Petroleum Res. J. Vol. 17 (2005); P. 59-66, 15 Figs.

Microbial Prevention of Wax Deposition During Transportation and Production of Crude Oil

Asma Attomi, I.EL Musrati, B. EL Gammoudi* and M. EL Behlil**

منع ترسب الشمع أثناء إنتاج ونقل النفط الخام بواسطة الأحياء الدقيقة

أسماء التومي وعزالدين المصراتي وبسام القمودي ومحمد البهيليل

يشكل الشمع والترسب البارافيني أحد المشاكل الحادة بحقول النفط المنتجة للنفط البارافيني. عليه تم دراسة هذه المشكلة بواسطة المعالجة الحيوية التي تعتمد على فعالية الأحياء الدقيقة المستوطنة طبيعيا بالبيئات النفطية المحلية ، حيث برهنت الدراسات السابقة على تأثير هذه الكائنات كبديل للطرق الكيميائية التقليدية وذلك لمنع وإزالة ترسب الشمع بمعدات وخطوط أنابيب النفط.

تم في هذه الدراسة عمليا عزل عدة سلالات بكتيرية من ترسبات بحرية ملوثة بالمشتقات النفطية ، وقد أظهرت هذه العزلات بوضوح قدرتها المتميزة على إفراز مركبات مصاحبة لنموها من خلال التكسير الحيوي للسلسلة الهيدروكربونية على هيئة جزئيات حيوية لها خاصية عالية في خفض التوتر والشد السطحيين بين الطبقات السائلة والصلبة مما يساعد المواد الشمعية على المزج والانتشار في الماء وغيره من السوائل الأخرى.

أجريت اختبارات تجريبية على معدلات نمو البكتيريا ودرجة ثبات المستحلب والتكسير الحيوي عن طريق التحاليل باستخدام جهاز الغازكروموتوجراف لخامة حقل السرير قبل وبعد المعالجة الحيوية . ولقد أظهرت النتائج أن التكسير الجزئي قد قلل من المحتوى البارافيني للخامات الثقيلة وزاد من المركبات الهيدروكربونية ذات السلاسل القصيرة خاصة من الالكينات العادية ، كما تم بنجاح عزل البكتيريا المسؤولة عن هذه التغيرات من البيئة المحلية وأعطيت الأسماء $PRCWB_1$, E_1, A1, B_2 هذا وسوف يتم اختبار تأثير هذه العزلات على عدة أنواع من الخامات البارافينية المحلية لغرض توضيح هذه المعطيات ودراسة إمكانية تطبيقها حقليا لمراقبة الترسبات البارافينية في الأنابيب والصهاريج النفطية.

Abstract: *One of the most severe problems at any oil field producing paraffin oils is the wax content and paraffin deposition. The microbial treatment, which is based on the activity of the naturally occurring selected isolated bacteria, proved to be an effective alternative method to the conventional chemical methods in preventing and removing wax deposition in oil field facilities and pipelines.*

Various bacterial species were isolated from different local sea water sediments contaminated by petroleum hydrocarbons. The isolated consortia were found to have the ability of producing biosurfactant in the form of biological molecules. The produced biosurfactant showed higher surface activities, capable of reducing surface and interfacial tension at the interfaces between liquids and solids. This phenomenon allows the mixing and dispersion in water and other liquids.

Experimental tests of specific growth rate (μ_{max}), emulsification stability and degradation strategies with G.C assay for Sarir crude oil were

* Petroleum Research Centre, P.O. Box 6431, Tripoli, Libya.
**Chemical Engineering Department, Al Fateh University.
E-mail: asma_etomi@lycos.com

performed before and after treatment. Results showed that biodegradation had minimized the paraffin contents with further expansion in light fraction of the hydrocarbon sequences, in particular the normal alkanes.

The bacterial isolates, responsible for these changes, were successfully isolated and nominated as PRCW B_1, E_1, A_1 and B_2. These isolates will be further tested on several types of paraffinic crude oils in order to verify such findings and support their application in the field to prevent and control paraffin deposition in oil field facilities and pipelines.

INTRODUCTION

Paraffinic hydrocarbons are organic compounds commonly found in crude oil, consisting of various forms and combinations of aliphatic hydrocarbons, aromatic hydrocarbons, napthenes, and asphaltenes. These different forms and combinations give paraffins their characterizations such as melting point, boiling point, solubility, pour point and cloud point.

