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TECHNICAL NOTE

Study on the role of *Nocardia farcinica* in enhancing the flow rate of crude oil

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ABSTRACT

Microbial degradation of paraffin wax is an efficient method of removing wax deposition from pipelines and enhancing the flow rate of crude oil. The present study was carried out to isolate a potential paraffin-degrading organism from oil wells of Gujarat. Screening for bacteria-utilizing paraffin wax as the sole source of carbon was carried out using 2,6-dichlorophenol indophenol (DCPIP) dye as redox indicator. The selected organism was identified as *Nocardia farcinica* by 16S rRNA sequencing. *Nocardia farcinica* showed 100% degradation of heneicosane, 65.99% degradation of docosane, and 50.59% degradation of tricosane, the major components of paraffin wax, in 8 days, which was observed by gas chromatography. Eicosane (86%) and heneicosane (80%) were utilized more by the selected organism compared with octacosane (61%) and triacontane (58%) (DCPIP dye method). Gas chromatographic analysis revealed that the selected organism degraded 50% of paraffin crude oil in 10 days. To determine the ability of the selected organism to enhance flow rate, parameters such as viscosity (cps), surface tension (d/cm^2), pour point (°C), and flow rate (min/2 ml) were determined, and the result showed significant reduction in all the parameters after the addition of *Nocardia farcinica*. The viscosity and surface tension of crude oil were reduced by 22 and 6.30 points, respectively, after the addition of *Nocardia farcinica*. Pour point and flow rate were reduced by 2 and 11 points, respectively, when compared with control. The above findings indicate that *Nocardia farcinica* isolated from crude oil plays a major role in enhancing the flow rate of crude oil.

KEYWORDS

Crude oil; 2,6-DCPIP; flow rate; gas chromatography; paraffin; pour point; surface tension; viscosity

Introduction

Crude oil is a naturally occurring, flammable liquid found in rock formations in the earth. It consists of various forms and combinations of aliphatic hydrocarbons, aromatic hydrocarbons, asphaltenes, resins, and naphthenes (Addison 1984). It has high melting points and cloud points. Waxes that are known as mostly heavy saturated paraffin tend to precipitate when the temperature and pressure of oil fields drop during the production and transportation. Wax appearance temperature (WAT) determines the deposition of the wax on the reservoir or pipelines when the temperature of the reservoir or production line falls below the WAT. WAT is regularly used to measure the tendency of a crude oil to produce wax in relation with pressure and temperature reduction. Wax deposition will be complex and costly. It will cause a lot of difficulties by blocking of transport equipments and pipelines. This can eventually decrease or in worst condition block the flow in the production line (Sanjay, Simantha, and Kulwant 1995; Merino-Garcia and Correra 2008; Al-Yaari 2011; Taraneh et al. 2008; Alghanduri, et al. 2010; Zhu, Walker, and Liang 2008; Elsharkawy, Al-Sahhaf, and Fahim 2000; Aiyejina et al. 2011). The flowability of crude oil is determined by the viscosity/surface tension of crude oil. Reduction of viscosity can be carried out by using microbes that have the ability to degrade hydrocarbons. Individual microbes or consortium of microbes can be used to reduce viscosity/surface tension of crude oil and thereby enhance the flow rate of crude oil.

Bacteria are the most active agents in petroleum degradation, and they play a major role as primary degraders of crude oil in the environment (Singh and Lin 2008). Several bacteria are even known to feed exclusively on hydrocarbons and produce various surface-active products (Satpute et al. 2010; Vasconcellos et al. 2011; Varjani et al. 2014). These surface-active materials increase the surface area of hydrophobic

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water-insoluble substrates by reducing surface tension and interfacial tension. This in turn increases their bioavailability, thereby enhancing the growth of bacteria, the rate of bioremediation, and oil recovery when applied in the field. Examples of oil-degrading bacteria are *Acinetobacter, Pseudomonas, Phenylobacterium, Stenotrophomonas, Gluconobacter, Agrobacterium, Vibrio, Micrococcus, Aeromonas, Beijerinckia, Flavobacterium, Corynebacterium, Sphingomonas, Microbacterium, Paracoccus, Burkholderia*, and many more (Jacques et al. 2007). The present study was carried out to analyze the role of *Nocardia farcinica* in enhancing the flow rate of crude oil.

