International Biodeterioration & Biodegradation 95 (2014) 338–345

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Comparison of bioremediation strategies for soil impacted with petrochemical oily sludge

Vanessa S. Cerqueira [a, *], Maria do Carmo R. Peralba [b], Flávio A.O. Camargo [c], Fátima M. Bento [a, *]

[a] Department of Microbiology, Federal University of Rio Grande do Sul, Sarmento Leite, 500, CEP 90050-170, Porto Alegre, RS, Brazil
[b] Department of Inorganic Chemistry, Federal University of Rio Grande do Sul, Bento Gonçalves, 9500, CEP 91500-970, Porto Alegre, RS, Brazil
[c] Department of Soils, Federal University of Rio Grande do Sul, Bento Gonçalves, 7712, CEP 91540-000, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form
7 June 2014
Accepted 24 August 2014
Available online 3 October 2014

Keywords:
Bioaugmentation
Biostimulation
Bioremediation
Oily sludge
Soil landfarming

ABSTRACT

Different bioremediation techniques (natural attenuation, biostimulation and bioaugmentation) in contaminated soils with two oily sludge concentrations (1.5% and 6.0%) in open and closed microcosms systems were assessed during 90 days. The results showed that the highest biodegradation rates were obtained in contaminated soils with 6% in closed microcosms. Addition of microbial consortium and nutrients in different concentrations demonstrated higher biodegradation rate of total petroleum hydrocarbons (TPH) than those of the natural attenuation treatment. Soils treated in closed microcosms showed highest removal rate (84.1 ± 0.9%) when contaminated at 6% and bacterial consortium and nutrients in low amounts were added. In open microcosms, the soil contaminated at 6% using biostimulation with the highest amounts of nutrients (C:N:P of 100:10:1) presented the highest degradation rate (78.7 ± 1.3%). These results demonstrate that the application of microbial consortium and nutrients favored biodegradation of TPH present in oily sludge, indicating their potential applications for treatment of the soils impacted with this important hazardous waste.

1. Introduction

Petroleum processing generates considerable amounts of a residue named oily sludge. One of the main issues faced by refineries and petrochemical industries is related to the safe disposal of this residue, since its destination and/or inappropriate treatment can cause serious impact to the environment and potential risk to human health (Cameotra and Singh, 2008; Xu and Lu, 2010). Oil sludge has been classified by the United States Environmental Protection Agency (US EPA) as a hazardous organic complex (Ubani et al., 2013). Usually, the oily sludge contains water, sand, oils, grease, organic compounds, chemical elements, and metals. Among the organic compounds present, the most common are alkenes, cyclic alkenes, benzene, toluene, ethyl-benzene, xylenes and polycyclic aromatic hydrocarbons (PAHs) and phenols (Kriipsalu et al., 2008). Polycyclic aromatic hydrocarbons (PAHs) consist in a serious environmental concern, as many of them are cytotoxic, mutagenic and potentially carcinogenic (Ubani et al., 2013).

Bioremediation of hydrocarbon-contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contaminants, has been established as an efficient, economic, versatile and environmentally safe treatment (Singh and Lin, 2008). In petroleum processing plants, when treating residues, mainly oily sludge, the landfarming process is a frequently chosen method, due to its operational simplicity, low cost (when compared to other techniques), high potential for contaminant removal and high rate of residue application, varying from 83 to 252 m^3/ha/year (Marín et al., 2005; Jacques et al., 2007). However, the method still shows several limitations especially to what regards to appropriate management and control, large areas requirements, the environmental unfavorable conditions of microbial growth and inefficiency in the reduction of inorganic contaminants level (Khan et al., 2004).

Bioremediation process efficiency of contaminated areas depends on several factors; the most important ones are the presence of microorganisms with appropriate catabolic capacity on the contaminated site, favorable environmental and nutritional

* Corresponding authors. Tel.: +55 51 33084497; fax: +55 51 3308 3665.
E-mail addresses: vanescerqueira@yahoo.com.br (V.S. Cerqueira), fatimabento@yahoo.com.br (F.M. Bento).

http://dx.doi.org/10.1016/j.ibiod.2014.08.015
0964-8305/

conditions to microbial growth and metabolism, contaminant composition and concentration and the pollutant bioavailability to microbial attack.

Two techniques have been proposed to increase the efficiency of bioremediation process: biostimulation and/or bioaugmentation. Biostimulation process consists in the introduction of nutrients, mainly nitrogen and phosphorus under the form of organic and/or inorganic fertilizers, in the contaminated system to stimulate the present microbial population growth and activity. The bioaugmentation process consists in the introduction of microorganisms, previously characterized as potential degraders to target contaminant, in the contaminated environment aiming an increase in the microbial population and, as a consequence, an increase in the biodegradation rate (Das and Chandran, 2011; Tyagi et al., 2011; Taccari et al., 2012).

