Original Research Article

EFFECT OF LINEAR ALKYLBENZENE SULPHONATE ON BACTERIAL POPULATION AND HYDROCARBON DEGRADATION IN CRUDE-OIL POLLUTED SOIL

ABSTRACT

Aim: The aim of this study was to determine the concentrations of LAS that are non-toxic to bacteria in crude-oil polluted soil, and also effective in enhancing biodegradation of the hydrocarbons.

Methodology: Seven concentrations (4, 8, 16, 32, 64, 128, and 256 mg/L) of LAS were prepared, and 50 ml of each were added to 500 g soils artificially polluted with crude-oil. Based on the quantity of soil used and the volume of LAS solutions added, the in-soil LAS concentrations were calculated to be 0.4 – 25.6 mg/Kg. A control was also set up were 50 ml sterile distilled water was used. The setups were maintained for seven days; on day 1, 3, and 7, the population of total heterotrophic bacteria (THB) were determined. Total hydrocarbon concentrations (THC) were determined on day 1 and 7.

Results: The results obtained showed that the extent of hydrocarbon degradation increased with increase in LAS concentration from 35.9 to 61.4 %, with the control having 10.9 %. The THB population strength in all the setups increased from 10^6 to 10^7; in the setup treated with the highest concentration of LAS, the increase was from 10^6 to 10^8. Bacteria isolated from the setups included *Bacillus*, *Micrococcus*, *Acinetobacter*, *Staphylococcus*, and *Pseudomonas* sp, which are known hydrocarbon degraders.

Conclusion: LAS concentration of up to 25.6 mg/Kg can lead to significant hydrocarbon biodegradation in crude-oil polluted soil with no adverse effect on bacteria present in the soil.

Keywords: Linear alkylbenzene sulphonate, petroleum hydrocarbons, total heterotrophic bacteria, crude-oil polluted soil, surfactant enhanced bioremediation.

INTRODUCTION

Linear alkylbenzene sulphonate (LAS) is a class of anionic surfactants [1]. They are part of the ingredients in many personal-care and household-care products such as detergents, soaps, shampoos, toothpaste, and spray cleaners. They are also been investigated for used in surfactant enhanced bioremediation of crude oil polluted environment.

Bioremediation of crude-oil polluted soil consists of nutrient addition, aeration, adjustment of moisture content, addition of hydrocarbon degrading microorganisms, and sometimes addition of a surfactant [2,3,4,5,6]. Crude-oil hydrocarbons are hydrophobic in nature; they thus adhere to soil particles and solid surfaces, and form large floating droplets on water surfaces. Due to this property, crude-oil hydrocarbons are not readily available for biodegradation. Biodegradation of crude-oil hydrocarbons can only take place after attachment of microorganisms to the oil-water interface or after transfer of hydrocarbon molecules from the oil phase into the aqueous phase [7]. Either of these processes can be instigated and enhanced by the presence of surfactants secreted by microorganisms (biosurfactant) or introduced artificially (chemically synthesized

surfactant). Surfactants are substances which lower the interfacial tension between gas-liquid, liquid-solid, and immiscible liquid-liquid interfaces [8]. By lowering the interfacial tension at oil-water interface, transport of hydrocarbon molecules from oil phase into aqueous phase is favoured thereby making the hydrocarbons available for biodegradation. With regards to crude-oil polluted soil, surfactant addition will facilitate desorption of petroleum-hydrocarbons bound to soil particles [9]. This increases the solubility (apparent-solubility) of the hydrocarbons, consequently making them available for biodegradation.

