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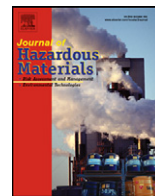
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## Prepared bed bioremediation of oily sludge in an oilfield in northern China

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### ABSTRACT

Field-scale bioremediation of oily sludge in prepared beds was studied at Shengli oilfield in northern China. The influence of manure, coarse sand, sawdust, a specialized microbial preparation and greenhouse conditions on the efficiency of removal of oil and grease was evaluated. After bioremediation for 230 d, oil and grease content fell by 32–42 g kg<sup>-1</sup> dry sludge in treated plots, indicating removal of 27–46% compared with only 15% in the control plot. Addition of manure, coarse sand, sawdust and greenhouse conditions significantly ( $p < 0.05$ ) increased the amount removed. Moreover, the physico-chemical properties of the sludge in all treated plots improved significantly after bioremediation. Microbial biomass in sludge and community-level physiological profiling examined using BIOLOG microplates was also studied. Total petroleum hydrocarbon degraders and polycyclic aromatic hydrocarbon degraders increased in all treated oily sludge. The activity of sludge microbial communities increased markedly in the treated plots compared with the control. Canonical correspondence analysis showed that differences in substrate utilization patterns were highly correlated ( $p < 0.05$ ) with sludge hydrolyzable N and oil and grease content. The biological toxicity of the oily sludge was lower following bioremediation in most of the treated plots as evaluated using *Photobacterium phosphoreum* T3.

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### 1. Introduction

Environmental contamination by petroleum and its derivatives is a serious problem worldwide. In addition to accidental contamination of the environment, vast amounts of oily sludge generated in water–oil separation systems in oil fields pose a problem as treatment/disposal methods are expensive [1]. Oily sludge contains hundreds of individual compounds and many of these components are potentially toxic, mutagenic and carcinogenic. They are therefore classified as priority environmental pollutants by the US Environmental Protection Agency [2] and their release into the environment is strictly controlled [1].

Oily sludge is currently treated using physical, chemical, and biological processes. Many of these technologies are either costly or do not result in complete decontamination. In contrast, biological treatments (bioremediation) appear to be among the most promising methods for dealing with a wide range of organic contaminants, particularly petroleum hydrocarbons. Biological techniques may also be environmentally friendlier by simulating natural processes [3]. A number of studies have been carried out on bioremediation of

petroleum-contaminated soils [4,5] but there have been few studies on bioremediation of oily sludge [6], especially at the field scale.

Shengli oilfield is the second largest oilfield in China and generates thousands of tonnes of oily sludge every year. In the present study the influence of manure, conditioner amendments (i.e. coarse sand and sawdust), a specialized microbial preparation and greenhouse conditions (covering the bed with plastic film) on oil and grease removal and the bacterial community structure of the sludge were evaluated during bioremediation in field plots.

### 2. Materials and methods

#### 2.1. Oil-degrading microbial preparation

A commercial oil-degrading microbial preparation ('Rhoder', Certificate number 77.99.04.515 D.004855.08.01, issued by the Russian Ministry of Health) was obtained from Moscow State University. The 'Rhoder' preparation consisted of two oil degrading strains of *Rhodococcus* spp. and represented a concentrated suspension of viable bacterial cells at a concentration of 10<sup>9</sup>–10<sup>10</sup> CFU g<sup>-1</sup>.

#### 2.2. Site selection and experimental design

A prepared bed 2400 m<sup>2</sup> in area (30 m × 80 m) was constructed at the Bingyi Joint Station of Shengli Oilfield, Shandong province,

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northern China (36°55'–38°10'N, 118°07'–119°10'E). The average annual rainfall in the region is 533 mm and the mean temperature is  $-4.1^{\circ}\text{C}$  in January and  $20.5^{\circ}\text{C}$  in May. The field study was conducted from October 2005 to May 2006.