Paraffins may be deposited throughout the oil production flow lines starting from the oil well reservoirs up to the storage tanks at the refineries. This deposition is due to the system temperature being lowered below the cloud point, and also due to the change in the crude oil composition. The changes in composition might be due to the evaporation of lighter hydrocarbon fractions (C_1-C_5) present in the crude oil.

According to Rocky Mountain Oil Field Testing Center (1997), there are two mechanisms for paraffin deposition. These mechanisms are the shear dispersion and molecular diffusion. The shear dispersion mechanism describes the relationship between deposition rate and the shear rate. The molecular diffusion mechanisms describe the diffusion process of the paraffinic hydrocarbon to the surfaces due to the temperature gradient of the liquid phase.

Since 1900, several methods were proposed and applied to overcome the problem of paraffin deposition. The injection of chemicals such as solvents and dispersants among the conventional methods is very successful for the control of paraffin precipitation and deposition. However, the chemical treatments proved to be costly and highly toxic (Sadeghazad and Ghaemi, 2003).

During the last decade, microbial remediation was found to be an available alternative method over the conventional methods. Microbial treatment can control paraffin deposition by reducing the length of the paraffin molecule and by producing by-products that act as surfactants and paraffin solvents. The cracking of long chain paraffin will produce an increasing in the API gravity and lowering in the cloud point. The bio-production of surfactants and solvents enables the fluid to solubilize the paraffins and remove paraffin-based skin damage from the well bore (Banat *et al*, 1999).

Remediation of Paraffin Precipitation and Deposition

The microorganisms contributing in the microbial treatment of crude oil, are generally live, naturally occurring, specifically isolated bacteria, and must have the following characteristics (Lazar *et al*, 1999).

- Facultative anaerobic, working in the presence or absence of oxygen.
- Non-pathogenic.
- Contain no sulphate-reducing bacteria or slime-forming bacteria.
- Not genetically altered.
- Water based and environmentally safe.

The advantages and benefits of using microbial treatment in biodegrading long chain paraffins are:

1- There will not be any paraffin precipitation or deposition problems along production flow line.
2- The viscosity of crude oil will be reduced and there will be an increase in API.
3- The amount of paraffin will be reduced.
4- Decrease in pour and cloud points of the crude oil at the same time.

MATERIAL AND METHODS

Bacterial Isolation Technique

The first experiments were performed in the chemostat by preparing two liters of enrichment salts media in culture vessel and autoclaving at 121° C for 30 min. After sterilization the system was started up with temperature adjusted to 37° C, pH = 7.3 and agitator 250 rpm.

These experiments were repeated several times with different seawater sediment samples. After a laboratory screening program, the naturally occurring bacterial isolates from waste hydrocarbon polluted site, as pure culture or natural bacteria consortia, were selected as the best degraders of paraffinic oil and used in the rest of the experiments.

Six pure bacterial strains were isolated from sea water sediment and one additional species was isolated from Sarrir crude sample.

Most of the isolates were grown in mineral salts media and produced biosurfactants on waxy crude, n-hexadecane was used as a source of energy and carbon.

The biosurfactants production has been verified by the kerosene tests. The performances in degradation of hydrocarbon contained in paraffinic oils or in paraffin depositions, have been investigated in aerobic conditions on a rotary shaker (in Erlenmeyers flasks of 250 ml) at 37° C for 7-10 days.

For the determination of bacterial ability in the degrading of hydrocarbons containing paraffin depositions, the crude oil extraction methods were implemented (ASTM D 5369-13).

Experimental Culture Conditions

The bacteria were cultivated in a medium containing either 1%, 5%, 10% (vol/vol) of n-hexadecane and crude oil as a carbon and energy source plus 1.5 g/l $(NH_4)_2SO_4$, 4.465 g/l K_2HPO_4, 1.5 g/l $NaH_2PO_4.H_2O$, 0.2 g/l $MgSO_4.7H_2O$, 2 ml trace element solution per litre of medium. Cultures were cultivated in 250 ml Erlenmeyer flasks with 50 ml of medium and incubated with shaking (150 rpm) at 37° C.

Optical Density

The turbidity of the cultures was determined by measuring the optical density (O.D) at a wave length of 540 nm in 2 ml cuvettes using a spectrophotometer.

