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Microbial enhanced separation of oil from a petroleum refinery sludge

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ABSTRACT

Petroleum refineries around the world have adopted different technological options to manage the solid wastes generated during the refining process and stocking of crude oil. These include physical, chemical and biological treatment methods. In this investigation bacterial mediated oil separation is effected. Two strains of *Bacillus* were isolated from petroleum-contaminated soils, and inoculated into slurry of sludge, and sludge–sand combinations. The bacteria could effect the separation of oil so as to form a floating scum within 48 h with an efficiency of 97% at ≤5% level of sludge in the sludge–sand mixture. The activity was traced to the production of biosurfactants by bacteria.

1. Introduction

Disposal of oil residues from storage, processing and transportation facilities have always been a major issue faced by Petroleum industries. Sludge is generated in significant amount in the refineries during crude oil processing. Crude oil is usually stored in storage tanks. Impurities present in the oil are deposited at bottom of the tank. During cleaning of the tank, the sludge is recovered, and is treated as waste. Sludge is also generated from the treatment plant of wastewater. In India, oil refineries generate approximately 28,000 tonnes of oily sludge (a mixture of hazardous hydrocarbon waste) per annum [1]. This waste residue is dumped into specially constructed sludge pit, consisting of a leachate collection system and polymer lining system to prevent the percolation of contaminants into ground water [1]. However these pits face the drawbacks of being rather expensive to construct and maintain, and increasingly more and more land is required for this purpose.

The available clean-up technologies are based on bioremediation principles and using physico-chemical treatment by washing the contaminated soil [2]. There has been a growing interest in surfactant applications in environmental remediation [3–7]. The mechanism behind surfactant-enhanced removal of oil from soil have been proposed to occur in two steps: mobilization and solubilization [8–11]. Biosurfactants are produced by many microbes in response to growth in petroleum hydrocarbons. These compounds offer greater potential over chemical surfactants in bioremediation of petroleum hydrocarbons (PHCs) as they exhibit lower toxicity, greater biodegradability and environmental compatibility [12]. In this investigation petroleum sludge generated in an oil refinery was used. The objective was to separate the oil from the petroleum sludge oil by induced biosurfactant production by bacteria.

2. Materials and methods

2.1. Estimation of total petroleum hydrocarbons (TPH)

The sludge used in this study was collected from the temporary storage site of a petroleum refinery. It is a composite of crude tank bottom sludge, product tank bottom sludge and effluent treatment plant sludge. Random samples of the sludge were collected monthly over a period of 6 months. A composite of each collection was air dried to a moisture content of 10% and stored at 4 °C in sealed glass containers for analysis. TPH of the sludge was estimated through soxhlet-extraction following the EPA method 3540C. The sample was mixed with anhydrous sodium sulphate prior to extraction and quantitatively transferred to extraction thimble. Consecutive extraction was done using *n*-hexane, dichloromethane and chloroform (100 ml each) as per [13]. All the three extracts were pooled and evaporated in a rotary vacuum evaporator to about 2 ml. The distilling head was removed, and dried in vacuum, cooled, and weighed. The concentration of TPH in the original sample was calculated as

$$\text{TPH (mg/kg dry weight)} = \frac{\text{Gain weight of the flask (mg)}}{\text{Weight of solid (g)}} \times 1000$$

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2.2. Isolation of bacterial strains

2.2.1. Culture medium

One hundred grams of sludge was weighed into a conical flask, added 100 ml of distilled water, stirred well and autoclaved. The sample was cooled and filtered through different grades of filters and finally through GF/C filter to get a brown clear filtrate. A mineral medium of composition 0.8 g/l K_2HPO_4, 0.2 g/l KH_2PO_4, 0.05 g/l $CaSO_4{\cdot}2H_2O$, 0.5 g/l $MgSO_4$ H_2O, 0.09 g/l $FeSO_4{\cdot}7H_2O$, and 1.0 g/l $(NH_4)_2SO_4$ was prepared. One hundred milliliters of the sludge extract was added to 1 l of mineral medium and sterilized by autoclaving. The bacteria were isolated and maintained in this liquid medium. Agar was added to this @ 15 g/l whenever solid media was required.

2.2.2. Isolation of microbes

Samples of soil were collected from petroleum-contaminated sites, dispersed in sterile distilled water and inoculated into test tubes containing the liquid culture medium. They were incubated at 28 °C for 5 days. Samples from the tubes that developed turbidity were inoculated into agar plates, and incubated as above. The colonies developed were individually separated to the test tube containing 15 ml liquid medium and incubated at 28 °C for 5 days. These were repeatedly plated to agar to select the most actively growing CFUs. Seven isolates were thus obtained.