Oily sludge was obtained from Bingyi Joint Station and selected physico-chemical properties were determined before the treatments were applied. The oily sludge had the following properties (dry weight basis): oil and grease content  $110\text{ g kg}^{-1}$ , pH 7.7, water-holding capacity (WHC) 5%, organic matter  $200\text{ g kg}^{-1}$ , hydrolyzable N  $41\text{ mg kg}^{-1}$ , available P  $2\text{ mg kg}^{-1}$  and available K  $278\text{ mg kg}^{-1}$ . Unamended original sludge (OS) from the Joint Station was used as control. OS was amended with about 5% (w/w) sawdust and 5% (w/w) manure separately then mixed to give pretreated sludge (PS) in the subsequent bioremediation treatments. The PS had the following properties (dry weight basis): pH 7.7, WHC 7%, organic matter  $205\text{ g kg}^{-1}$ , hydrolyzable N  $145\text{ mg kg}^{-1}$ , available P  $207\text{ mg kg}^{-1}$  and available K  $1591\text{ mg kg}^{-1}$ . The bed was divided into 5 plots. The treatments applied to the individual plots are described below. Plot RCPG: 1 kg of 'Rhoder' preparation per tonne and about 15% coarse sand were added to PS and a plastic greenhouse was built over the plot to increase the ambient temperature; Plot RCP: 1 kg of 'Rhoder' preparation per tonne and about 15% coarse sand were added to PS; Plot PS: PS without further amendment; Plot CP: 15% coarse sand was added to PS; Plot OS (control): OS without further amendment.

After the above treatments were applied the oil and grease concentrations in plots RCPG, RCP, PS, CP, and OS were 90, 90, 101, 90 and  $110\text{ g kg}^{-1}$ , respectively. Sludge with the various amendments was placed on a plot of the prepared bed to a depth of 30 cm and separated from adjacent plots by a 50 cm gap. The areas of plots RCPG, RCP, PS, CP, and OS were about 100, 1100, 1000, 100 and  $100\text{ m}^2$ , respectively. Plots RCPG, CP, and OS were subdivided into four equal sub-plots and plots RCP and PS into five sub-plots. The sludge in the plots was thoroughly tilled using a tractor fitted with a harrow with the exception of plot RCPG which was tilled by hand and all plots were watered at 3-day intervals to maintain adequate aeration and moisture levels during bioremediation.

### 2.3. Sampling

Sludge samples were taken at 0 (before bioremediation), 40, 100, 160 and 230 d from all sub-plots. Five samples were taken randomly from the prepared bed (0–30 cm depth) in each sub-plot and combined to give four composite samples for plots RCPG, PS, and CP and five composite samples for plots RCP and PS. The composite samples were stored at  $4^{\circ}\text{C}$  for a maximum of 7 d.

### 2.4. Analysis of sludge properties

Physico-chemical properties of the sludge samples from each sub-plot were characterized at the end of the experiment. Sludge was analyzed for pH, organic matter, WHC, hydrolyzable N and available P and K using standard methods [7]. The sludge was air dried and sieved through a 2 mm mesh before analysis.

### 2.5. Extraction of oil and grease from oily sludge

10-g sub samples of air-dried and pulverized sludge were mixed with an equal volume of anhydrous  $\text{Na}_2\text{SO}_4$ . The mixtures underwent Soxhlet extraction with dichloromethane (DCM) for 24 h. The extracts were concentrated to dryness with a rotary evaporator. The flasks were reweighed to determine the oil and grease contents.

### 2.6. Microbial counts and community level physiological profiling (CLPP) analysis

Microbiological analyses of the sludge samples from three sub-plots of each plot were carried out at 0, 40, 100, 160 and 230 d. The heterotrophic bacteria were enumerated on nutrient agar plates. Total petroleum hydrocarbon (TPH) degraders and PAH degraders were enumerated by the most-probable-number (MPN) technique [8]. The community level physiological profiling (CLPP) of the sludge microbial communities was examined using Gram-negative microplates (BIOLOG Inc.) according to the method of Garland and Mills [9]. For the BIOLOG data, average well color development (AWCD) and Shannon's diversity index were calculated according to Zak et al. [10].

### 2.7. Toxicity assay

DCM and dimethylsulfoxide (DMSO) were used to extract the sludge according to the method of Plaza et al. [11]. The biological toxicity of the DCM/DMSO extracts and DMSO (control) were tested based on the measurement of reduction in light emission by *Photobacterium phosphoreum* T3 under toxic stress. All bioassays were carried out in triplicate and light output was measured using a portable luminometer (Model DXY-2). The luminometer and *P. phosphoreum* T3 were obtained from the Institute of Soil Science, Chinese Academy of Sciences, Nanjing. Toxicity results are presented as the effective concentration promoting a 50% (EC50) reduction in light emitted by the bacteria. The full experimental procedure has been described previously by Bundy et al. [12].