Biomass (dry weight)

The net dry weight for the dry biomass was determined. 1 ml of culture broth was centrifuged at 1500 rpm for 30 minute then washed with distilled water, vortexes and poured in certain container with a known weight then dried over night at 90° C to constant weight, and cooled for reweighing.

Relationship between the Optical Density and the Dry Weight Biomass

During the batch experiments, the optical density and the dry mass were determined simultaneously. The linear relation between OD_{540} and dry mass was obtained.

Biosurfactant Detection

To detect the presence of the biosurfactant the following tests must be conducted:

1. Xylene or oil emulsification assays.
2. Thin-layer chromatography (TLC)
3. Surface and interfacial tension measurements.

Emulsification Activity and Measurements

There are two procedures performed in this study to measure the emulsification activities as follows:

1. Culture broth was made cell free by centrifuging. 3.5 ml of the broth. Then it was vigorously shaken with 2 ml of toluene and xylene on a vortex shaker and left undisturbed. After one hour, optical density of the oil-in-water emulsion phase was measured at 610 nm wave length. The O.D. was reported as the emulsification activity. After 24 hours the height of the emulsion layer (water-in-oil) was measured and emulsification activity was expressed in cm. The test tubes utilized for activity measurements have diameters of 1 cm, capable of handling a 2 cm substrate layer, which corresponds to 2 mls of tested substrate (Banat, *et al* 1991).

2. Emulsification activities were also measured using the method of Cooper and Goldenberg. In this method; 2 ml of kerosene are added to 2 ml of the culture broth in a screw cap test tube and vortexes at high speed for 2 minutes using VORTEX TOP MIX. The emulsion stability was determined after 24 hours and the measured height of the emulsion layer was divided by the mixture total height and multiplied by 100 (Benbatla, 1995, Bicca, *et al*. 1999).

Gas Chromatographic Analysis

The gas chromatography was used in this research for the determination of the residual content, and the degree of biodegradation on crude oil effluents.

- **Residual Oil**

The residual oil was collected by extraction with a mixture of n- hexane and dichloromethane (1:1). All samples were collected and analyzed by G.C for n-alkanes before and after treatments.

- **Crude Oil Effluent Biodegradation**

The degree of biodegradation of the crude oil

effluents was determined using the chrompack model 439 capillary gas chromatograph equipped with a flame ionization detector (FID), a split / splitless injector and cp-sil 5 CB column (50 m, 0.32 mm, 0.12 μm film thickness). The gas chromatographic conditions were realized with a 300° C detector, 300° C injector, split ratio on 100:1, carrier gas helium flow rate of 62 Kpa and samples of 0.1μl injection. The column temperature of 40° C held 2 minutes, and then increased at a rate 5° C/min and final temperature 300° C for 30 minutes

Future Research Work

The laboratory pilot tests were designed as" flow equipment" simulating the alternation of stationary and flowing periods of the paraffinic crude oils during the production process.

At the end of tests on "flow equipment" samples of the circulated mixture will be collected and analyzed for fluid rheology, viscosity, pour point, wax content and API gravity. This research work investigates the following aspects:

1. The degradation of heavy fractions of crude oil into light fractions.
2. The reduction in viscosity and pour point tempeature of the crude oil.
3. The production of biosurfactents and organic acid to lower the surface tension and interfacial tension.

RESULTS AND DISCUSSIONS

The individual community members which were isolated from the local environment are found and considered to be Actinomycetes, Pseudomonas and Bacilli. This identification was achieved by direct analysis with epifluorescence microscopic observation. All genera display much variation in morphology and capsulation.

The specific growth rate and net dry weight of the different isolates were determined and illustrated in Figures 1 and 2. These figures indicate the effluence of the specific growth rate and biomass precipitation on a period of bacterial cultivation in mineral salts medium containing 0.5% (vol/vol) n-hexadecane. The highest specific growth rate was observed on PRCW B_1, PRCW E_1 with μ_{max} (0.243 h^{-1}) and (0.1926 h^{-1}) respectively. These bacterial isolates were selected for further investigations, since the PRCW B_1 and PRCW E_1 have optimum E_{24} and growth rate over the other isolates (Table 1).

The emulsification index results showed the ability of the isolates to grow with water immiscible hydrocarbon such as kerosene, toluene, xylene and crude oil. This indicates the production of surface-active compounds by the microbial culture, which has been shown to aid the metabolism of the substrate and stimulate microbial growth.

Table 2 shows emulsification index of six isolates on different source of hydrocarbon where all values

Table 1. Maximum growth rate of six isolates.

