Desalination and Water Treatment
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doi: 10.1080/19443994.2012.698838

Taylor & Francis
Taylor & Francis Group

48 (2012) 329–334
October

Bioremediation of waste drilling fluid: comparison of biostimulation and bioaugmentation

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Received 20 February 2012; Accepted 30 April 2012

ABSTRACT

In this study, waste drilling fluid was remediated by both biostimulation with the inorganic nutrient addition and bioaugmentation with the inoculation of a selected and well-adapted microbial consortium. The results showed that, compared to the non-bioaugmented system, the bioaugmented system exhibited considerably stronger pollution disposal abilities, with 95.2% total organic carbon degradation (TOC) and 91.2% total petroleum hydrocarbon (TPH) removal within 120 h. In contrast, in the non-bioaugmented system, the corresponding TOC and TPH removal efficiencies were 82.9% and 58.3%, respectively, within 120 h. The active role of the microbial inoculum in pushing the entire community towards an effective bioremediation has been proven through hydrocarbons analysis and metabolic profiling at community level (Biolog system).

Keywords: Drilling fluid; Bioremediation; Petroleum hydrocarbon; Microbial activity

1. Introduction

Drilling fluid is widely used to lubricate and cool down the drill bit during petroleum drilling operations and also to carry the drill cuttings to the surface for further screening and disposal.

Drilling fluid is stable suspensions and contains numerous components such as mineral, oil, organics, viscosifiers such as clays, polymers, cellulose, xanthan gum, and guar, weighting agents such as barytine, carbonate, filtrate reducers such as starch, carboxy methyl cellulose, resins, and clays swelling inhibitors such as KCl, and glycol [1]. After the completion of drilling actions, drilling fluid became waste material. Disposal of waste drilling wastes constitutes one of the most significant waste discharges associated with oilwell drilling.

Currently, Bioremediation is one of the most widely used and cost-effective treatment technologies for oil-contaminated water and soil [2]. As drilling fluid consists of numerous components, it is very difficult to define the mechanisms involved in their degradation. It has also been suggested that the pollutants rather than the geographical origin of the soil sample were more important in determining the functional or species diversity within bacterial communities [3].

The aim of this study is to test the feasibility of remediation of waste drilling fluid. The treatment efficiency of biostimulation and bioaugmentation was examined and compared. The persistence of the

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inoculated specialized bacteria and the activity of the biological community were investigated.

2. Materials and methods

2.1. Waste drilling fluid

Waste drilling fluid to be tested was obtained from an active drilling operation in Sichuan, China. The base fluid used in the original drilling fluid was polysulfonate potassium drilling fluid. The drilling fluid on site was stored in a storage pool. The fluid has the characteristics of sticky dark-brown fluid and offensive odor. Samples were stored in uncontaminated polyethylene containers and immediately transported back to the laboratory and maintained at 4°C until used. The main physical, chemical, and biological characteristics of the studied sample are shown in Table 1.

2.2. Mixed bacteria for bioaugmentation

Mixed bacteria were enriched from petroleum-contaminated soil obtained from an oilfield in the Sichuan Province of China, by repeating selection and batch cultivation with target pollutants. The medium used for the enrichment and acclimation was mineral salts medium (MSM), containing (g/L): 10.0 g NaCl; 0.42 g $MgSO_4 \cdot 7H_2O$; 0.29 g KCl; 0.83 g KH_2PO_4; 1.25 g Na_2HPO_4; 0.42 g $NaNO_3$; 1 L deionized water. The pH of the medium was adjusted to 7.5.

Table 1
Characteristics of the drilling fluids used in this study

Parameter	Value
pH	9.5
Density (g/cm^3)	1.71
Electrical conductivity (μS/cm)	1050
Moisture content (%)	31.3
TPH (mg/kg)	15,300
Cl^- (mg/kg)	1980
Total carbon (mg/kg)	106,200
Total organic carbon (mg/kg)	89,800
Total nitrogen (mg/kg)	500
THBC (CFU/kg)	5.24×10^6
Fe (mg/kg)	132
Mn (mg/kg)	45
Cu (mg/kg)	65
Ca (mg/kg)	198

Ten grams samples of soil were added to 90 mL of sterile MSM solution and shaken at 150 rpm for 30 min. After settling for 10 min, 5 mL of the suspension was transferred to a flask containing 100 mL of sterilized MSM solution, supplemented with sterile fluid (2%, v/v) as the sole carbon source. After 14-day incubation on a rotary shaker at 150 rpm and 30°C, 5 mL of the turbid culture broth was serially transferred into 100 mL of fresh MSM solution to re-establish selective circumstances. The sterile drilling fluid was added after medium sterilization and its concentration was gradually increased up to 6% (v/v) from 2% (v/v) in steps of 2% (v/v). Enrichment procedure with the same conditions was repeated three times, and then the resultant culture was fermented using the MSM plus 10 g/L glucose. The fermentation broth was centrifuged at 10,000*g* for 5 min and the pelleted cells were rinsed three times with phosphate-buffered saline, stored at 4°C until used. The dominant bacteria were characterized using standard methods for bacterial identification [4], viz. morphological features of colonies and cells, catalase, oxidase, OF glucose and carbon utilization patterns. The species identified were *Acinetobacter* spp., *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Alcaligenes*, *Nocardia*, *Actinomyces*, *Enterobacter* and *Klebsiella* sp.