### 2.8. Statistical analysis

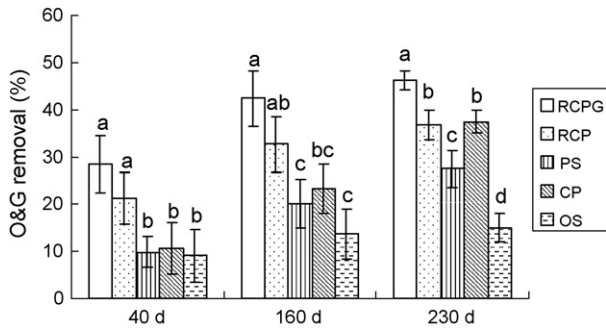
The data were analyzed for significant differences between treatments using two-way analysis of variance. The relationships between the BIOLOG results and measured environmental variables were analyzed by Canonical correspondence analysis (CCA) using the CANOCO software program (CANOCO 4.5, Microcomputer Power, Ithaca, USA). Actual absorbance values after correction of the absorbance from the control well in BIOLOG were used in the analysis. All negative absorbance values were set to zero and the 95 BIOLOG substrates were considered as individual species. The physico-chemical properties and oil and grease contents of sludge (Table 1) were tested as environmental variables for significant contribution to observed changes in substrate utilization patterns with the forward selection subroutine available in CANOCO. The statisti-

**Table 1**  
Oil and grease content and selected physico-chemical properties of the oily sludge after bioremediation for 230 days

Treatment	pH <sup>a</sup> (in water)	Water-holding capacity <sup>b</sup> (%)	Organic matter <sup>b</sup> ( $\text{g kg}^{-1}$ )	Hydrolyzable N <sup>b</sup> ( $\text{mg kg}^{-1}$ )	Available P <sup>b</sup> ( $\text{mg kg}^{-1}$ )	Available K <sup>b</sup> ( $\text{mg kg}^{-1}$ )	Oil and grease <sup>b</sup> ( $\text{g kg}^{-1}$ )
RCPG	7.7–7.8	71 ± 2	194 ± 13	134 ± 16	192 ± 32	1390 ± 380	49 ± 2
RCP	7.5–7.7	62 ± 5	185 ± 16	81 ± 11	100 ± 59	782 ± 307	57 ± 3
PS	7.5–7.6	49 ± 21	202 ± 15	80 ± 14	42 ± 40	712 ± 205	74 ± 4
CP	7.7–7.8	51 ± 21	174 ± 22	48 ± 5	100 ± 3	341 ± 34	57 ± 2
OS (control)	7.2–7.8	13 ± 3	200 ± 33	44 ± 2	0 ± 0	265 ± 5	94 ± 4

<sup>a</sup> The range of pH.

<sup>b</sup> Means and standard deviations.



**Fig. 1.** Oil and grease removal of RCPG, RCP, PS, CP, OS after 40, 160 and 230 d remediation. Values are means of quadruplicates for plots RCPG, CP, and OS and quintuplicates for plots RCP and PS. Error bars:  $\pm 1$ S.D. Different letters on the error bars indicate significant differences between treatments in oil and grease removal at  $p < 0.05$ .

cal validity of the association between environmental variables and variance in BIOLOG substrate data was tested by the Monte Carlo permutation test [13]. The Monte Carlo tests were based on 499 random permutations of the data.

### 3. Results

#### 3.1. Oily sludge properties

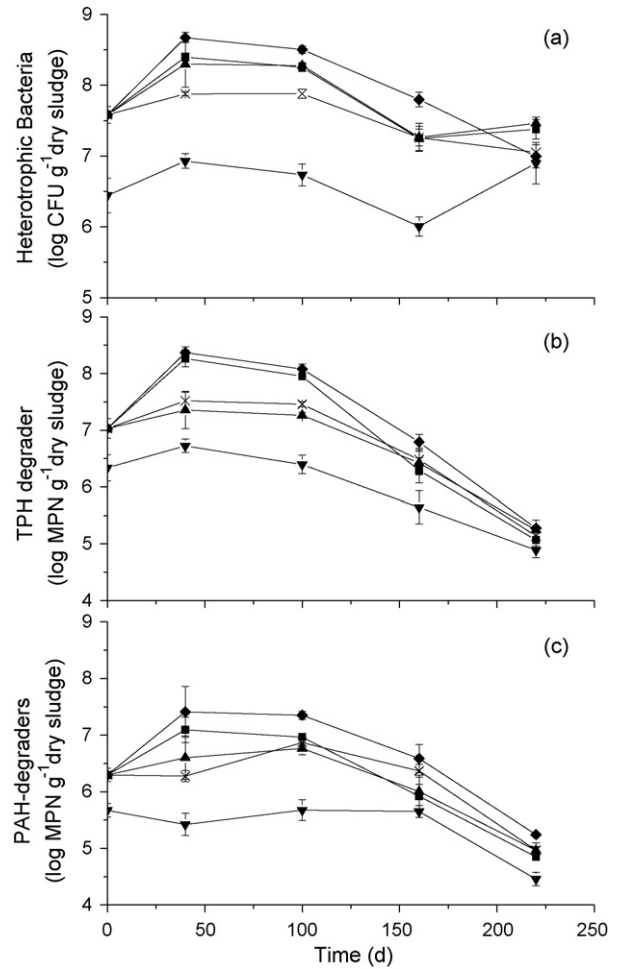
The physico-chemical properties of the sludge after bioremediation are presented in Table 1. There were no significant changes in organic matter or pH after bioremediation in any of the plots. However, WHC in all treated plots increased significantly during bioremediation and the highest WHC occurred in plot RCPG at the end of the experiment. As for other properties such as hydrolyzable N, available P and available K, manure-amended plots were significantly higher than the unamended control plot but there were no significant differences among plots RCPG, RCP, PS and CP.