Bacterial ID	Growth rate (μ_{max}), hr-1
PRCW A_1	0.0673
PRCW A_2	0.1365
PRCW B_1	0.2430
PRCW E_1	0.1926
PRCW E_2	0.0562
PRCW B_2	0.0385

Table 2. Emulsification index of six isolates.

Emulsification index percent				
Bacterial ID	Toluene	Xylene	Crude	Kerosene
PRCW B_1	83	93	100	90
PRCW E_1	30	60	93	66
PRCW A_1	25	50	83	36
PRCW B_2	40	42	100	50
PRCW A_2	50	50	83	53
PRCW E_2	0	0	90	0

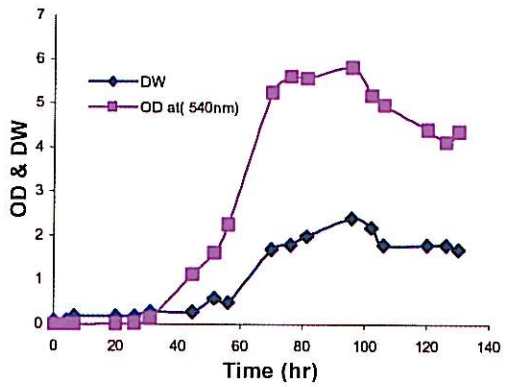


Fig. 1. Growth rate of bacteria type PRCW A_2 (OD = optical density; DW = dry weight (mg/ml)).

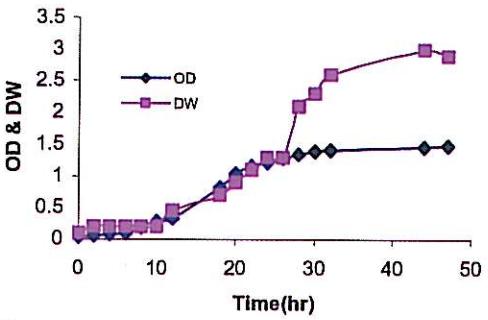


Fig. 2. Growth rate of bacteria type PRCW B_1 (OD = optical density; DW = dry weight (mg/ml)).

of emulsification index on crude oil are greater than values which was obtained on kerosene and other hydrocarbon source, these values reached to (E_{24}=100%) for PRC W B_1, PRC W B_2 and (E_{24}=93%) for PRCW E_1 at about 120 h, Figure 3 shows these results.

A measure of E_{24} on kerosene showed small quantity of surfactant was initially present in the culture broth. After approximately 48 hours of growth, the E_{24} value increases and reaches its maximum value at about 120 h (E_{24}=90%) for the isolate PRC W B_1. There is no any emulsification activity on PRCW E_2 {PRC W E_2 source isolation crude oil}.

Optical density and dry mass were determined simultaneously. The linear relation between OD_{540} and dry mass was obtained as shown in Figures 4 and 5.

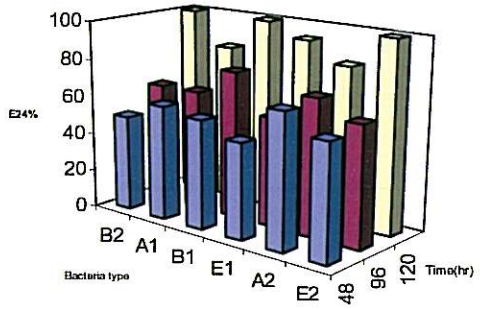


Fig. 3. Emulsification index on crude oil.

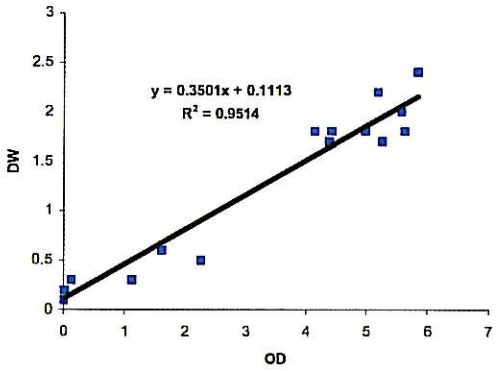


Fig. 4. Relation between biomass and optical density for bacteria PRCW A_2.

