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Short communication

Large Scale Bioaugmentation of Municipal Waste Water Contaminated with Petroleum Hydrocarbons

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Abstract

The effect of addition of a microbial consortium (A) with 22 micro-organisms on the large scale (400 m^3) bioremediation of municipal waste water contaminated with petroleum hydrocarbons was evaluated. Our results showed that addition of Consortium A was effective in reducing the level of contamination of total petroleum hydrocarbons (TPH) from 1086 mg L^{-1} to 56 mg L^{-1} after just 21 days and to 18 mg L^{-1} in 104 days. Phytotoxicity assays using *Brassica rapa* and bacterial viable counts confirmed the efficacy of the bioaugmentation agent. Overall, these results represent a promising and cost effective approach to the remediation of contaminated wastewater.

INTRODUCTION

Industrial effluents from the petroleum related industry contain a mixture of chemicals such as phenolic compounds, polycyclic aromatic hydrocarbons, heterocyclic compounds and aliphatic hydrocarbons [1-3]. Most of these compounds are toxic and threaten both human and environmental health. Among the cleanup methods employed to remove the contaminants, bioremediation or the use of microbes to degrade petroleum hydrocarbons represents a promising technology.

Bioaugmentation is one bioremediation approach that, in this case involves the addition of hydrocarbon degrading microorganisms to the contaminated effluents. The rationale for using bioaugmentation relies on the introduction of organisms capable of degrading the contaminants thereby enhancing the remediation But despite its apparent simplicity there have been some failures and these have been well detailed and reviewed [4-8]. In contrast, reports of the successful application of a microbial consortia for bioremediation are limited [9], resulting in the conclusion that bioaugmentation is an unproven, inconsistent technology [6-8,10]. Despite the fact that pure microbial cultures have been shown to be effective in laboratory conditions, it is recognized that a mixed community of microbes would be needed for the complete mineralization of the various hydrocarbons found in wastewaters. In addition, it has been argued that real-life conditions cannot be perfectly mimicked in the laboratory and consequently a gap exists between reported success in the laboratory and the reported lack of success in field work [11-13]. What is required are specific scale-up experiments specifically designed to meet the needs of the industry, carried out *in situ* in order to truly access the potential of this technology [14].

Therefore the aim of this study was to evaluate the efficacy of bioaugmentation in a large scale project (up to 400 m^3) *in situ* using petroleum industry wastewaters and a microbial consortium with mixed species capable of degrading a wide range of hydrocarbons.

MATERIALS AND METHODS

Bioaugmentation agents

The hydrocarbon degraders, 22 bacterial isolates from different species (mostly *Bacilli* sp.) were used as the bioaugmentation treatment (Consortium A) (Table 1). The bacteria were previously isolated from a municipal wastewater treatment plant and their ability to degrade hydrocarbons was confirmed in a previous study [15].

Laboratory experiments

A preliminary set of experiments was conducted to assess the efficacy of Consortium A for the biodegradation of petroleum

Table 1: List of bacteria used in this study (Consortium A).

No	Gram	Bacteria	No	Gram reaction	Bacteria
1	+	*Bacillus lentus*	12	-	*Acinetobacterhaemo-lyticus*
2	-	*Pseudomonas aeru-ginosa*	13	+	*Bacillus cereus*
3	-	*Pseudomonas stutzeri*	14	+	*Bacillus sphaericus*
4	-	*Pseudomonas stutzeri*	15	+	*Bacillus cereus*
5	+	*Bacillus megate-rium*	16	+	*Bacillus megaterium*
6	-	*Pseudomonas stutzeri*	17	+	*Bacillus licheniformis*
7	+	*Arthrobacter*sp.	18	+	*Bacillus cereus*
8	+	*Bacillus pumilus*	19	-	*Acinetobacterbau-mannii*
9	+	*Bacillus cereus*	20	-	*Acinetobacterbau-mannii*
10	+	*Bacillus subtilis*	21	-	*Alcaligenesfaecalis* type II
11	+	*Bacillus subtilis*	22	+	*Brevibacillusbrevis*

hydrocarbons prior to field experiments. A sample (1.0 L) of high TPH (about 250,000 mg L_{-1}) wastewater (WW) was filtered using Whatman No. 2 filter paper and adjusted to pH 7.0; aliquots (50 mL) were placed into sterile glass tubes maintained at 37ºC without stirring or aeration. The bioaugmentation agent was prepared as previously described [15].