#### 3.2. Removal of oil and grease from sludge

The efficiency of removal of oil and grease from oily sludge at 40, 160 and 230 d are shown in Fig. 1. Significant increases ( $p < 0.05$ ) in removal of oil and grease were obtained only in plots RCPG and RCP compared with the control at 40 d. However, the efficiency of removal in all treated plots was significantly higher than the control at the end of the experiment (230 d). The efficiency of removal of oil and grease was 46, 37, 28, 37 and 15% in plots RCPG, RCP, PS, CP and OS, respectively. Greenhouse conditions and addition of coarse sand and sawdust had significant effects but the 'Rhoder' preparation had no detectable effect.

#### 3.3. Microbial biomass and CLPP analysis

After addition of manure to the oily sludge the heterotrophic bacterial count was  $3.9 \times 10^7$  CFU  $g^{-1}$  dry sludge, which was significantly higher than the control count of  $2.4 \times 10^6$  CFU  $g^{-1}$  dry sludge ( $p < 0.05$ ). All treatments led to increases in heterotrophic bacterial counts in the sludge during first 40 d (ranging from  $7.5 \times 10^7$ – $4.8 \times 10^8$  CFU  $g^{-1}$ ) but bacterial counts in all treated plots gradually decreased from 40 d until the end of the remediation period. Although somewhat variable, the number of heterotrophic bacteria in the sludge in the control (OS) remained relatively constant throughout the experiment at about  $5 \times 10^6$  CFU  $g^{-1}$ . The heterotrophic bacterial counts in the treated plots were always higher than in the control plot throughout the experiment (Fig. 2a) and followed the sequence: RCPG > CP > RCP > PS > OS although the

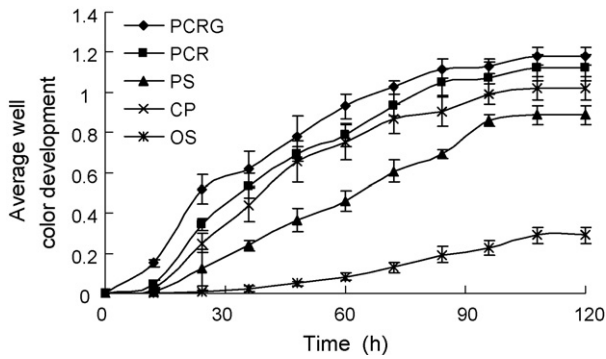


**Fig. 2.** Changes in counts of total heterotrophic bacteria (a), TPH degraders (b) and PAH degraders (c) in the sludge during bioremediation. Values are means of triplicates for all plots. Error bars:  $\pm 1$ S.D. Symbols: (□) RCPG; (■) RCP; (▲) PS; (×) CP; (▼) OS.

differences were not always statistically significant (Fig. 2a). The TPH degrader counts in the sludge in all four treatments increased by 99, 80, 12 and 14 times over the first 40 d experiment in plots RCPG, RCP, PS, and CP, respectively, but only about 2 times ( $5.28 \times 10^7$  CFU  $g^{-1}$ ) in the control. The TPH degrader bacterial counts in all four treatments subsequently decreased but remained above the control values (Fig. 2b). The bacterial counts in plot RCPG were the highest over the duration of the experiment (Fig. 2b) and were usually about two orders of magnitude higher than the control values. As is shown in Fig. 2c, the PAH degraders in the sludge showed similar trends to the TPH degraders.

