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Soil mesocosm studies on atrazine bioremediation

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ABSTRACT

Accumulation of pesticides in the environment causes serious issues of contamination and toxicity. Bioremediation is an ecologically sound method to manage soil pollution, but the bottleneck here, is the successful scale-up of lab-scale experiments to field applications. This study demonstrates pilot-scale bioremediation in tropical soil using atrazine as model pollutant. Mimicking field conditions, three different bioremediation strategies for atrazine degradation were explored. 100 kg soil mesocosms were set-up, with or without atrazine application history. Natural attenuation and enhanced bioremediation were tested, where augmentation with an atrazine degrading consortium demonstrated best pollutant removal. 90% atrazine degradation was observed in six days in soil previously exposed to atrazine, while soil without history of atrazine use, needed 15 days to remove the same amount of amended atrazine. The bacterial consortium comprised of 3 novel bacterial strains with different genetic atrazine degrading potential. The progress of bioremediation was monitored by measuring the levels of atrazine and its intermediate, cyanuric acid. Genes from the atrazine degradation pathway, namely, *atzA*, *atzB*, *atzD*, *trzN* and *trzD* were quantified in all mesocosms for 60 days. The highest abundance of all target genes was observed on the 6th day of treatment. *trzD* was observed in the bioaugmented mesocosms only. The bacterial community profile in all mesocosms was monitored by LH-PCR over a period of two months. Results indicate that the communities changed rapidly after inoculation, but there was no drastic change in microbial community profile after 1 month. Results indicated that efficient bioremediation of atrazine using a microbial consortium could be successfully up-scaled to pilot scale.

1. Introduction

Herbicides help increase crop yield, but their accumulation in soil and water resources results in environmental pollution and health hazards (Kanissery and Sims, 2011). Besides agricultural land, the most affected areas are the pesticide manufacturing and storage sites. The triazine herbicides are amongst the most widely used pesticides in agriculture, wherein, atrazine is a broad-leaf weed control herbicide, widely used in agriculture of sugarcane and corn. Atrazine (2-chloro, 4-ethylamino, 6-isopropylamino, 1,3,5-triazine), is reported to be an endocrine disrupter, affecting the endocrine system, the central nervous system and the immune system (Lasserre et al., 2009). It is also reported to cause sex reversal in male frogs (Hayes et al., 2003). Additionally, depending on the physicochemical properties of triazine herbicides, they can accumulate in sediments, severely affecting the organisms living in the benthic zone (Sánchez-Sánchez et al., 2013). Because of its wide application and slow rate of natural degradation, it is detected in soil, sediments and groundwater at concentrations well above the permitted limits. The European Union has banned atrazine, based on its persistent contamination of groundwater (Prosen and Zupancic-Kralj, 2005).

Removal of herbicides from soil is mostly dependent on the catabolic capacity of the soil microflora. Yet, bioremediation, a sustainable method for clean-up, has not yet been fully exploited. Despite decades of research on bioremediation, it continues to be an area of priority research, since pollution levels are still high in many locations and success stories taking lab-scale research to the field are few (Röling et al., 2004; Jørgensen et al., 2000). Especially field scale studies of bioaugmentation with efficient contaminant degraders are rare (Jorgensen, 2007). We have earlier shown that

Abbreviations: BS, Basal salt; CA, Cyanuric acid; LH-PCR, Length heterogeneity PCR; HEX, Hexachloro fluorescein; FAM, Fluorescein amidite.

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atrazine degradation in tropical agricultural soil can be enhanced significantly by biostimulation and by augmentation with known atrazine degrading bacterial strains (Sagarkar et al., 2013).

Designing a successful bioremediation program involves comparison of different treatment strategies for removal of the pollutant in a defined time scale. However, if degradation is not complete, it could lead to the accumulation of intermediates which may also be toxic. Hence, it is not only important to monitor the disappearance of the pollutant but also to monitor the intermediates of the degradation pathway. In the case of atrazine degradation, the most widely reported process is the dechlorination route, where atrazine is converted to hydroxyatrazine. This is initiated by the action of *atzA* gene (atrazine chlorohydrolase) or *trz*N gene (triazine hydrolase) (Krutz et al., 2010). Atrazine is degraded to cyanuric acid by *atzABC* genes in gram-negative bacteria (Iwasaki et al., 2007) and *trz*N-*atz*BC genes in gram-positive bacteria (Vibber et al., 2007). The intermediate cyanuric acid is further degraded by the *atz*D,E,F or *trz*D-*atz*E,F gene cluster to ammonia and carbon dioxide (Krutz et al., 2010).

This study attempts to bridge this gap by scaling up bioremediation studies from microcosm level (Sagarkar et al., 2013) to mesocosm levels using 100 kg soil mesocosms for bioremediation. Atrazine was used as model pollutant and natural and enhanced bioremediation was compared in treatments that mimicked history of pollutant use versus contaminated soil without history of herbicide use. We monitored the presence of atrazine and its intermediate, cyanuric acid in the mesocosms. The mesocosms were divided into two groups, each containing three treatment options: natural attenuation (capacity of inherent soil microflora) and two enhanced bioremediation strategies, biostimulation (enhancing concentration of nutrients in the soil) and bioaugmentation (introduction of additional gene pool using bacterial inoculum in the soil). The mesocosms were placed outdoors so they would be exposed to environmental conditions in the field. Bioaugmentation was done with a bacterial consortium isolated from contaminated agricultural soil. Analytical tools were used to measure pollutant levels while molecular tools were used to study microbial population dynamics and gene profile during the course of treatment.