A single inoculation (3.0% v/v) of Consortium A, containing around 10^8 bacteria mL^{-1} was performed at time 0, with no further inoculation in subsequent weeks. A control (C) was included using a single 3.0% (v/v) addition of RO water at time 0 to monitor changes in phenol concentration due to evaporation. The experiment was conducted in duplicate over a period of 57 days. The mean TPH levels at the start were 246,542 mg L^{-1} for Consortium A, and 247,108 mg L^{-1} for Control C.

Field experiments

The preparation for translation and scale-up required the production of large volumes which requiring a modified protocol to produce Consortium A. Each pure culture was prepared using a loop of pure culture taken from a sub-culture plate of Nutrient Agar and inoculated into 50 mL of PB media that was incubated overnight at 37°C and shaken at 150 rpm. These were then used to inoculate 500 mL of production broth medium contained glucose (10 g), yeast extract (8 g) and NaCl (5 g) and incubated in a shaker incubator at 500 rpm at 37°C for 24 to 48 hours depending on which cultures were grown. These cultures were then stored in 4 L carboys at 4°C. Each carboy of pure culture was then placed in a pre-sterilized 20 L Braun™ fermenter which was stirred at 150 rpm and aerated for better mixing. A 500 mL of sterile phosphate buffer was added to the resulting mixture and allowed to mix for 5 minutes. This was then harvested to produce 4 L carboys of Consortium A which was then stored at 4°C until required.

In regards to the scale-up experiment, a biocluster bioreactor (750 litres) and a treatment tank were used. The treatment tank was a holding tank made of steel, concrete and fibreglass with a holding capacity of 400 m^3 and an aeration grid to provide mixing and aeration at minimal cost. The consortium was prepared as described above but for the pilot scale experiment at the wastewater treatment plant(WWTP)due to scale limitations the inoculum was approximately 0.03% (v/v), added once a week into the biocluster as seed for the treatment tank. After addition of Consortium A, wastewater was then pumped from the biocluster into the treatment tank. Samples were collected at two weekly intervals from the treatment tank for further analyses

Total petroleum hydrocarbon (TPH) analysis

A modified standard protocol US EPA method (8015a) based on gas chromatography (GC) analysis was used for TPH measurement in both laboratory and field experiments. The GC system used was an HP 5890 Series II coupled with Agilent GC Chemstation software. The GC was equipped with a Flame Ionisation Detector (FID) for the detection of solids and liquids with boiling points below 2,500°C with a limit of detection of 1 mg L^{-1}. The standards used were Alphagaz PIANO Calibration Standards from Supelco ®. Standard QC parameters were included throughout.

Phytotoxicity assay and enumeration of total viable bacteria: Phytotoxicity assays were conducted using *Brassica rapa* according to the methods of Khan, Waqas et al. (2015) [16], for each sampling time point (end day 104). The plate count technique was used to enumerate total viable bacteria using Plate Count Agar medium (Oxoid LTD, UK).

Data analysis

Data were analyzed using T test methods using IBM SPSS (Version 21). Standard error was shown where required.

RESULTS AND DISCUSSION

The TPH concentrations decreased to 0 from an initial 240,000 mg L^{-1} in just 43 days in the laboratory (Figure 1). TPH

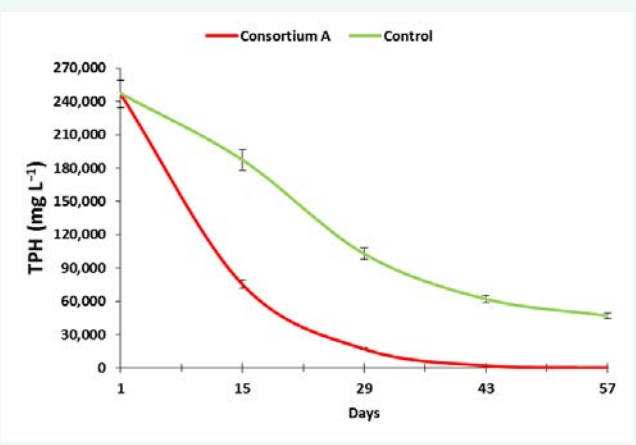

Figure 1 Laboratory experiments. Effect of bioaugmentation on the degradation of hydrocarbons using Consortium A in wastewater with high Total Petroleum Hidrocarbons (TPH)content (250,000 mg L^{-1}) compared to control over 57d at 30°C, n=2. No mixing or aeration, pH adjusted to pH 7 at time 0.

analysis was performed to establish the treatment with the highest efficacy to reduce the petroleum hydrocarbon content in the wastewater to below 1,000 mg L^{-1} without any agitation or aeration. A paired T-test confirmed a significant difference between the remaining TPH concentration in the control wastewater and that inoculated with Consortium A (P value = 0.03). Following successful laboratory trials, translational work was carried *in situ* in a field study. The addition of Consortium A into wastewater led to a significant and rapid reduction in the levels of TPH, from 1056 mg L_{-1} to 56 mg L^{-1} in only 21 days. Overall, the degradation of hydrocarbons decreased from 1086 to 18 mg L^{-1} in 104 days (Figure 2).