Chemical synthesized surfactants including Triton X-100, Tween 80, sodium dodecyl sulphate (SDS), and linear alkylbenzene sulphonate have been shown to increase the apparent aqueous solubility of hydrocarbons thereby leading to enhanced biodegradation [7,10,11,12]. There are however concerns about the use of chemically synthesized surfactants with regards to inhibition, toxicity and degradability [7, 11]. This favours the argument for the use of biosurfactant over chemically synthesized surfactant; biosurfactants are said to be biodegradable and of lower toxicity [13]. However, the cost of producing biosurfactants is 3-10 times higher than that of chemically synthesized surfactants [14, 15], thereby making the use of biosurfactants for use in large scale bioremediation of crude oil polluted environment economically unfeasible. Some chemically synthesized surfactants such as Tween 80 and LAS may not pose a significant threat to terrestrial soil ecosystems at low concentrations as suggested by studies of Asok & Jisha [16], and Galvez *et al* [17]. Linear alkylbenzene sulphonate undergo biodegradation easily under aerobic conditions, with half-life of 1 to 3 weeks [18]. Such surfactant can thus be used within a safe limit in lieu to biosurfactants where the cost of bioremediation becomes economically challenging.

The aim of this study is to determine the concentrations of Linear alkylbenzene sulphonate that are non-toxic to total heterotrophic bacterial population in crude-oil polluted soil, and also effective in enhancing biodegradation of the hydrocarbon pollutants.

MATERIALS AND METHODS

Preparation of different concentrations of linear alkylbenzene sulphonate

Different concentrations of linear alkylbenzene sulphonate (LAS) was prepared by dissolving 1 g of a powdery detergent containing 30 % LAS in 1 L of sterile distilled water. Seven concentrations (4, 8, 16, 32, 64, 128, and 256 mg/L) of LAS were then prepared from the detergent solution using sterile distilled water and the dilution formula M1V1=M2V2 [19]. The preparation is presented in Table 1.

Table 1: Preparation of LAS solutions of different concentrations from the detergent

Vol. of DS (ml)	Vol. of SDW (ml)	Total vol. (ml)	DC (mg/L)	LAS.C (mg/L)
1.3	98.7	100	13	$3.9 \approx 4$
2.7	97.3	100	27	$8.1 \approx 8$
5.3	94.7	100	53	$15.9 \approx 16$
10.7	89.3	100	107	$32.1 \approx 32$
21.3	78.7	100	213	$63.9 \approx 64$
42.7	57.3	100	427	$128.1 \approx 128$
85.3	14.7	100	853	$255.9 \approx 256$

LAS: Linear alkylbenzene sulphonate, DS: the detergent solution (1000 mg/L), SDW: sterile distilled water, DC: detergent concentration, LAS.C: Linear alkylbenzene sulphonate concentration (0.3×DC).

Experimentation

About 5 Kg soil was placed in a glass trough, and artificially polluted with 750 ml of crude-oil. The polluted soil was allowed undisturbed for a week, after which duplicate samples were collected for determination of total hydrocarbon concentration (THC) and population of total heterotrophic bacteria (THB). After collection of the samples, the polluted soil was divided into eight (8) glass troughs (500 g per trough). The troughs were labeled 0, 4, 8, 16, 32, 64, 128, and 256 mg/L LAS. About 50 ml sterile distilled water was added to the polluted soil in the trough labeled 0 mg/L LAS, while 50 ml of the prepared LAS solutions of different concentrations were added to the polluted soils in the corresponding labeled troughs. Based on the quantity of soil used and the volume of LAS solutions added, the in-soil LAS concentrations were calculated to be 0.4 – 25.6 mg/Kg. The setups were maintained for seven days. On day 1, 3, and 7, the populations of THB in the setups were determined. Also on day 7, THC was determined.

Determination of population of total heterotrophic bacteria

The population of total heterotrophic bacteria (THB) was determined using spread plate technique, and nutrient agar as the culture medium. About 1g of soil sample was placed into 10 ml sterile normal saline to obtain 10^{-1} mixture. The mixture was agitated, and then 1 ml of it was transferred into 9 ml sterile normal saline to obtain 10^{-2} dilution. This process was repeated till a dilution of 10^{-5} was obtained. Aliquot of 0.1 ml of the different dilutions were spread inoculated on plates of nutrient agar in duplicates with the aid of a sterile bent glass rod. Inoculated plates were incubated at ambient temperature (29 - 31° C) for 24 hours. After incubation, counts of ensuing colonies on the plates were used to calculate the population of THB.