AWCD and diversity index were analyzed using BIOLOG data to detect microbial differences among treatment. The activity of soil microbial communities evaluated by AWCD values were ranked as follows: RCPG > RCP > CP > PS > OS (Fig. 3). The mean Shannon indices were 4.38, 4.30, 4.28, 4.25 and 4.20 in plots RCPG, RCP, PS, CP and OS, respectively, which showed similar trends to the AWCD values. Analysis of the Shannon diversity indices showed significant increases ( $p < 0.05$ ) in all treatments compared with the control plot (OS) but no marked differences were found among the treatments.

To investigate relationships between microbial community composition and measured environmental variables, the BIOLOG results were also analyzed using CCA. The CCA of the combined data from the sludge of different plots separated all treatment plots clearly on the basis of physico-chemical properties and oil

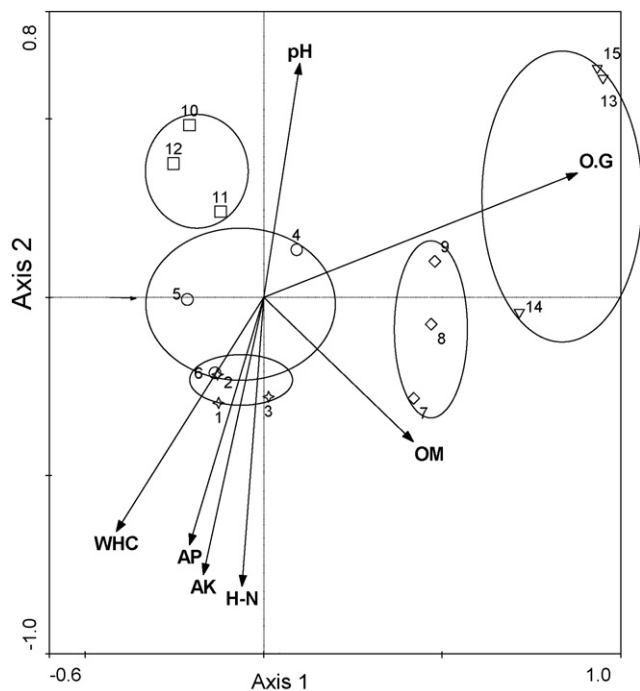


**Fig. 3.** Average well color development (AWCD) in Biolog plates for the microbial community of different treated oily sludge during incubation. Symbols: (◻) RCPG; (◼) RCP; (▲) PS; (×) CP; (\*) OS. Error bars:  $\pm 1$ S.D. ( $n=3$ ).

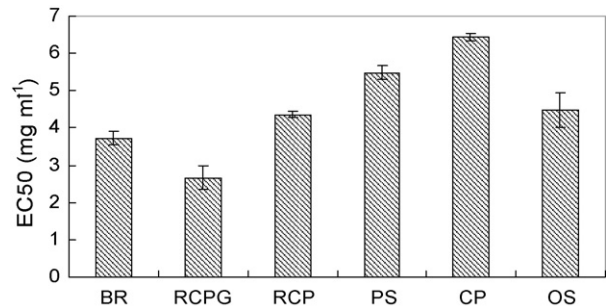
and grease contents of the oily sludge as environmental variables (Fig. 4). The influence of the environmental variables on the CCA biplot is indicated by arrows in which lengths are proportional to their importance. As shown in Fig. 4, oil and grease contents had the longest arrow, indicating that it was most important in influencing the microbial community in the oily sludge ( $p=0.01$ ). Among other sludge properties, only hydrolyzable N showed the significant effect in terms of explaining the variation substrate utilization data ( $p=0.04$ ) by means of the Monte Carlo permutation.

#### 3.4. Biological toxicity assay

The toxicities of the DCM/DMSO extracts of the sludge at the end of the experiment as evaluated with *P. phosphoreum* T3 are presented in Fig. 5. The EC<sub>50</sub> of the oily sludge from all plots except plot RCPG decreased after bioremediation compared with the sludge



**Fig. 4.** CCA ordination biplot of treatment plot scores and significant environmental variables of oily sludge under different treatments. O.G., OM, H-N, AP, and AK represent oil and grease, organic matter, hydrolyzable N, available P, and available K. Concerning the variance of data, the first axis explained 30.1% of the total variation, the first and the second axes explained 47.5%; Treatments: (◊) RCPG; (○) RCP; (◻) PS; (◻) CP; (◻) OS.



**Fig. 5.** Biological toxicity of the oily sludge under different treatments. BR denotes the sludge before bioremediation. Error bars:  $\pm 1$ S.D. ( $n=3$ ).

at the onset of the experiment and the control at the end of the experiment (Fig. 5). However, the toxicities of the extracts were not correlated with their oil and grease concentrations.