2. Materials and methods

2.1. Chemicals

Analytical-grade atrazine (purity, 99%; water solubility at 20 °C, 33 mg L^{-1}), cyanuric acid (purity, 99%) were purchased from Sigma, USA. [^{14}C] ring labelled atrazine (specific activity 160 mCi/mmol; radiochemical purity, 99%) was purchased from Larodan Fine Chemicals Ab, Sweden.

2.2. Consortium designing and mineralization of [U-ring-^{14}C] atrazine

Isolation of bacteria was carried out from a soil sample collected from an agricultural field Yadamavala, Maharashtra, India, where sugarcane was cultivated and atrazine was used for the last three years. The site is described in Sagarkar et al. (2013). Based on preliminary growth data on BS media (Basal media) 0.9 g L^{-1} KH_2PO_4, 6.5 g L^{-1} Na_2HPO_4:$12H_2O$, 0.2 g L^{-1} $MgSO_4$:$7H_2O$ (Cai et al., 2003) containing atrazine, 40 isolates were selected for screening for atrazine degradation. Degradation was monitored by HPLC (data not shown) and five isolates were selected for consortium design. Mineralization of atrazine using ^{14}C ring-labelled atrazine was tested for all five isolates and based on growth rate and degradation profile (data not shown); a three-membered consortium was designed. The isolates were identified by sequence analysis of their 16S rDNA as *Arthrobacter* sp. AK_YN10 (Accession no. HE716859), *Pseudomonas* sp. AK_AAN5 (Accession no. HE716858), *Pseudomonas* sp. AK_CAN1 (Accession no. HE716855).

Ring-labelled ^{14}C – atrazine mineralization potential of the consortium was checked by radiorespirometric analysis. The method involved incubation of 10^6 cfu ml^{-1} g^{-1} of each bacterial strain with 2 kBq of ring-labelled ^{14}C-atrazine [specific activity 160 mCi/mmol; 99% radiochemical purity (Larodan Fine Chemicals Ab, Sweden)] in 10 mL liquid BS media in 100 mL infusion bottles. In order to maintain a higher atrazine concentration, 100 mg L^{-1} of pure non-labelled atrazine was also added to the bottles. Along with this, the mineralization potential of the consortium was also checked in 10 g soil. 2 kBq of ^{14}C ring-labelled and 100 mg kg^{-1} of pure non-labelled atrazine was added to this soil. All the bottles were sealed and incubated at 30 °C in the dark for up to 30 days at 100 rpm. The production of ^{14}C labelled CO_2 in the bottles was trapped in 1 mL of 1 M NaOH, which was analyzed in a Winspectral 1414 Liquid scintillation counter (Wallac Oy, Turku, Finland) using Ultima Gold high flash point LSC-scintillation cocktail (PerkinElmer, MA, USA). The counting efficiency of the instrument was determined at the start of the experiment by an external standard method and determined to be 91.5% ± 0.5%. Bottles were aerated after every two days with sterile air. The experiment was carried out in triplicate.

2.3. Soil sampling and characterization

Soil samples were obtained from NEERI campus garden premises, Nagpur, India. Soil was characterized as silty clay loam. 700 kg soil was collected from three sites in the garden; each site had three sub-sampling sites from which soil was mixed after removing stones and roots to represent a homogenous sample. This soil was then used for the mesocosm setup. Soil properties: 0.82% organic carbon, 0.81% total nitrogen, 0.005% total phosphate, 7.67 kg $hectare^{-1}$ available phosphates, pH-6.8.

2.4. Mesocosms assemblement

Mesocosms were setup using garden soil as described in Section 2.3. Soil was sieved through 2 mm sieve into plastic trays. In this way a very homogenous batch of soil was obtained. The sieved soil was separated into two sets of 300 kg each. One set was mixed with commercial grade atrazine (Dhanuka, India) at a concentration of 300 mg kg^{-1} of dry soil. From here, 100 kg soil aliquots were transferred into three wide mouth plastic tanks (700 mm diameter and 600 mm height) having 200 L water holding capacity (to give 100 L headspace to carry out aerobic biodegradation). These three mesocosms will be henceforth referred to as 'History' mesocosms. The other set of 300 kg soil was further divided into 100 kg aliquots and transferred into three plastic tanks as above. No atrazine was mixed here at this time point. These are the 'No-History' mesocosms. All six mesocosms were kept in the garden premises for a period of one month, under ambient environmental conditions (about 30 °C during the day and 20 °C during the night; post-monsoon season in central India). Moisture content of the soil was monitored every week and water content was maintained at 40% until the end of the experiment with sterile water.

After a period of one month, the bioremediation program was initiated by mixing commercial grade atrazine at a concentration of 300 mg kg^{-1} of dry soil in each of the six mesocosms. The two groups, i.e. 'History' and 'No-History' consisted of three mesocosms each, which were subjected to three different treatments as follows:

1. BA: Bioaugmentation, by addition of a consortium comprising of; *Arthrobacter* sp. AK_YN10, *Pseudomonas* sp. AK_AAN5 and