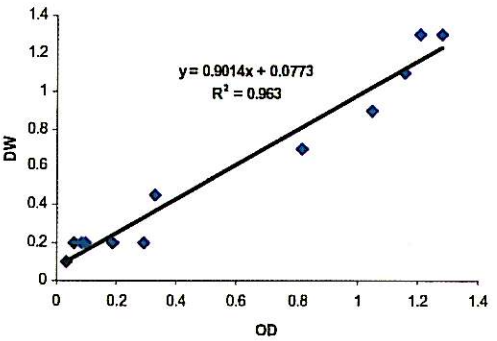


Fig. 5. Relation between biomass and optical density for bacteria PRCW.

Different isolates were able to produce a significant amount of biosurfactants as detected by water-in-oil and oil-in-water emulsions, during their growth on 1% of n-hexadecane. PRCW B_1 showed better performance (height of emulsion 4 cm) when grown for 120 hours in toluene and xylene. These bacterial isolates have good emulsification activities which appeared and were detected by absorbency, as showed in Figures 6 through 9.

The effects of hydrocarbon concentration on different isolates were studied in a batch culture system with different concentration 10 vol%, 5 vol%, 1 vol % of crude oil, Table 3 showed optimum growth rate at 1% with μ_{max} = 0.113hr^{-1} for PRCW E_1 and faster growth shown on PRCW

Table 3. Growth rate of six isolates on different concentrations of crude oil.

	Growth rate , hr^{-1}		
Bacterial ID	10%	5%	1%
PRCW A_1	0.028	0.0373	0.040
PRCW B_1	0.100	0.0852	0.093
PRCW B_2	0.027	0.0241	0.020
PRCW E_1	0.110	0.0500	0.113
PRCW E_2	0.027	0.0430	

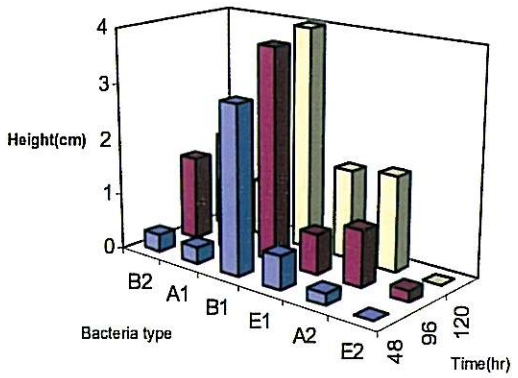


Fig. 6. Water-in-oil emulsification activities in the cell-free culture fluids of the bacterial strains grown and tested on toluene.

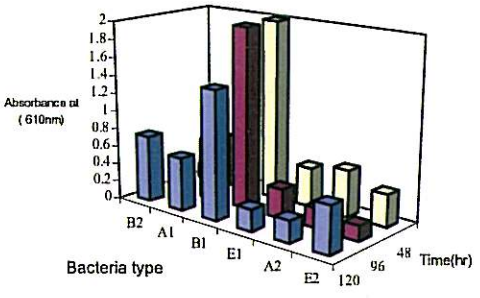


Fig. 7. Oil-in-water emulsification activities in the cell-free culture fluids of the bacterial strains grown and tested on xylene.

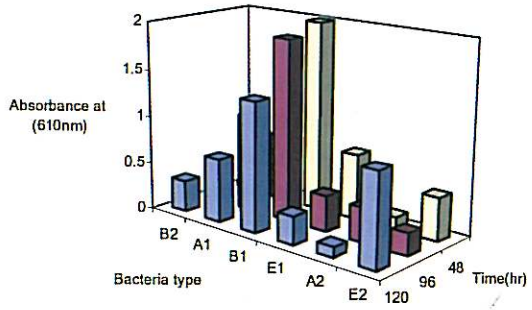


Fig. 8. Oil-in-water emulsification activities in the cell-free culture fluids of the bacterial strains grown and tested on toluene.

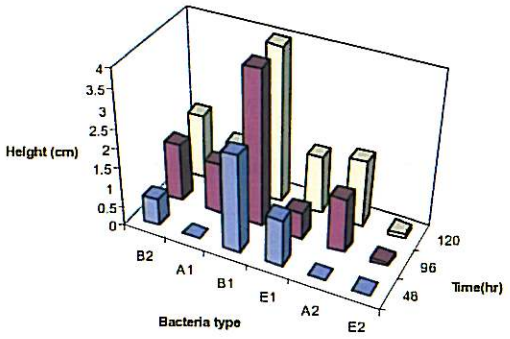


Fig. 9. Water-in-oil emulsification activities in the cell-free culture fluids of the bacterial strains grown and tested on xylene.

