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An effective soil slurry bioremediation protocol for the treatment of Libyan soil contaminated with crude oil tank bottom sludge

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ABSTRACT

Petroleum hydrocarbons are the most widespread contaminants in the environment. Soil contamination with crude oil tank bottom sludge (COTBS) represents a significant risk to both human and environmental health. Current traditional approaches only partially resolve the issue as they are inefficient, expensive, not environmentally friendly and leave behind unwanted products; hence, there is an urgent need to develop an environmentally friendly and cost effective solution to address this issue, especially in developing countries. The aim of this study was to develop a slurry phase bioremediation protocol for the treatment of Libyan soil contaminated with crude oil tank bottom sludge (COTBS) at a minimum soil:-water ratio using indigenous bacterial isolates. Two hydrocarbonoclastic bacterial isolates, *Pseudomonas* spp. (4M12) and *Pseudomonas xanthomarina* (4M14), were used in three different strategies, namely:-bioaugmentation (BA), biostimulation (BS) and biostimulation-bioaugmentation (BS-BA) to assess their ability to reduce the total petroleum hydrocarbon concentration (TPH) in COBTS contaminated Libyan soil. The results indicated that a substantial reduction in TPH was observed in all three treatments, with the BS-BA treatments showing the highest reduction (96–97%, from 30,703 to 860 and 1020 mg kg^{-1}), followed by the BS treatment (92–93% reduction). In contrast, control microcosms showed only a 17.15% reduction (30,667 mg kg^{-1}) in TPH concentration. The results from CO_2 respiration, community fingerprinting (DGGE) and metabolic profiling (Biolog assay) confirmed increased activity in the BS/BA treatment. For example, BS-BA treatments produced the highest levels of CO_2 after 90 d incubation (0.075 mg day^{-1} g $soil^{-1}$ and 0.072 mg day^{-1} g $soil^{-1}$) respectively, some 244–258% higher than the control (0.020 mg day^{-1} g $soil^{-1}$). This study confirms the potential benefit of the BS-BA approach and the ability of *Pseudomonas* spp. isolates to significantly reduce the TPH concentration in soil contaminated with COTBS. This approach can be utilized at the commercial scale, as it has lower water requirements than traditional slurry treatments.

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1. Introduction

In the first quarter of 2015, the total world production of petroleum crude oil was 95.24 × 10^6 barrels per day while the total world consumption was 93.30 × 10^6 barrels per day (EIA, 2015), with an expected 38% increase in the consumption of oil and other liquid fuels expected between 2010 and 2040. Fuels obtained from refined petroleum crude oil supply more than 50% of the world's energy and heating services (Speight, 2014). In modern society petroleum crude oil is one of the most important materials, used as a source of raw materials for many industries (Speight, 2014). However, serious environmental problems are associated with the oil industry worldwide. The oil industry is a potential source of air, soil and water pollution and contamination (Costa et al., 2012). In addition to the accidental release of different hydrocarbon contaminants into the environment, significant quantities of oily sludges are generated from oil industry facilities. Of these, the largest source of sludge is crude oil storage tanks where vast amounts of oily crude oil tank bottom sludge (COTBS) are generated

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every year resulting in enormous pollution, causing a threat when released into the environment (Liu et al., 2010). Improper handling or disposal of untreated COTBS represents a serious human and environmental disaster (Hu et al., 2013). Remediation of soil contaminated with COTBS is, therefore, essential (Juan et al., 2008). Soil contaminated with COTBS is usually treated using one of three methods: (1) physical, (2) chemical, and (3) bioremediation treatments. In comparison to bioremediation, many of the physical and chemical technologies are either costly, do not remove contaminants completely or are not environmentally accepted (Liu et al., 2010). Moreover, unwanted chemical materials are generated or released during or after the physical and chemical treatment technologies. Bioremediation, the utilization of the hydrocarbon contaminants by microorganisms, converting the pollutants to harmless products (mineralization) can be considered as an effective and cost effective approach. However, effective bioremediation requires significant optimization of key environmental parameters to permit the growth and activity of microorganisms leading to faster degradation rates (Sharma, 2012). Failure of commercial bioremediation projects in the past has largely been due to poor preparation and pre-optimization, resulting in inappropriate selection of micro-organisms for the pollutant or environmental conditions (Tyagi et al., 2011). While inoculation of soil with hydrocarbon degrading microorganisms (bioaugmentation) has resulted in increased rates of bioremediation in some cases (Ma et al., 2015), the use of indigenous bacterial isolates has proved to be best, resulting in significant reductions in hydrocarbon concentration in contaminated soil (Silva et al., 2009). Karamalidis et al. (2010) conducted a study on the bioremediation of soil contaminated with hydrocarbon materials. The authors reported that the selected indigenous isolates were able to reduce 94% of the hydrocarbons present after 191 days. However, poor selection of microorganisms may significantly reduce the effectiveness of the approach. Teng et al. (2010) tested the biodegradation potential of *Paracoccus* strain HPD-2 on an aged hydrocarbon contaminated soil. After 28 days, the inoculated strain showed only a 23.2% decrease in soil total hydrocarbon concentrations (Teng et al., 2010). Other studies have also reported the ineffectiveness of a bioaugmentation strategy (Abdulsalam et al., 2011).