Isolation and identification of bacteria

Bacterial colonies on selected enumerated nutrient agar plates were isolated and sub-cultured onto sterile NA and coded. Isolated bacteria were subjected to Gram staining & microscopic examination, and the following biochemical/physicochemical tests: catalase, oxidase, motility, citrate utilization, indole production, Methyl red (MR), Vogues-Proskauer (VP), starch hydrolysis, and fermentation tests using glucose, lactose, maltose, sucrose, mannitol, xylose, and glycerol. Reaction pattern to these tests in addition to microscopic morphology were used in identifying the isolates.

Determination of total hydrocarbon concentration and extent of hydrocarbon degradation

The total hydrocarbon concentration (THC) was determined using a spectrophotometric method. In the method, 5 g of polluted soil was placed in 250 ml capacity beaker, followed by addition of 10 ml N-hexane. The resulting mixture was agitated for about 30 seconds, and then filtered through Whatman No. 1 filter paper held in glass funnel into clean 150 ml capacity conical flask. The filtrate was subjected to absorbance measurement using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China) set at 420 nm. Absorbance reading was used to determine THC through extrapolation from a previously obtained plot of concentrations of

crude-oil against the absorbance at 420 nm. On deriving the THC concentrations, the extent of hydrocarbon degradation (EHD) in each setup were then determined using the equation below:

$$\text{EHD (\%)} = \frac{(\text{Initial THC} - \text{Final THC}) \times 100}{\text{Initial THC}}$$

RESULTS

Hydrocarbon concentration and extent of hydrocarbon degradation in the setups

The concentrations of hydrocarbon in the setups at the beginning and end (day 7) of the experiment, and extent of hydrocarbon degradation are presented in Table 2. In the Table, it can be seen that the extent of hydrocarbon degradation increased with increase in *l*inear alkylbenzene sulphonate (LAS) concentration. The highest degradation value obtained was 61.4 %, for the highest concentration of LAS (256 mg/L) added to the polluted soil.

Table 2: Extent of hydrocarbon degradation in the setups

Setup (LAS conc.)	THC (mg/Kg)		EHD
	Initial THC	On day 7	(%)
0 mg/L	54985 ± 265	49009 ± 492	10.9
4 mg/L		35242 ± 492	35.9
8 mg/L		29342 ± 492	46.6
16 mg/L		23120 ± 756	58.0
32 mg/L		22363 ± 1513	59.3
64 mg/L		21985 ± 378	60.0
128 mg/L		21607 ± 756	60.7
256 mg/L		21228 ± 378	61.4

LAS: linear alkyl benzene sulphonate, THC: total hydrocarbon concentration, EHD: extent of hydrocarbon degradation.

Population of total heterotrophic bacteria in the setups

The population of total heterotrophic bacteria (THB) in the polluted soil before experimentation, and on day 1, 3, & 7 of the experimentation is presented in Table 3. In the Table, it can be seen that the population strength of THB in all the setups increased from 10^6 to 10^7; in the setup

treated with the highest concentration of LAS solution (256 mg/L), the increase was from 10^6 to 10^8.

Identity of isolated bacteria

Bacteria isolated from enumerated nutrient agar plates were coded as L1-L11. Their reaction pattern to the biochemical/physicochemical tests used is presented in Table 4. Comparing the reaction pattern of the isolates to information on "identification of bacteria" in Madigan *et al*. [20], Prescott *et al*. [21], and Skerman [22], the identity of the isolates is suspected as follows: L1 – *Aeromonas* sp., L2 & L10 – *Bacillus* sp., L3 & L7 – *Micrococcus* sp., L4 – *Acinetobacter* sp., L5 & L8 – *Staphylococcus* sp., L6 – *Citrobacter* sp., and L9 & L11 – *Pseudomonas* sp.

