paleru near the wastewater discharge point. The sediment consisted of light gray, calcareous particles and had a brown colour. After mixed adequately with 100 mg/L nitrobenzene, the sediments were paved (about 0.17 m in height) on the bottom of each container with the same volume of river water on the top.

For biostimulation, nutrients were added into the container to make the same N and P concentrations as those in mineral solution. For bioaugmentation, bacterial grown in 25 mg/L nitrobenzene was added into the container with a volume ratio to water inside of 1:100. No measures were taken in the natural attenuation experiment. The experiments were conducted in triple at a temperature of 15°C which is close to the daily average temperature in the Krishna River. Samples were taken at appropriate time for analysis. Nitrobenzene degrading bacteria was enumerated using a tailored version of the Brown and Braddock sheen screen MPN method [6]. An initial dilution of ca. 1 g soil in 10 mL basal nitrobenzene medium was used for a 6 tube MPN, with seven 1:10 serial dilutions in a 96 well micro-titre plate. After serial dilution, 5 μL of filter-sterilised nitrobenzene was applied to the surface of the medium in each well. The last row only contained medium and nitrobenzene as an un-inoculated control. Micro-titre plates were incubated for up to 30 days and examined every 5 days. Tubes were scored as positive if the hydrocarbon sheen was disrupted. Cell concentration was determined by measuring the absorbance at a wavelength of 550 nm using a UV-Visible spectrophotometer. Cell density was obtained from the formula: DCW (mg L^{-1}) = 314.5* OD_{550}. For analysis of NB concentrations, the sample was centrifuged and extracted with methyl-t-butyl ether. A 1 μL extract was then analyzed for NB using a capillary gas chromatograph with an ECD detector. The GC temperature program was as follows: the initial temperature was 85°C (2 min) and it increased at 10°C/min to 165°C (5 min). The detector and injector temperatures were 310°C and 280°C, respectively. The measured limit of detection for nitrobenzene in water is 0.1 mg/L [7-9].

Table 1: Chemical parameters of water and sediment from Krishna River

S.No.	Parameter	Water	Sediment
1	Temperature(°C)	15	N.A
2	pH	7.5-7.8	N.A
3	Dissolved Oxygen	8 ppm	N.A
4	Organic Carbon (%)	N.A	1.26
5	COD(mg/L)	25	N.A
6	TN	1.2	2.52
7	TP	0.23	1.02
8	Electrical Conductivity	194.5μS to 1030 μS	N.A.
9	Total hardness	42.4 ppm	N.A.

RESULTS

Bacteria growth in river water and sterilized culture media was investigated and compared to evaluate the potential of bacteria inoculation in field conditions. Temporal profiles of nitrobenzene concentration and bacterial growth are shown in Fig. 1.

As can be seen from Fig. 1, nitrobenzene degraded quickly both in river water and culture media. It took about 12 h and 20 h, respectively for the 20 mg/L NB to completely degrade in the two systems. The final cell density in both systems was all about 26 mg/L. Bacterial growth in river water followed the batch growth curve of a short lag phase, followed by an exponential biodegradation phase and ended in stationary phase. Only about 3% of the initial NB was removed in the control experiment and no increase in cell density was observed. The results showed that there was no difference of bacterial growth in river water compared with that in culture media. On one hand, this means the nutrient concentration in river water was not the growth-limiting factor for cell growth. On the other hand, the cells can also be inoculated directly in river water for large-scale remediation of NB-contaminated river water or sediment. This is very useful in practical remediation work whereas disinfection of contaminated environment is impossible. Figure 2 shows NB concentration profiles in sediment and Figure 3 shows NB concentration profiles in covered water under different remediation strategies.