Optimizing key physical and chemical parameters such as temperature, aeration and supplying the required nitrogen and phosphorus (biostimulation) in some cases accelerates the bioremediation mechanism by enhancing the growth of the indigenous hydrocarbon degraders (Milić et al., 2009). Recent research in China studied the effect of biostimulation on the bioremediation of soil contaminated with hydrocarbons and found that after 30 days the removal of total petroleum hydrocarbon (TPH) reached 88.3% (Dong et al., 2013). In this case, the limiting factors preventing the bioremediation of the contaminant by the indigenous microbial population was a lack of nitrates (NO_3^-), phosphates (PO_4^{3-}), and iron (Fe) (Atlas and Hazen, 2011). However, biostimulation has also been reported to be an ineffective treatment. Bento et al. (2005) studied the effect of biostimulation on the degradation rate of Hong Kong soil contaminated with diesel. The authors reported only a 35.5% reduction of diesel concentration in the soil after 12 weeks. Another study on the effects of biostimulation on the degradation rate was conducted by Couto et al. (2010) on a soil contaminated with petroleum hydrocarbon obtained from an oil refinery in Portugal. After 9 months, the results indicated that TPH reduction reached only between 10 and 35% in soil at a depth of 5–10 cm, while no TPH reduction was observed in contaminated soil obtained from depths below 20 cm (Couto et al., 2010).

The success of bioremediation of soil contaminated with hydrocarbons depends on the selection of the appropriate hydrocarbon degrading microorganisms and supplying the degraders with the required nutrients (bioaugmentation/biostimulation). For this reason, a number of researchers have conducted bioremediation tests on soils contaminated with petroleum hydrocarbon materials using a combined bioaugmentation/biostimulation strategy. For example, Xu (2010) carried out a bioremediation study on a soil contaminated with petroleum crude oil using BS/BA as a remediation option. After 12 weeks, the results indicated that 61% TPH reduction was achieved (Xu and Lu, 2010). In another study carried out on soil contaminated with hydrocarbon where BS/BA, BS and BA strategies were chosen, after 5 weeks, BS/BA showed the highest percentage biodegradation (87.3%) compared to the other techniques (BS and BA). Moreover, highest bacterial growth was also observed with the BS/BA treatment (Agarry et al., 2010).

Many studies have also used slurry phase bioremediation of soil contaminated with hydrocarbons (Machín-Ramírez et al., 2008; Kriipsalu et al., 2007; De-qing et al., 2007). Soil slurry bioreactors offer maximum control and have shown a significant enhancement to both the initial rates and overall extent of hydrocarbon mineralization. Soil slurry phase bioreactors ensure an effective contact between contaminant and hydrocarbon degrading microorganisms obtaining a significant enhancement of hydrocarbon degradation in a shorter time (Mohan et al., 2009). Devi et al. (2011) conducted a bioremediation study using different techniques and found that by using BS/BA with indigenous hydrocarbonoclastic microorganisms in slurry phase bioreactors, the maximum TPH removal was observed by integrating biostimulation with bioaugmentation (BS/BA) (44.01%) (Devi et al., 2011). Another study carried out on long term hydrocarbon contaminated soil used a BS/BA slurry phase with indigenous hydrocarbonoclastic bacteria (Aburto-Medina et al., 2012) showed that after 42 days of incubation, 51.6% removal of TPH was observed.

Libya is one of the major oil producing countries in North Africa and generates considerable amounts of sludge every year. At Azzawiya Oil Refining Company (Azzawiya-Libya), over the last 6 years, about 18,000 T of untreated COTBS were collected. To date very few studies have been carried out on soils contaminated with COTBS and to the authors knowledge there have been no studies carried out on COTBS contaminated soils from the Middle East and North Africa. Early work investigated the potential of three bioaugmentation agents (*Pseudomonas* spp. (4M12), *Pseudomonas xanthomarina* (4M14) and *Arthrobacter nitroguajacolicus* (1B16A) to degrade the TPH within COTBS contaminated Libyan soil in a 5:1 water:soil ratio. Because Libya is among the most arid countries in the world, the purpose of the present study was to investigate and evaluate the effects of biostimulation/bioaugmentation, selecting the best two indigenous individual bacterial isolates in slurry phase bioreactors on the reduction of total petroleum hydrocarbon in Libyan soil contaminated with COTBS at lower water:soil ratios.

2. Materials and methods

2.1. Contaminated soil sampling

The COTBS contaminated soil samples used in this study were collected from Azzawiya Oil Refinery in Libya. Contaminated soil samples (~5 kg) were collected from the top 0–30 cm of historically stockpiled soil. Soils were homogenized, sieved to 2 mm mesh size in zip lock plastic bags, transported to RMIT University, Melbourne, Australia, coded and stored in a Quarantine facility. The sample was air-dried for 7 days at room temperature.

2.2. Preparing bacterial isolates for microcosms

Two bacterial isolates from these contaminated Libyan soils, previously isolated and identified (Mansur et al., 2014a) were

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