#### 4. Discussion

There was a distinct decline in the oil and grease content in all plots during bioremediation, ranging from 15% in the control (OS) to 46% in plot RCPG, and the efficiency of removal was more rapid during the first 40 d. A similar pattern with faster removal of hydrocarbons during the early stages of bioremediation has been reported in other studies [1,6,14]. One explanation may be that different components of the hydrocarbons had different degrees of degradability [15]. In fact, almost all of the C<sub>13</sub>–C<sub>30</sub> alkanes (measured by GC-FID) in the sludge were degraded in the RCPG plot and over 90% were degraded in plots RCP, PS, and CP at the end of the experiment (data not shown). Another explanation may be that parts of contaminants which are 'locked up' in particle pores may show poor bioavailability [16]. The changes in the microbial community in the sludge also may be associated with this phenomenon. As shown in Fig. 2, the TPH and PAH degrader counts decreased after the period of bioremediation. With the declining of oil and grease content the properties of the oily sludge after bioremediation also improved in all treated plots. A marked increase in the WHC of the sludge at the end of the study can be attributed to the substantial reduction of hydrophobic hydrocarbon compounds in the sludge due to degradation and the plot which has the highest removal efficiency has the largest WHC. As for the nutrient components of the sludge addition of manure to the treatment plots significantly increased ( $p < 0.01$ ) the concentration of nutrient elements in the sludge. And these nutrient components in the RCPG plots were highest at the end of the experiment which may result from the decreased leaching of the nutrients by rain in the greenhouse.

The oil and grease removal efficiency from oily sludge in different plots at 40 d followed the order: RCPG > RCP > CP > PS > OS. Addition of 'Rhoder' preparation was the only treatment to have a marked effect ( $p < 0.05$ ) at that time. However, at the end of the experiment, no significant effect of the preparation was observed. On the contrary, addition of manure, coarse sand, sawdust and greenhouse conditions showed significant effects on oil and grease removal ( $p < 0.05$ ). In this study, the initial indigenous population of TPH degraders was found to be about  $2.4 \times 10^6$  MPN  $g^{-1}$  in the control. It has been reported that when the population of indigenous microorganisms capable of degrading the target contaminant is less than  $10^5$  CFU  $g^{-1}$  of soil, bioremediation will not occur at a significant rate [17]. After addition of manure, coarse sand, sawdust and greenhouse conditions the indigenous population of TPH degraders increased quickly (Fig. 2b). In fact, the indigenous bacteria which are able to degrade oil hydrocarbons are ubiquitous and manure itself contains a diverse range of micro-organisms in addition to being a

nutrient source but the environmental conditions affect the growth of the bacteria in some oily sludge. Adding the preparation acted only as a 'booster' at the start of biodegradation but after 100 d the TPH degrader count was the same whether supplementary organisms had been added or not, thus addition of the preparation had no significant effect at the end of the bioremediation period. Previous study has also shown the similar results [18].

In the literature bioremediation techniques usually include fertilizer application in terms of the ratio of C (in the crude oil) to N and P. One optimum fertilizer ratio often quoted is 100:10:1 [19] for bioremediation of hydrocarbon-contaminated soils. However, it is rarely necessary to add the total amount of N and P called for by this theoretical ratio because nutrients are recycled during the course of treatment as microorganisms turnover, especially in the case of oily sludge which has high concentrations of C. It has been demonstrated that excess nitrogen can depress the rate of microbial activity and petroleum degradation in contaminated soils due to depression of osmotic soil water potential [20]. In this study, manure addition significantly increased the counts of TPH and PAH degraders in the sludge compared with the control (Fig. 2b and c). Temperature is an important factor controlling microbiological activity and the rate of organic matter decomposition. Microbial growth usually doubles for every 10 °C increase. There is also a decrease in adsorption with rising temperature, which makes more organics available for the microorganisms to degrade. It was observed that the temperatures under greenhouse conditions were about 10 °C higher than outdoors and this led to significantly higher removal of oil and grease (Fig. 1).

CLPP is a fast screening method to detect differences among treatments and AWCD is an important indicator reflecting sole C source utilization ability (Fig. 3). The activities of soil microbial communities in plots RCPG, RCP, PS, and CP increased markedly in AWCD compared with the control plot (OS). This indicates that the temperature and amendments (coarse sand, sawdust, and manure) had important influences on the microbial activity of oily sludge. There were also similar treatment effects on metabolic diversity in different plots. The Shannon index provides information on the distribution of C source utilization by microbial communities and potential metabolic diversity of the communities [21]. Thus, it can be concluded that not only did oil and grease decrease after about 230 d of bioremediation in the oily sludge but microbial activity and community diversity also increased. It must be emphasized that CLPP is not a culture-independent method and is somewhat biased towards fast-growing and easily cultivable species [22]. Thus, CLPP should not be seen as a stand-alone method, but it can be a useful adjunct to other approaches (e.g. classical and molecular) in the polyphasic analysis of microbial communities.