B_1, as explained in Figure (10). PRCW B_1 showed nearly the same growth on crude oil concentration 10% by volume, 1% by volume, with $\mu._{max} = 0.1hr^1$ and $\mu._{max} = 0.093hr^1$ respectively, PRCW A_1 showed best growth on 10 vol%, with $\mu._{max} = 0.04hr^{-1}$ and PRCW B_2 best growth on 1 vol%, with $\mu._{max} = 0.027hr^{-1}$.

The laboratory analysis was performed on treated and untreated crude oil samples using gas chromatography. The spectrums of GC analysis have been illustrated in Figures 11 through 13. The results showed a change in the distribution of alkanes and a decrease in the long chain carbon paraffins and an increase in the lighter fraction on isolates PRCW A_1, B_1, B_2 and E_1, while the other

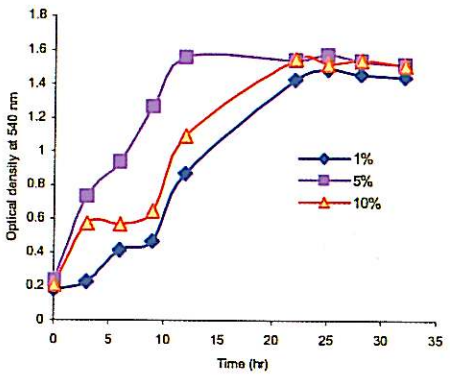


Fig. 10. Growth rate of bacteria PRCW B_1 on different concentrations of crude oil.

species did not show the same effect. Moreover, the concentration changes of n-alkanes obtained from GC analysis have been shown respectively in Figures 14 and 15.

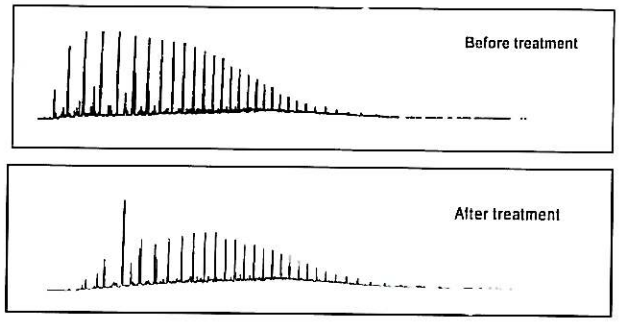


Fig. 11. Comparison of GC spectrums of crude oil before and after treatment with bacterial type PRCW A_1.

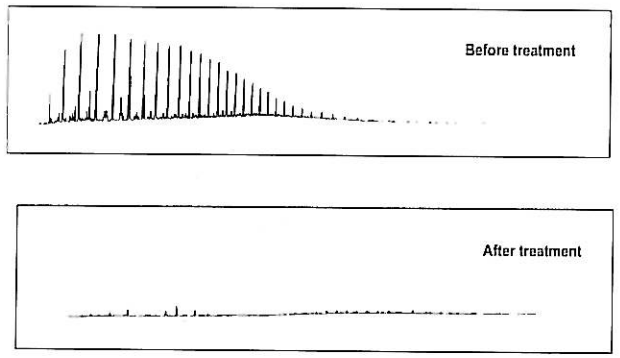


Fig. 12. Comparison of GC spectrums of crude oil before and after treatment with bacterial type PRCW B_1.

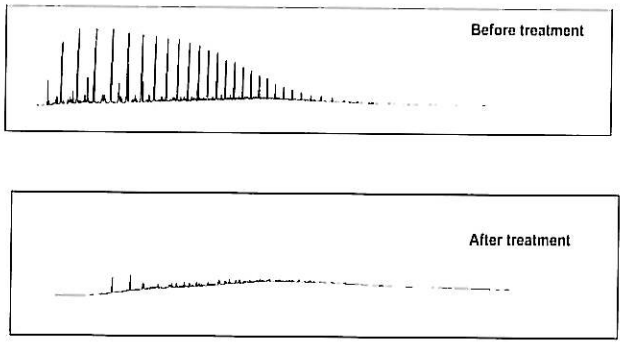


Fig. 13. Comparison of GC spectrums of crude oil before and after treatment with bacterial type PRCW E_1.