Plate count results showed that the number of total viable bacteria increased during the bioremediation process from 8.8 $\log_{10}$CFU/dry g soil at day 0 to 9.4 $\log_{10}$ CFU/dry g soil at day 21 (~ 4 fold increase) before reducing to 7.6 $\log_{10}$CFU/dry g soil at the end of experiment. These results confirm the efficacy of Consortium A in terms of both survival and contaminant degradation *in situ* at a large scale.

The historical prevalence of hydrocarbons in the environment has inevitably led to the acclimation and adaptation of microorganisms that appear to have substantial hydrocarbonoclastic activity [17]. This has been supported by the large numbers of organisms that have been reported to be involved in the bioremediation of crude [17-20], polycyclic aromatic hydrocarbons (PAHs) [21,22], diesel [11,23], diesel and fuel oil [24] and soil contaminated with weathered hydrocarbons [25], aircraft fuel [26], waste engine oil [27], waste sludge [28], weathered crude oil [29], oily sludge [30] and oil tank bottom sludge (OTBS) [31,32]. However unlike this study, many of the publications previously cited have included reports of the effectiveness of only pure cultures isolated and identified as part of the investigations with much of the work carried out at a laboratory scale. There is a need for reliable and predictable microbial consortium rather than individual organisms to handle the fluctuations commonly observed in wastewater with high concentrations of toxic components that can be effectively translated and scaled-up [33]. It has been argued that there was a gap between reported successes in the laboratory and the seemingly lack of reported success in field work [34]. This translation and scale-up closed this perceived gap in this study [14].

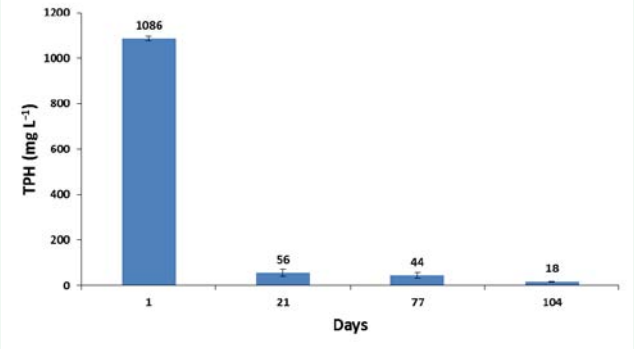

Figure 2 Effect of bioaugmentation on the degradation of total petroleum hydrocarbons (TPH) using Consortium A in field conditions.

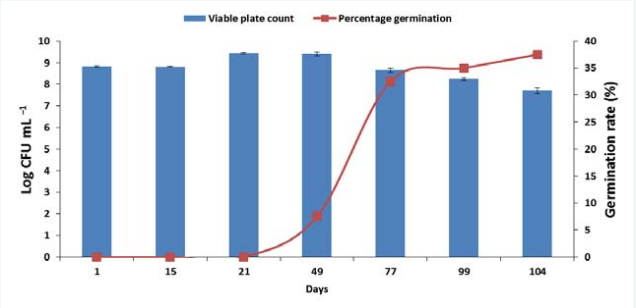

Figure 3 Viable plate count and germination rate of Brassica rapa during the field experiment run after 104 d.

The use of chemical analyses does not measure the overall toxicity of effluents; the use of biological toxicity testing is intended to compensate for limitations arising from traditional chemical analyses [35]. In the current study, the percentage of *B. rapa* germination increased from 1 to 39% in wastewater sampled at time 0 and after 104 days, indicating that the degradation of the hydrocarbons decreased the toxicity (Figure 3). Plants are a key component in the terrestrial ecosystem and are a useful monitoring tool for evaluating adverse environmental impact by pollutants that are not evident by chemical measurements alone [36,37]. Quantitative measurements based on seed germination and seedling growth have been used as the basis of plant bioassays to evaluate the biotoxicity of hydrocarbons in the environment [38-44].

CONCLUSION

In the present study, the *in situ*, large scale remediation of wastewater contaminated with petroleum hydrocarbons bioremediation was achieved, with a 98% reduction in the contaminant and a much reduced toxicity. In this instance bioaugmentation using a microbial consortium represents an appropriate technology for use in a full scale treatment facility. This work is among the first to demonstrate its application at a biological wastewater treatment plant.

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