Table 3: Population of total heterotrophic bacteria (THB) before and during experimentation

Setup (LAS conc.)	Total heterotrophic bacteria population (CFU/g)			
	Initial THB	Day 1	Day 3	Day 7
0 mg/L	$3.23\pm2.14 \times 10^6$	$5.95\pm4.31 \times 10^6$	$5.49\pm3.56 \times 10^6$	$2.84\pm1.75 \times 10^7$
4 mg/L		$5.40\pm5.09 \times 10^6$	$4.60\pm1.98 \times 10^6$	$4.77\pm1.03 \times 10^7$
8 mg/L		$5.60\pm4.81 \times 10^6$	$5.70\pm1.84 \times 10^6$	$8.17\pm1.32 \times 10^7$
16 mg/L		$3.04\pm0.76 \times 10^6$	$6.30\pm3.82 \times 10^6$	$2.09\pm0.83 \times 10^7$
32 mg/L		$2.10\pm0.14 \times 10^6$	$3.32\pm1.24 \times 10^6$	$2.35\pm0.35 \times 10^7$
64 mg/L		$5.75\pm4.60 \times 10^6$	$5.50\pm4.95 \times 10^6$	$7.00\pm1.13 \times 10^7$
128 mg/L		$3.75\pm3.18 \times 10^6$	$4.55\pm3.46 \times 10^6$	$5.02\pm4.92 \times 10^7$
256 mg/L		$5.80\pm3.11 \times 10^6$	$6.05\pm4.17 \times 10^6$	$8.44\pm1.36 \times 10^8$

LAS: linear alkyl benzene sulphonate,

DISCUSSION

Due to the bioavailability problem of petroleum hydrocarbons in polluted soil matrix and thus the subsequent limitation in hydrocarbon biodegradation, surfactants have been investigated and shown to be very helpful in solving these problems [7,10,23]. There are however concerns of surfactant toxicity to microorganisms and soil ecosystems. Studies on the anionic surfactants linear alkylbenzene sulphonate (LAS) have shown that it does not pose significant threat to terrestrial soil ecosystems at low concentrations [16, 17]. LAS concentration of 2 to 8 mg/Kg soil was shown by Asok and Jisha [16] to only slightly decrease bacterial population, and the bacterial population subsequently increased with time. The in-soil concentrations of LAS (0.4 – 25.6 mg/Kg) achieved in this study had no effect on the total heterotrophic bacterial populations in the hydrocarbon polluted soil as seen in Table 3; the populations even increased by a factor of 10 – 100 by day 7. This shows that beyond 8 mg/Kg as obtained in Asok and Jisha [16] to 25.6 mg/Kg as obtained in this study, LAS will be non-toxic to bacteria present in petroleum hydrocarbon polluted soil. In another related study, 50 % microbial growth inhibition in mineral

medium containing 1 g/L glucose was observed when LAS reached a concentration of 8.22 mg/L [24]. The inhibition however diminished when anthracene (a polycyclic aromatic hydrocarbon derived from crude-oil) was added and kept constant at 0.16 mg/L, while LAS concentration was increased from 0.16 to 70.11 mg/L. This suggests that LAS will only be toxic to microorganisms in the absence of petroleum hydrocarbons. The result on effect of increasing LAS concentration on bacterial population obtained in this study is thus in agreement with what has been observed.