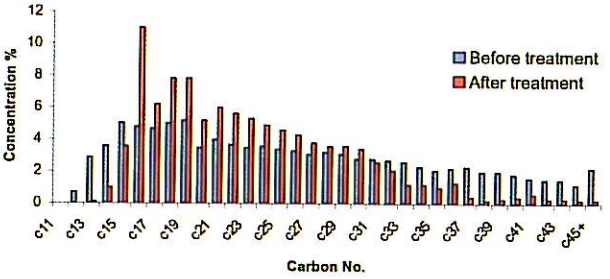


Fig. 14. GC. analysis of treated crude oil (5% concentration) with isolate PRCW A_1 after 21 days.

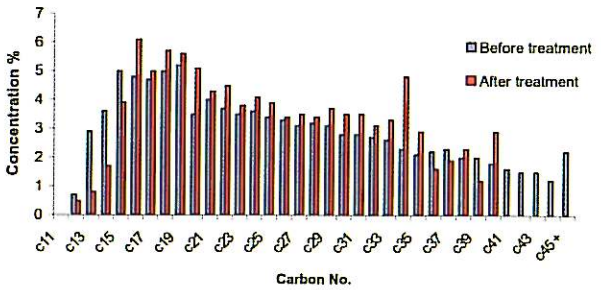


Fig. 15. GC. analysis of treated crude oil (5% concentration) with isolate PRCW B_2 after 21 days.

CONCLOUSIONS

1. Several different bacterial hydrocarbon degraders were detected in pure consortium from sea water sediment contaminated by waxy crude oil.

2. Specific bacterial cells PRCW B_1 have shown emulsification activity up to 100%, while the other species such as PRCW E_1 shows 93% emulsification activity. These two isolates can be targeted for selection in preventing or controlling wax deposition in field application.

3. After microbial treatment degradation rate by PRCW B_1 isolates on Sarir crude samples showing the highest values of n-alkanes reduction when exposed to PRCW B_1.

4. The same growth rate was achieved with PRCW B_1 in the concentration of 1% and 10% of crude oil which means that this type of isolates could be used in the field trial to prevent and control wax deposition.

5. PRCW A_1, B_1, B_2 and E_1 isolates have shown greater reduction rate of the heavy fraction of the original waxy crude oil sample (Sarir crude) due to their higher emulsification performance.

RECOMMENDATIONS

1. Biodegradation of bacteria for higher concentration of crude oil has to be verified specifically for 30% or higher.

2. Perform rheology tests for examining the isolates to determine the API gravity, cloud, pour point temperatures and viscosity.

3. Dynamic flow test experiments need to be carried out for simulating the field conditions.

REFERENCES

American Standard Test Method. ASTM D 5369-13.

Banat. M., Samarh. N., Murad. M., Horne. R. and Banerjee. S. 1991. Biosurfactent production and use in oil tank clean-up. Wld J. Microbiol. Biotechnol. 7,80-88.

Banat, I.M., Makkar. R.S., Cameortra S.S. 1999. Potential commercial applications of microbial surfactants. Appl. Microbiol. Biotechnol .53, 495-508.

Benbatla, S. 1995. Isolation of biosurfactent producing bacteria from oil contaminated soils. November Boumerdes 35000 Alger, 289-294.

Bicca. F. C., Fleck L.C., M.A. and Zachia. 1999. Production of biosurfactant by hydrocarbon degrading *Rhodococcus* rubber and *Rhodococcus erythropolis*. Rev.microbiol.30, 0001-3714.

Cooper, D.G. 1982. Biosurfactents and Enhanced Oil Recovery, pro.int. Conf microbial enhanced oil recovery, Afton, ok, doe conf-85051 .40, 112-114.

Rocky Mountain Oil Field Testing Center. December 17, 1997. Chemical and microbial paraffin control project.

Lazar, I., Voicu, A., Nicolescu, C., Mucenica, D., Dobrota, S., Petrisor, G. I., Stefanescu, M. and Sandulescu, L., 1999. The use of naturally occurring selectively isolated bacteria for inhibiting paraffin deposition, Journal of Petroleum Science and Engineering. 22, 161-169.

Sadeghazad, A. and Ghaemi, N., 2003. Microbial prevention of wax precipitation in crude oil by biodegradation mechanism. Society of petroleum engineers. SPE 80529, 1-11

Technical memorandum B-1.July 10, 2002. Microbial technology for improvement of oil quality.