2.3. Bioremediation experiment

All bioremediation experiments were carried out in a series of identical stainless steel-made vessels which has a total volume of 100 L. Each reactor received 50 L drilling fluid. A mechanical mixer set at 600 rpm provided mixing. Aeration was via a diffuser stone situated at the bottom of the reactor. Together with water, NH_4NO_3 and KH_2PO_4 were added to achieve a C:N:P ratio of 100:10:1 [5,6]. The pH of the fluid was adjusted to 7.5. After harvesting by centrifugation, the cell pellet was added to the fluid to give an inoculum of 10^6 bacterial cells per mL. Water was supplied to the microcosm to increase the water content to 40% (w/w). The experiment was conducted at room temperature (25°C).

There were three trials: (A) abiotic control (drilling fluid with 2 wt.% $HgCl_2$, without inoculation); (B) fluid without inoculation (biostimulation); (C) fluid with inoculation (bioaugmentation). Each treatment was prepared in triplicate.

2.4. Analytical methods

At regular intervals, the drilling fluid was withdrawn and an aliquot of the sample was centrifuged at 8000*g* for 10 min. The resulted supernatant was subjected to the analysis of chroma and inorganic ions. The

other analysis was performed on the uncentrifuged fluid. The pH and electrical conductivity of drilling fluid were measured in 1/10 (dry fluid/water) suspensions using a digital pH meter and an electric conductivity meter, respectively. Total carbon and total organic carbon (TOC) were quantified by using the Walkley Black method [7]. Total nitrogen was extracted by Kjeldhal digestion, and determined in the form of NH_4-N by the indophenol blue method [8]. Total petroleum hydrocarbon (TPH) was determined by USEPA Method 1664A (modified from EPA 821/B-94-004b) using *n*-hexane as the extraction solvent [9]. The extracts were rotary dried and dehydrated with anhydrous sodium sulfate. Total heterotrophic bacterial counts (THBC) were determined by spread plating method [10]. Chroma was determined according to the method proposed by Hongve and Akesson [11]. The concentration of inorganic ions was analyzed using an ion chromatography system (HIC-10A, Shimadzu, Japan).

2.5. Biolog® ECOPlates

The metabolic profiles of microbial communities were obtained by means of the Biolog® Microstation System 4.2 (Biolog Inc., CA, USA) using ECOPlates. Ten grams of soil were added to 100 mL distilled water in a flask and shaken at 200 rpm for 10 min. Tenfold serial dilutions were made and the 10^{-3} dilution was added into the Biolog ECOplate. Then the plates were incubated at 28°C in the dark and analyzed by the Microplate Reader (dual wavelength data: OD590–OD750) at regular 24-h intervals, until the color development of the plates reached the plateau. Kinetic analysis was performed using average well color development (AWCD) as a parameter according to Garland et al. [12].

2.6. Data analysis

The data are presented in terms of arithmetic averages of three replicates values ± standard deviation. Results were expressed on an oven-dry (105°C, 24 h) mass basis or on a volume basis. The comparisons between treatments were done using the one-way analysis of variance (ANOVA). The statistical significance in this study was defined at $p < 0.05$.

3. Results and discussion

3.1. TOC removal

TOC was selected as the principal parameter for analysis in the present study, because the time required for the determination of the organic content in a sample is only 3–4 h. In addition, TOC can reflect overall organic contamination degree of water, soil, and sediment. As such, the results of TOC removal are particularly useful in determining the performance of remediation system.

The TOC removal efficiency of the bioremediation system is presented in Fig. 1. The results showed that, after 8 h, the removal efficiency of TOC was 19.5%, 20.4% and 20.5% for trials A, B and C, respectively. This indicates that the waste drilling fluid contained a large amount of volatile substances, which escaped from the drilling fluid through aeration. In addition, there was little difference in TOC removal efficiency among the three trials in the first 8 h. This is because that the microbial community needed time to propagate.

