Bioresource Technology 100 (2009) 164–172

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Ex situ slurry phase bioremediation of chrysene contaminated soil with the function of metabolic function: Process evaluation by data enveloping analysis (DEA) and Taguchi design of experimental methodology (DOE)

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ARTICLE INFO

Article history:
Received 21 February 2008
Received in revised form 3 June 2008
Accepted 4 June 2008
Available online 25 July 2008

Keywords:
Polycyclic aromatic hydrocarbons (PAH)
Anoxic
Microenvironment
Optimization
Bioaugmentation

ABSTRACT

Bioremediation of chrysene in soil matrix was evaluated in soil slurry phase bioreactor in conjugation with metabolic functions (aerobic, anoxic and anaerobic), microenvironment (single and mixed) conditions and nature of mixed consortia (native/resident mixed microflora and bioaugmented inoculum). Twelve experiments were operated independently in agitated-batch reactor keeping all other operating conditions constant (substrate loading rate – 0.084 g chrysene/kg soil-day; soil loading rate – 10 kg soil/m^3-day (3:25 soil water ratio); operating temperature – 35 ± 2° C). Data envelopment analysis (DEA) procedure was employed to analyze the performance of experimental variations in terms of chrysene degradation and pH. The efficacy of anoxic metabolism over the corresponding aerobic and anaerobic metabolic functions was documented. Aerobic metabolic function showed effective degradation capability under mixed microenvironment after augmentation with anaerobic inoculum. Anaerobic metabolic function showed lowest degradation potential. Application of bioaugmentation showed positive influence on the chrysene degradation rate. Design of experimental methodology (DOE) by Taguchi approach was applied to evaluate the effect of four selected factors (native soil microflora, microenvironment, metabolic function and bioaugmentation) on the chrysene degradation process. The optimized factors derived from analysis depicted the requirement of native soil microflora under anoxic metabolic function using mixed microenvironment after augmenting with anaerobic inoculum for achieving effective chrysene degradation efficacy.

1. Introduction

Anthropogenic sources like gasoline and diesel combustion, oil spills, former gas plant facilities, etc., contribute to the generation of PAH (Kanaly and Harayama, 2000; Juhasz and Naidu, 2000; Meckenstock et al., 2004; Johnsen et al., 2005) and their ubiquitous distribution, environmental persistence and potentially deleterious effect on human health manifests its remediation. Polycyclic aromatic hydrocarbons (PAH) compose of fused aromatic rings representing a large and diverse group of organic molecules having a broad range of properties, differing in molecular weight, structural configuration, water solubility, number of aromatic rings, volatility and sorption coefficients (Kanaly and Harayama, 2000; Maliszewska-Kordybach and Smreczak, 2000; Harmsen, 2004; Venkata Mohan et al., 2006a). Biochemical persistence of PAH arises from dense clouds of p-electrons on the ring structures which make them resistant to nucleophilic attack and possess low aqueous solubility and high solid-water distribution ratios (Johnsen et al., 2005). The complex and diverse structural configurations of PAH, combined with their low-bioavailability, hydrophobic nature, strong sorption phenomena, and high persistence in soil make the design of effective bioremediation methodologies a challenge.

Bioremediation is gaining wider approval as a feasible and alternative technology for the remediation of PAH contaminated soils employing various technologies such as solid phase treatment, land treatment/farming, composting, bioreactors, phytoremediation, enzyme catalyzed bioremediation and combined/integrated methods (chemical pre-treatment followed by bioremediation), in addition to the application of strategies such as biostimulation, microbial adaptation, bioaugmentation, bacterial chemotaxis, etc., for enhancing bioremediation rates (Field et al., 1995; Antizar-Ladislao et al., 2007; Gaskin and Bentham, 2005; Rodrigo et al., 2005; Valentın et al., 2006; Mancera-Lopez et al., 2008; Vanessa et al., 2007; Prasanna et al., 2008; Venkata Mohan et al., 2006a, 2008a). Soil slurry phase bioreactors were also used to treat PAH contaminated soil which facilitate effective contact between contaminant and microflora resulting in significant enhancement in both initial rates and overall extent of mineralization in short time (Doick and Semple, 2003; Prasanna et al., 2008; Venkata Mohan

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doi:10.1016/j.biortech.2008.06.020

et al., 2006a). Soil slurry phase reactors for PAH remediation were operated in both aerobic (Kim et al., 2001; Seung et al., 2004; Lei et al., 2005; Prasanna et al., 2008; Venkata Mohan et al., 2008a) and anaerobic (Bosma et al., 1997; Kuwano and Shimizu, 2006) microenvironments.