The bacteria isolated at the end of the study including *Bacillus*, *Micrococcus*, *Acinetobacter*, *Staphylococcus*, and *Pseudomonas* have been isolated as members of hydrocarbon utilizing/degrading bacteria in other studies [25,26,27]. Their presence thus indicates that the reduction in total hydrocarbon concentration as observed in Table 2 was as a result of biodegradation of hydrocarbons which were made bio-available due to the presence of the surfactant LAS. Also, it can be seen in Table 2 that there was increase in the extent of hydrocarbon degraded with increase in LAS concentration. This indicates that with increase in LAS concentration, the more hydrocarbons were made bio-available for degradation. In a related study, 90 and 92 % polycyclic aromatic hydrocarbons (PAHs) degradation was obtained with the use of two surfactants, sodium dodecyl benzene sulphonate and Tween 80, at application concentration of 50 mg/kg [28]. It was also shown that in the presence of the surfactants the number of the operational taxonomic units associated with *Bacillus* and *Pseudomonas* increased from 2–3 to 15–30 %. Similar observations with regards to increases in hydrocarbon degradation and bacteria genera associated with hydrocarbon-degrading capability resulting from the application of surfactants were recorded in Cecotti *et al* [29], Cueva *et al*. [30], and Tsai *et al* [31]. The high extent of hydrocarbon degradation with in-soil LAS concentration of 1.6 – 25.6 mg/Kg and presence of hydrocarbon degrading bacteria obtained in this study is thus in agreement with what has been observed in other studies with other similar surfactants.

Table 4: Reaction pattern of isolated HUB to selected biochemical/physicochemical tests

	Isolates										
Tests	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**	**L7**	**L8**	**L9**	**L10**	**L11**
GS	-	+	+	-	+	-	+	-	-	+	-
MM	R	R	C	R	C	R	C	R	R	R	R
CT	+	+	+	+	+	+	+	+	+	+	+
OX	+	+	+	-	-	-	+	-	+	+	+
MT	+	+	-	-	-	+	-	-	+	+	+
CU	+	+	-	-	+	+	-	+	+	+	+
IN	+	-	-	-	-	+	-	-	-	-	-
MR	+	+	-	-	-	+	-	-	-	+	-
VP	-	+	+	-	+	-	+	+	-	+	-
SH	-	+	-	-	-	-	-	-	-	+	-
GF	A	A	A	A	A	AG	A	AG	A	A	A
LF	0	A	0	A	AG	AG	0	AG	0	A	0
MLF	A	A	0	0	A	A	0	A	0	A	0
SF	A	A	0	0	A	A	0	A	0	A	0
MnF	A	A	A	0	A	A	A	A	A	A	A
XF	0	A	0	0	0	A	0	A	A	A	A
GyF	A	A	0	0	A	A	0	A	A	A	A

SO	*Aeromonas*	*Bacillus*	*Micrococcus*	*Acinetobacter*	*Staphylococcus*	*Citrobacter*	*Micrococcus*	*Staphylococcus*	*Pseudomonas*	*Bacillus*	*Pseudomonas*

GS: Gram stain, MM: microscopic morphology, R: rods, C: cocci, CT: catalase, OX: oxidase, MT: motility, CU: citrate utilization, IN: indole production, MR: Methyl Red, VP: Vogues Proskauer, SH: starch hydrolysis, GF: glucose fermentation, LF: lactose fermentation, MLF: maltose fermentation, SF: sucrose fermentation, MnF: mannitol fermentation, XF: xylose fermentation, GyF: glycerol fermentation, A: acid produced, AG: acid and gas produced, 0: no acid nor gas produced, SO: suspected organism.

CONCLUSION

This study reveals that in-soil linear alkylbenzene sulphonate (LAS) concentration of up to 25.6 mg/Kg can lead to significant hydrocarbon biodegradation in crude-oil polluted soil with no adverse effect on bacteria present in the soil. A high extent of hydrocarbon degradation of 61.4 % was obtained within 7 days, even though the starting crude-oil concentration was quite high (about 55, 000 mg/Kg). The use of LAS solution in bioremediation of crude-oil polluted soil can thus achieve high hydrocarbon reduction within a short time frame. Further investigation is suggested with increase in LAS concentration until an inhibitory value is observed. This will enable a safe limit to be prescribed for the use of LAS in surfactant enhanced bioremediation of crude-oil polluted soil.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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