Significant differences in TOC removal were observed after 24 h of treatment ($p < 0.05$) (Fig. 1). TOC evaporation efficiency was 26.8% after 120 h for trial A, and most of the TOC loss occurred in the first 24 h. After 48 h, significant differences in TOC removal were observed between trials B and C. At the end of test, the initial TOC contamination level, i.e., 89,800 mg/kg, was decreased to 15,356 mg/kg in trial B, corresponding to a decontamination percentage of 82.9%. In contrast, TOC was reduced to 4310 mg/kg in trial C, corresponding to a removal efficiency of 95.2%. This result demonstrates that the inoculated bacteria performed well in the treatment system.

Bioaugmentation is an efficient approach to improve the removal of recalcitrant compounds in polluted sites or bio-treatment systems through the addition of some microbial cultures [13,14]. These special cultures were some specific microorganisms capable of degrading target compounds. However, bioaugmentation is not an universal method and the microorganisms introduced usually fail to enhance degradation of the pollutants due to the poor survival or low activity of these microorganisms caused by abiotic and biotic stresses [15,16].

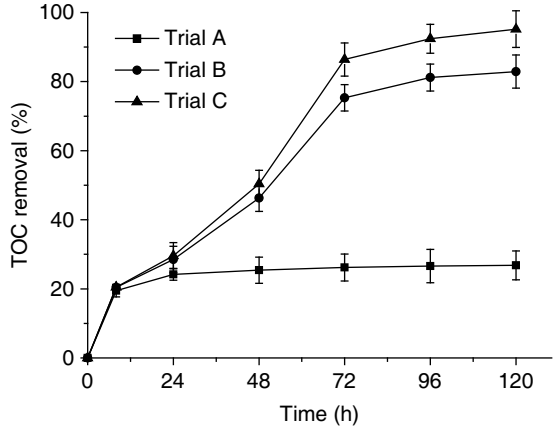

Fig. 1. Time course of TOC removal efficiency during bioremediation. Trial A: abiotic control; trial B: biostimulation; and trial C: bioaugmentation.

3.2. Chroma removal

The chroma removal during bioremediation is shown in Fig. 2. As can be seen, chroma removal efficiency reached 9.3% in trial A after 120 h, which was far below the corresponding TOC loss (26.8%) (Fig. 1). This may be due to that most of the colored substances in the drilling fluid were difficult to volatilize under experimental conditions. From the 72nd to 96th hour, a decrease in chroma removal was observed for both trials B and C. This can be attributed to that some colored substances were generated during biodegradation reactions. After 120 h, the total chroma removal was 89.8% and 88.1% for trials B and C, respectively. No significant difference in chroma removal was observed between biostimulation and bioaugmentation. This indicates that the colored substances were mainly degraded by the indigenous microorganisms.

3.3. Anion concentration changes

Polysulfonate potassium drilling fluid contains quite complex components; therefore it would be very difficult to determine concentration changes of different components. In this study, concentration changes of Cl^-, NO^{3-} and SO_4^{2-} were monitored during treatment because these anions were the metabolic products of corresponding organic groups. As shown in Table 2, the concentrations of Cl^-, NO^{3-} and SO_4^{2-} ions increased with time. It can be inferred that the microflora in the drilling fluid could degrade various pollutant components. In addition, the sharp increase in the concentration of SO_4^{2-} indicates that the microorganisms could utilize sulfonate groups well. At the 72nd hour, the NO^{3-} concentrations declined somewhat, which may be due to that the consumption rate of NO^{3-} ions by the microorganisms as a nitrogen source was greater than the generation rate of NO^{3-} ions during biodegradation of nitrogen-containing compounds.

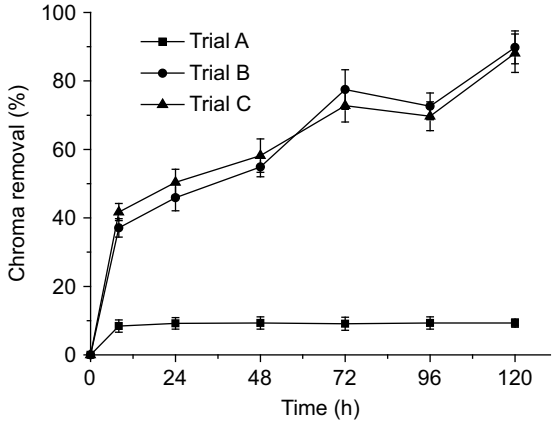

Fig. 2. Time course of chroma removal efficiency during bioremediation. Trial A: abiotic control; trial B: biostimulation; and trial C: bioaugmentation.