Biological degradation is the primary dissipation mechanism for most of the organic pollutants in the soil environment, but the activity of degrading microorganisms is dependent upon many factors, including contaminant uptake and bioavailability, concentration, toxicity, mobility, access to other nutrients, and activated enzymes (Cerniglia, 1992). Multi-phasic nature of the bioremediation process, restricts mass transfer and non-availability of degrading soil microflora further compound the problem. Microbial ecology, system microbiology, process operation conditions (microenvironment and configuration) and the presence/absence of indigenous activity will inturn govern the overall bioremediation process efficiency (Venkata Mohan et al., 2006a). Some factors alter the rate of microbial uptake and metabolism (the intrinsic activity of cell) and other factors change the rate of contaminant transport to the microorganisms (bioavailability) (Johnsen et al., 2005). PAH bioremediation in contaminated soil is considered to be a complex phenomenon due to the toxic and hydrophobic nature of the contaminants, soil composition, heterogeneity of microbial environment, the multi-phase nature of the bioremediation process and the environmental factors governing the process (Venkata Mohan et al., 2006a). Therefore, the aim of the present work is to study chrysene bioremediation process in contaminated soil using slurry phase bioreactor with respect to microenvironment, metabolic function and application of bioaugmentation. Further the experimental data was evaluated by adopting two mathematical approaches viz., data envelopment analysis (DEA) and Taguchi design of experimental (DOE) methodology. DEA is a frontier analysis technique used to measure the performance by evaluating the relative efficiency adopting graphical approach (Charnes et al.,1994; Ram Mohan, 2005; Venkata Mohan et al., 2008b,c). This model offers an easy approach to analyze results obtained by interpreting different ratios based on relative efficiency. DEA was employed to analyze the relative performance of experimental variations in terms substrate (chrysene) degradation rate (SDR) and output pH. Design of experimental (DOE) methodology by Taguchi orthogonal array (OA) a factorial based approach involves establishment of large number of experimental situations described as orthogonal array (OA) to reduce errors and to enhance the efficiency and reproducibility of the laboratory experiments (Mitra, 1998; Venkata Mohan et al., 2005a, 2007, 2008b,c). DOE methodology was adopted in this study to evaluate the process of chrysene degradation in soil slurry phase reactor and to study the relative role of selected factors with the final aim of optimizing the process.

2. Methods

2.1. Soil

Soil used for horticulture activity at our institute was used for the bioremediation experiments. The selected soil belongs to silt-loam category as per US department of agriculture triangular soil classification chart (23% of clay, 24% of sand, 1.1% of organic fraction and 51.9% of silt). The soil on the whole had 76% of finer particles (altogether silt, clay and organic fraction). Prior to experimentation, the soil after passing through a 2 mm sieve to remove debris was partially air-dried (in fume hood for 24 h) and stored at 2 °C to maintain its in situ biological activity prior to use. The soil was having bulk density of 1.54 g/cm^3 and distribution coefficient (k_{SD}) was found to be 25.451×10^{-7} m^3/g. The air-dried soil was spiked with known concentration of chrysene (>98% purity Sigma) at concentration of 1 g chrysene/kg soil dissolved in dichloromethane. Chrysene impregnated soil after evaporation (12 h; room temperature) was used for slurry preparation at soil loading rate of 10 kg soil/m^3-day (3:25 soil water ratio).

2.2. Experimental design

Twelve experimental variations as described in Table 1 were studied to understand the role of reactor microenvironment and metabolic functions on slurry phase degradation of chrysene. Soil slurry bioremediation experiments were performed in 350 ml reactors (perspex) with provision to create required microenvironment during operation (length, 20 cm; depth, 20 cm; diameter, 30 cm). Aerobic [A] experiments (Reactors A, B, G and H) were performed by exposing the reactors to atmospheric environment followed by agitation on the temperature controlled orbital shaker (100 rpm; 35 ± 2 °C). Agitation ensured continuous suspension of soil matrix and provided dissolved oxygen (DO) at 2.5 ± 0.2 mg/l. Anaerobic [An] systems (Reactors C, D, I and J) were operated by closing the reactor with rubber septum after sparging with oxygen free nitrogen gas for 5 min to maintain anaerobic microenvironment. Subsequently, reactors were placed on agitator (100 rpm; 35 ± 2 °C) for proper suspension and mixing of the soil matrix. In the case of anoxic [Ax] experiments (Reactors E, F, K and L), the reactors without sparing N_2 gas were kept for agitation (100 rpm; 35 ± 2 °C) after closing with rubber septum. Control (killed) [C] experiments were operated separately for each of the metabolic function studied [Reactor A (aerobic), Reactor C (anaerobic), Reactor E (anoxic)] by using sterilized soil (20 min, 120 °C) impregnates with chrysene. Control reactors help to understand the volatile behavior of the chrysene (if any) during the reactor operation.

Table 1
Details of experimental variations studied

S.no.	Reactor	Experimental conditions adopted	Microenvironment	Metabolic function
1	A	Killed control-sterile [A_c]	Aerobic	Aerobic
2	B	Native soil microflora [A]	Aerobic	Aerobic
3	C	Control-sterile (killed) [An_c]	Anaerobic	Anaerobic
4	D	Native soil microflora [An]	Anaerobic	Anaerobic
5	E	Killed control-sterile [Ax_c]	Anoxic	Anoxic
6	F	Native soil microflora [Ax]	Anoxic	Anoxic
7	G	Soil microflora augmented with domestic sewage [A D_S]	Mixed [M]	Aerobic
8	H	Soil microflora augmented with anaerobic sludge [A Ai]	Mixed [M]	Aerobic
9	I	Soil microflora augmented with domestic sewage [An D_S]	Mixed [M]	Anaerobic
10	J	Soil microflora augmented with anaerobic sludge [An Ai]	Mixed [M]	Anaerobic
11	K	Soil microflora augmented with domestic sewage [Ax D_S]	Mixed [M]	Anoxic
12	L	Soil microflora augmented with anaerobic sludge [Ax Ai]	Mixed [M]	Anoxic

A – aerobic metabolic function; Ax – anoxic metabolic function; An – anaerobic metabolic function; M – mixed microenvironment; Ds – domestic sewage (bioaugmentation); Ai – anaerobic mixed inoculum (bioaugmentation); Ac – control aerobic metabolic function; Axc – control anoxic metabolic function; Anc – control anaerobic metabolic function.