The initial concentrations of NB in the sediment and water were 8 mg/kg and 56 mg/L. It took about 10 and 8 days for the NB to degrade in sediment and water with bacterial augmentation. NB degradation with

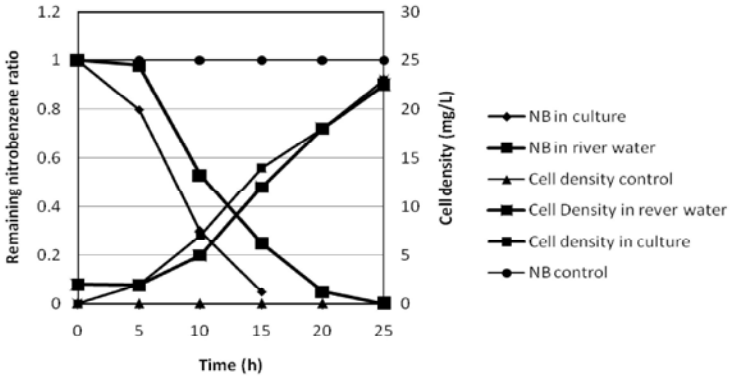

Fig. 1: Temporal profiles of bacterial growth and Nitrobenzene (NB) degradation in river water and culture media

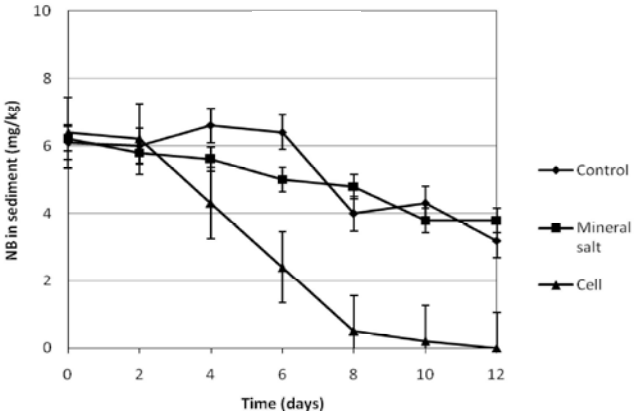

Fig. 2: Temporal profile of nitrobenzene in the sediment

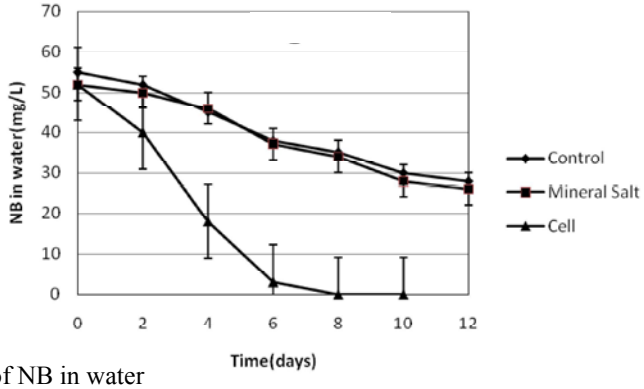

Fig. 3: Temporal profiles of NB in water

bioaugmentation was much faster than that in the other two systems. There was no distinct performance difference between natural attenuation and biostimulation. Until the end of the experiment (12 days), about 52% and 44% NB remained in the sediment and water without bacterial augmentation.

DISCUSSION

There is no discussion without references. Please insert some relevant references here The results affirmed that the added bacterial can grow quickly in the field condition, even at a low temperature of 10°C. As the external bacterial was initially isolated from the sediment and later inoculated in river water, the competition between external and indigenous cell could be alleviated to a minimum level, resulting in a quick remediation process. The bacterial abundance as estimated by MPN was highly variable, resulting in high standard deviations in each microcosm and at each time of sample collection. The initial culturable NB degrading microbial population were low (between 3 X 10^3 g dry soil^{-1} and 9 X 10^3 g

dry soil^{-1}). With the bacterial augmentation, there was an exponential increase in bacterial number for the first 4 days of bioremediation. The culturable bacterial population then remained relatively stable at 5 X 10^8 g dry soil^{-1}, with no statistical significant difference in the microbial numbers. Without bacterial addition, there was also a slow increase in bacterial numbers for the 12 days of remediation.

In conclusion, the persistence and effectiveness of the bacterial during bioremediation is anticipated. It can also be concluded that nutrients were not the limiting factors in the system since the sediment and water were taken from the polluted Krishna River. However, it is necessary to investigate the additional limiting factor with further study. These increases may be attributed largely to the growth of indigenous bacterial. Actually there were about seven different bacteria existing in the polluted sediment. Although the completion of NB degradation could finally be anticipated even without bacterial addition, bioaugmentation is still necessary for a rapid bioremediation procedure.

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