The objective of the present work was to evaluate bioremediation potential in contaminated soils with different oily sludge concentrations in open and closed microcosms using biostimulation and bioaugmentation techniques.

2. Material and methods

2.1. Sampling

Dystrophic Red Argisoil soil samples (depth of 0–30 cm) were collected randomly in 5 points in landfarming cells, which have received petrochemical oily sludge for three decades. The sampling was made at the end of the treatment period and before applying more oily sludge. The samples were sent to the laboratory, homogenized and the soils sieved to 2 mm. Representative samples of soil and oily sludge were submitted to physical chemical characterization (Table 1).

2.2. Bioremediation experiments

For soil bioremediation, two contamination levels (1.5% and 6%) of oily sludge were tested in open and closed microcosms. The soil contamination was executed in the laboratory at the experiment start. For each different combination of contamination level and microcosm condition, the following treatments were carried out in triplicate:

1) Natural attenuation (NA): soil + oily sludge
2) Biostimulation BS(N+P+): soil + oily sludge + nutrients with high concentration
3) Bioaugmentation BA(N+P+): soil + oily sludge + nutrients with high concentration + bacterial consortium

Table 1
Physical chemical analyses of Landfarming soil and oily sludge.

Parameters	Landfarming soil	Oily sludge	Methodology
Humidity	15%	ND	Gravimetry/105 °C
pH	6.4	ND	pH in water 1:1
SMP index	6.8	ND	–
Oil and greases	1.75%	48%	Soxhlet extraction
Organic carbon	9.5%	88%	Humid combustion/Walkey Black
Nitrogen	0.14%	0.06%	Kjeldahl
Total Phosphorus	0.08%	0.01%	Humid digestion nitric-perchloric/ICP; OES
Total Potassium	0.08%	<0.01%	Humid digestion nitric-perchloric/ICP; OES

ND: not determined.

4) Biostimulation BS(N–P–): soil + oily sludge + nutrients with low concentration
5) Bioaugmentation BA(N–P–): soil + oily sludge + nutrients with low concentration + bacterial consortium

2.3. Bacterial consortium

On the bioaugmentation experiments a bacterial consortium of 5 bacteria with biodegradation potential for oily sludge and production of biosurfactants isolated directly from the oily sludge (*Stenotrophomonas acidaminiphila*, *Bacillus megaterium and Bacillus cibi*) and soil (*Pseudomonas aeruginosa* and *Bacillus cereus*) was used. The isolates were identified based on gene partial sequencing 16S rRNA (Cerqueira et al., 2011).

The bacterial inoculum was prepared through the transference with a platinum wire from the isolate (stock culture) to flasks containing the sterile nutritive broth, which were kept in an orbital shaker at 100 rpm, 30 °C for 24 h. Thereafter the cells were centrifuged at 4 °C, 9000 rpm during 15 min, washed with sterile saline solution (0.85%) and the processes was repeated 3 times. The cells were suspended in sterile saline solution (0.85%). The inoculum of each isolate was standardized and the consortium added to the soils of each experimental unit in order to inoculate 10^8 cells/g of soil.

2.4. Experimental design

Experiments were carried out during 90 days in 1.0 L microscoms (glass flasks). In experiments with closed microcosm the flasks were kept tightly closed and in open microcosm experiments the flasks were kept without lids. Experiments in open microcosms were performed to represent field conditions, where the humidity adjustment was more frequent. The amount of soil in each flask was calculated in dry basis (200 g). The soils were contaminated with 1.5% and 6% w/w of petrochemical oily sludge.

In the biostimulation and bioaugmentation experiments, nitrogen and phosphorus were added using solutions of $(NH_4)_2SO_4$ and KH_2PO_4 respectively. The C:N:P ratio was adjusted to 100:10:1 and 100:0.4:0.2 for experiments that corresponds to high and low amounts of nutrients. Control experiments were carried out with soil without applying contaminants and treatments.

Soil humidity was periodically adjusted by adding sterile distilled water, after the weighing of flasks, in order to keep the field capacity in 70%. The field capacity was determined according Silva et al. (2007). The soils were periodically revolved in order to increase the aeration rate. The flasks were maintained indoor and exposed to temperature variations to represent field conditions. Room temperature was monitored as a function of time.

2.5. Soil analyses

Soils were sampled at 0 and 90 days for quantification of total petroleum hydrocarbons (TPH) and after 7, 14, 30, 60 and 90 days for microbiological analyses.

2.5.1. Counting of hydrocarbon degrading microorganisms and total heterotrophics

The counting of microorganisms in soils was performed in each of the triplicate samples for every treatment at the corresponding sampling time by the Most Probable Number Method (MPN) (Braddock and Catterall, 1999). Initially, the soil sample was submitted to decimal dilution with a saline solution (0.85%) in test tube and homogenized in a vortex for 30 s. Dilutions in 24-well microtitre plates were then made.