2.3. Microbial aided oil recovery

2.3.1. Screening test

The seven isolates were screened in a stirred bioreactor to identify their potential to separate oil from the sludge. The sludge was mixed with washed and sterilized river sand to effect sludge concentrations 2.5%, 5%, 10%, 20%, 40%, and 80% by w/w. A set of un-amended sludge was also taken. Two hundred and fifty grams of the samples were taken in 1 l borosil beakers and was stirred with 200 ml of distilled water. The stirrer was specially modified to effect thorough mixing of the sludge with water; still it was not possible to produce a homogeneous slurry at sludge levels >5%. The test samples were enriched with urea and diammonium phosphate to provide a C:N:P ratio of 100:5:1, based on carbon content in original sludge. Ten milliliters each of the respective inoculums having a count of 10^8 CFU/ml was added to the test jars. Controls of un-inoculated sludge were also maintained. The test beakers were placed on the jar test apparatus and stirred continuously for 7 days @ 5 min per hour at ambient temperature (28 ± 2 °C). It was then allowed to settle for a few hours. The oil separation was observed visually. The test jars with 5% sludge were retrieved, oil scum removed and the supernatant poured off. The TPH of the residue was estimated and expressed as residual TPH.

Based on the above results, two isolates were selected for further investigation. These were identified as *Bacillus* strain SEB2 and *Bacillus* strain SEB7 by standard tests of colony morphology on nutrient agar, staining and biochemical tests. The sludge concentration was limited to 5% for further investigation.

2.3.2. Confirmatory test

The test was set up similar to the above experiment. Each of the test jars containing the enriched 5% sludge–sand slurry was inoculated with either of the *Bacillus* sp. and kept stirred in the Jar test apparatus for 7 days, taking observations every 24 h. The formation of oil scum was observed visually. Test jars were removed every 24 h and allowed to settle. The supernatant was poured off. The TPH of the residue was estimated. The experiment was repeated twice.

Table 1
Oil separation and mean residual TPH (g/kg) after 7 days (initial TPH 42.52 g/kg)

Isolate	Visual observation	TPH (5% sludge)	
		g/kg	% reduction
SEB1	+	3.04	92.85
SEB2	+	1.10	97.41
SEB3	+	2.05	95.18
SEB4	+	2.85	93.30
SEB5	+	3.54	91.67
SEB6	+	2.85	93.30
SEB7	+	1.08	97.46

(+) Occurrence of oil separation.

2.3.3. Demonstration of biosurfactant activity

Assuming that the oil separation occurred due to surfactants produced by the *Bacillus* sp. the above experiment was again set up for a running time of 48 h. Test jars were removed every 4 h, allowed to settle and supernatant poured off after removing oil scum. It was filtered through membrane filter and surface tension measured using Tensiometer (Du-Nouy, CSC No. 70535). Presence of biosurfactants in the supernatant was further demonstrated by inoculating the filtered supernatant of the *Bacillus* SEB2 inoculated reaction vessel (200 ml) to a fresh set of test jars containing enriched sludge (5%) slurry. Microbial inoculation was not done. The experiment was repeated for 48 h, retrieving test jars every 8 h to estimate the residual TPH. The results were compared to those inoculated with *Bacillus* SEB2.

3. Results and discussion

The sludge used for the investigation was black in colour with a sticky solid consistency. The TPH content was estimated to be 850 ± 150 g/kg. When the different concentrations of sludge were inoculated with the seven bacterial isolates and incubated for 7 days there occurred a clear separation of oil and water, the former forming a distinct floating scum in the test jars of 2.5% and 5%. Oil layer separation did not occur in higher concentrations of sludge; they did not form homogeneous slurry. The respective un-inoculated controls also remained unchanged. The residual TPH of the 5% sludge is given in Table 1. The efficiency of removal of the various isolates ranged from 91.67% to 97.46%. The isolates SEB2 and SEB7 were found to be most efficient. These were identified as *Bacillus* sp. based on their characteristics (Table 2).

The rate of separation of oil by *Bacillus* sp. was highest during the first 48 h (Fig. 1). Beyond 48 h the rate was nearly constant. The surface tension of the bio-slurry was also observed to decrease as reaction time progressed indicating the production of biosur-

Table 2
Culture characteristic of the isolate SEB2 and SEB7

Tests	SEB2	SEB7
Colony size	Large	Small
Pigmentation	White	Cream
Form	Irregular	Round
Margin	Serrate	Entire
Elevation	Raised	Raised
Gram staining	Gram positive long rods	Gram positive short rods
Spore staining	Gram positive rods with terminal endospores	Gram positive rods with terminal endospores
Acid from Mannitol	Positive	Negative
Voges Proskauer	Positive	