3.4. Petroleum hydrocarbon biodegradation

In general, waste drilling fluid contains a certain amount of petroleum hydrocarbons. The degradation of TPH was monitored during the whole treatment period (120 h) (Fig. 3). In the control, the loss of TPH after 120 h was about 2400 mg/kg, corresponding to a removal efficiency of 15.7%. Biostimulation (trial B) resulted in a rapid degradation of TPH within 96 h, followed by a marked slowdown of the degradation. At the end of process (120 h), the total removal efficiency of TPH for trial B was 58.3% (from 15,300 mg/kg at t = 0 h to 6380 mg/kg at t = 120 h). The effect of adding a consortium (trial C) promoted significantly the degradation from the 48th hour to the 120th hour (91.2% TPH removal, from 15,300 mg/kg at t = 0 h to 1240 mg/kg at t = 120 h). This treated waste drilling fluid can be emitted into municipal wastewater treatment plants for further treatment. These results show that bioaugmentation with selected strains could lead to more effective treatment than the application of uncharacterized communities [17]. Recently, there are growing evidences that bioaugmentation using pre-selected microorganisms is a good

Table 2
Anion concentration changes during bioremediation. Trial B: biostimulation; trial C: bioaugmentation. The data for the control was not presented due to very little changes in the anion concentration.

Treatment duration (h)	Anion concentration (mg/L)					
	Cl^-		NO_3^-		SO_4^{2-}	
	Trial B	Trial C	Trial B	Trial C	Trial B	Trial C
0	1840	1840	15	15	243	243
24	1952	1938	86	72	1253	1327
72	2425	2516	64	58	1974	2156
120	3241	3295	124	142	2215	2375

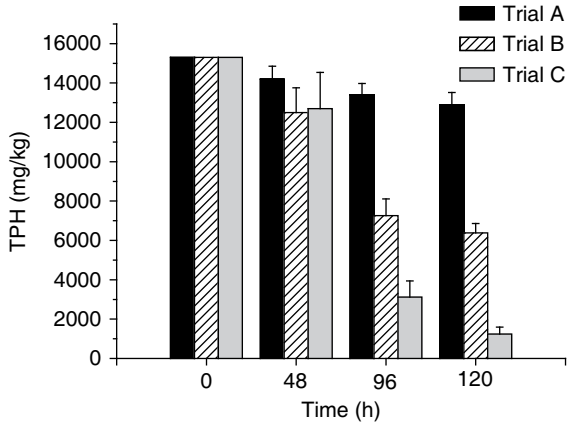

Fig. 3. TPH degradation during 120 h of treatment. Trial A: abiotic control; trial B: biostimulation; and trial C: bioaugmentation.

approach for bioremediation [14,18–22]. In this study, the enriched microorganisms have greater degradation ability of TPH than the indigenous microbial community present in the drilling fluid.

3.5. Analysis of microbial community

The AWCD measure gives a general indication of the metabolic capacity of the community with respect to substrate utilization. In this study, data are expressed as AWCD in Biolog ECOPlates incubated up to 240 h. As shown in Fig. 4, the comparison of AWCD curves in microcosms shows an evident decrease of the general metabolic capacity in both trial B and C after 120 h of treatment, due to the toxicity exerted by the pollutants and/or metabolites on the microbial community. As can be observed, the addition of the microbial inoculum (trial C) to the reactor alleviated the decrease in the metabolic activity compared with the biostimulation treatment (trial B). These results confirm that the enriched microflora could better trap the energy flux, driving the entire microbial community towards biodegradation.

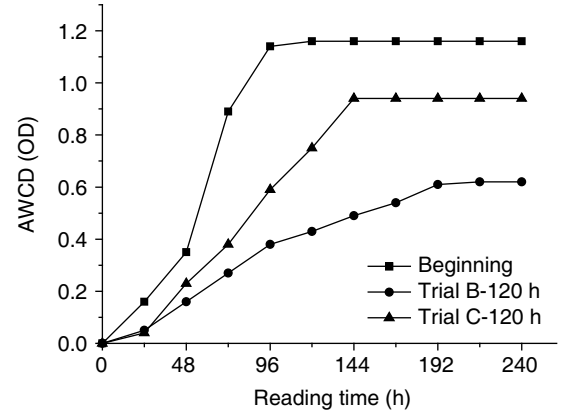

Fig. 4. Comparison of the metabolic activity of the microbial community at the beginning of the experiment and after 120 h under different conditions. Trial B: biostimulation; and trial C: bioaugmentation. The data are expressed as AWCD in ECOPlates incubated up to 240 h

4. Conclusions

The results of this study confirmed the positive effects, in terms of physical and chemical improvement, nutritional factors and microbial presence, of adding nutrients for drilling fluid bioremediation. The combined action of nutrient addition and bioaugmentation by inoculating a selected and well adapted microbial consortium provided good results for both contaminant removal and microbial diversity in waste drilling fluid even if this behavior needs further confirmation at the field-scale. The finding in this study is of ecological significance, since spillage of waste drilling fluid into the environment cannot be completely avoided, the application of bioremediation will help to rid the environment of the drilling wastes.

Symbols

TOC	total organic carbon
TPH	total petroleum hydrocarbons
MSM	mineral salts medium
THBC	total heterotrophic bacterial counts
AWCD	average well color development

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