The strength of association between microbial community properties and specific environmental variables were also quantified with a statistical approach using CCA, a technique originally developed for evaluating environmental effects on plant community structure. As with AWCD and the Shannon index, all treatments on oil sludge had an influence on the microbial community. However, the relationships between microbial community (BILOG data) and sludge physico-chemical changes effected by the experimental treatments have not been previously evaluated simultaneously to separate out their relative importance. On the CCA biplot, the influence of environmental variables is indicated by arrows whose lengths are proportional to their importance. As shown in Fig. 4, oil and grease content with longest arrows are significant ( $p = 0.01$ ) in terms of explaining variation in substrate utilization data.

There are no universal TPH cleanup standards for soils contaminated with crude oils [23]. Some guidelines have been developed for oil content. Petroleum contains hundreds of individual compounds with varying toxicity, mutagenicity and carcinogenicity

and its composition varies according to the production area, the source strata, and even within different parts of the same geological strata. It is therefore impossible to evaluate the toxicity of soil pollutants based solely on the oil and grease content. Ecotoxicity bioassays should therefore be used as supplementary tools for monitoring treatment effects [11]. In our study *P. phosphoreum* T3 was used to test the biotoxicity of the oily sludge before and after bioremediation. The EC50 of the oily sludge from all plots except RCPG increased after bioremediation at the end of the experiment (Fig. 5). Contrary to expectations, the sludge in plot RCPG showed an increase in biological toxicity. One explanation may be that less toxic components of the hydrocarbons had been biodegraded, leaving the more toxic components intact. It has been reported that the initial degradation products of hydrocarbons, usually carboxylic acids [24], are more toxic than the parent hydrocarbons [25]. This may well be due to their increased solubility and hence availability. The toxicity of sludge in plot RCPG would be expected to decrease faster than that of other plots since carboxylic acids are more readily degradable than oil and grease. Previous microbial toxicity studies have also shown an increase in toxicity during the initial phase of bioremediation, followed by toxicity reduction to non-toxic levels [26–28].

## 5. Conclusions

In our study the indigenous TPH degraders, and hydrolyzable N and P were unable to stimulate degradation of oil and grease in the oily sludge. Amendment of the oily sludge with coarse sand, sawdust and manure and the construction of a greenhouse over the sludge bed led to a significant increase in the removal of oil and grease. Of these, the greenhouse (mainly temperature) effect was the most important. After 230 d of bioremediation, oil and grease in the four amended plots had removal efficiency ranging from 28 to 46% compared with only 15% in the unamended control. Marked increases in the WHC and microbial activity and diversity of the sludge were also observed after the experiment in the treated plots. Although there were marked decreases in oil and grease and distinct improvements in the physico-chemical properties and microbial activity of the sludge after bioremediation, the oil and grease content was still as high as 49 g kg<sup>-1</sup> in plot RCPG in which the highest removal occurred and further remediation was required even in this treatment.

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## References

- [1] B. Mryayan, M.N. Battikhi, Biodegradation of total organic carbons (TOC) in Jordanian petroleum sludge, *J. Hazard. Mater.* 120 (2005) 127–134.
- [2] USEPA, Test Method for Evaluating Solid Waste, SW-846, vol. 1A, third ed., USEPA, Washington, DC, 1986.
- [3] M.T. Balba, N. Al-Awadhi, R. Al-Daher, Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation, *J. Microbiol. Methods* 32 (1998) 155–164.
- [4] K.S. Jorgensen, J. Puustinen, A.M. Suortti, Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles, *Environ. Pollut.* 107 (2000) 245–254.
- [5] J. Sabate, M. Vinas, A.M. Solanas, Laboratory-scale bioremediation experiments on hydrocarbon-contaminated soils, *Int. Biodeter. Biodegr.* 54 (2004) 19–25.
- [6] S. Mishra, J. Jyot, R.C. Kuhad, B. Lal, Evaluation of inoculum addition to stimulate in situ bioremediation of oily sludge-contaminated soil, *Appl. Environ. Microb.* 67 (2001) 1675–1681.

- [7] R.K. Lu, Analytical Methods of Soil Agricultural Chemistry, China Agricultural Science and Technology Press, Beijing, China, 1999 (in Chinese).
- [8] B.A. Wrenn, A.D. Venosa, Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most-probable-number procedure, *Can. J. Microbiol.* 42 (1996) 252–258.
- [9] J.L. Garland, A.L. Mills, Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level, sole-carbon-source utilization, *Appl. Environ. Microb.* 57 (1991) 2351–2359.
- [10] J.C. Zak, M.R. Willig, D.L. Moorhead, H.G. Wildman, Functional diversity of microbial communities: a quantitative approach, *Soil Biol. Biochem.* 26 (1994) 1101–1108.
- [11] G. Płaza, G. Nałęcz-Jawecki, K. Ulfig, R.L. Brigmon, The application of bioassays as indicators of petroleum-contaminated soil remediation, *Chemosphere* 59 (2005) 289–296.
- [12] J.G. Bundy, G.I. Paton, C.D. Campbell, Combined microbial community level and single species biosensor responses to monitor recovery of oil polluted soil, *Soil Biol. Biochem.* 36 (2004) 1149–1159.
- [13] D.A. Bossio, K.M. Scow, Impact of carbon and flooding on the metabolic diversity of microbial communities in soils, *Appl. Environ. Microb.* 61 (1995) 4043–4050.
- [14] D. Sarkar, M. Ferguson, R. Datta, S. Birnbaum, Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition, carbon supplementation, and monitored natural attenuation, *Environ. Pollut.* 136 (2005) 187–195.
- [15] R.M. Atlas, *Petroleum Microbiology*, Macmillan Publishing Company, New York, USA, 1984.
- [16] M.H. Huesemann, Incomplete hydrocarbon biodegradation in contaminated soils: limitations in bioavailability or inherent recalcitrance? *Bioremed. J.* 1 (1997) 27–39.
- [17] J.V. Forsyth, M. Tsao, R.D. Bleam, Bioremediation: when is augmentation needed? in: *Bioaugmentation for Site Remediation*, Battelle Press, OH, USA, 1995.
- [18] E. Riser-Roberts, *Remediation of Petroleum Contaminated Soils: Biological, Physical and Chemical Processes*, CRC Press LLC, Florida, 1998, pp. 59.
- [19] J.P. Obbard, K.L. Ng, R. Xu, Bioremediation of petroleum contaminated beach sediments: use of crude palm oil and fatty acids to enhance indigenous biodegradation *Water Air Soil Pollut.* 157 (2004) 149–161.
- [20] J. Walworth, A. Pond, I. Snape, J. Rayner, S. Ferguson, P. Harvey, Nitrogen requirements for maximizing petroleum bioremediation in a sub-Antarctic soil, *Cold Reg. Sci. Technol.* 48 (2007) 84–91.
- [21] B.D. Harch, R.L. Correll, W. Meech, C.A. Kirkby, C.E. Pankhurst, Using the Gini coefficient with BIOLOG substrate utilization data to provide an alternative quantitative measure for comparing bacterial soil communities, *J. Microbiol. Methods* 30 (1997) 91–101.
- [22] K. Smalla, U. Wachtendorf, H. Heuer, W.T. Liu, L. Forney, Analysis of BIOLOG GN substrate utilization patterns by microbial communities, *Appl. Environ. Microb.* 64 (1998) 1220–1225.
- [23] J.P. Salanitro, P.B. Dorn, M.H. Huesemann, K.O. Moore, I.A. Rhodes, L.M.R. Jackson, T.E. Vipond, M.M. Western, H.L. Wisniewski, Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment, *Environ. Sci. Technol.* 31 (1997) 1769–1776.
- [24] J.G. Leahy, R.R. Colwell, Microbial degradation of hydrocarbons in the environment, *Microbiol. Rev.* 54 (1990) 305–315.
- [25] S.C. Long, C.M. Aelion, Metabolite formation in evaluating bioremediation of a jet fuel contaminated aquifer, *Appl. Biochem. Biotech.* 76 (1999) 79–97.
- [26] X.P. Wang, R. Bartha, Effects of bioremediation on residues, activity and toxicity in soil contaminated by fuel spills, *Soil Biol. Biochem.* 22 (1990) 501–505.
- [27] J. Shen, R. Bartha, On-site bioremediation of soil contaminated by no. 2 fuel oil, *Int. Biodeter. Biodegr.* 33 (1994) 61–72.
- [28] V. Riis, M. Stimming, D. Miethel, W. Babel, Investigations into the toxicity of persistent fractions of mineral oils, *Chemosphere* 32 (1996) 1435–1443.