Materials and methods

Sample collection

Crude oil samples were collected from different oil wells of an oil field in Limbodara Village, Gandhinagar of Gujarat State, India. Crude oil samples contained asphaltenes, resins, and wax in the range of 0.4–2.4%, 7.28–10.58%, and 15.06–21.45%, respectively. The crude oil sample used for isolating paraffin-degrading microorganisms had a density of 915.8 kg/m^3 (15°C), American Petroleum Institute (Gravity Scale; API) of 22.93, viscosity of 996 cps (30.1°C), and pour point of 30°C.

Chemicals and media

Minimal salts medium was used for bacterial isolation and cultivation (Lal and Khana 1996). The minimal salts medium is a mixture of two solutions, B_1 and B_2. Solution B_1 contains $MgSO_4{\cdot}7H_2O$ 0.5 g, KNO_3 0.5 g, NH_4Cl 0.5 g, trace element solution 10 ml, and vitamin solution 1 ml in distilled water 900 ml (pH 7.2). Solution B_2 contains KH_2PO_4 1 g and K_2HPO_4 1 g in distilled water 100 ml. Solutions B_1 and B_2 were autoclaved separately and then mixed aseptically after cooling. Steam-sterilized paraffin was added as the carbon source to this medium.

Trace element solution consisted of nitrilotriacetic acid 1.5 g, $MgSO_4{\cdot}2H_2O$ 3.0 g, $MnSO_4{\cdot}2H_2O$ 0.5 g, NaCl 1.0 g, $FeSO_4{\cdot}7H_2O$ 0.1 g, $CoCl_2$ 0.1 g, $CaCl_2{\cdot}2H_2O$ 0.1 g, $ZnSO_4$ 0.01 g, $CuSO_4{\cdot}5H_2O$ 0.01 g, $Alk(SO_4)_2$ 0.01 g, H_3BO_3 0.01 g, and Na_2MoO_4 0.01 g in distilled water 100 ml (pH to 6.5).

Vitamin solution consisted of biotin 2.0 mg, folic acid 2.0 mg, vitamin B_{12} 0.1 mg, pyridoxine HCl 10.0 mg, thiamine HCl 5.0 mg, riboflavin 5.0 mg, nicotinic acid 5.0 mg, calcium pentothionate 5.0 mg, *p*-aminobenzoic acid 5.0 mg, and lipoic acid 5.0 mg in distilled water 100 ml.

Chemicals obtained from HiMedia (Mumbai, India) and the paraffin wax (melting point 58–60°C) used in the study was from Merck India (Mumbai, India). All other chemicals used for the study were of standard analytical grade.

Enrichment and isolation of paraffin wax–degrading bacteria

The paraffin wax–degrading bacteria were isolated by enrichment culture technique from paraffin crude oil samples. For enrichment, 5 ml paraffin crude oil samples were inoculated into 100 ml of minimal salts medium (MSM) (Lal and Khanna 1996) and incubated at 37°C (temperature selected based on paraffin wax formation) on a rotary shaker (150 rpm) for 7 days. After three cycles of enrichment, 0.1 ml was plated on MSM agar plates with paraffin wax (0.1% *w*/*v*) as the carbon source. Morphological characters of bacterial colonies formed on the paraffin-containing MSM plates were noted. Colonies with different morphological characters were purified by subculturing onto paraffin-containing MSM.

Screening of paraffin wax–degrading bacteria

Screening of potential paraffin wax–degrading bacteria was carried out by modified Hanson, Desai, and Desai (1993) and Bidoia, Montagnolli, and Lopes (2010) methods, using 2,6-dichlorophenol indophenol (DCPIP) as redox indicator. By incorporating an electron acceptor such as DCPIP to the culture medium, it is possible to ascertain the ability of the microorganisms to utilize hydrocarbon substrate by observing the color change of DCPIP from blue (oxidized) to colorless (reduced), which was monitored at 600 nm wavelength.

Inoculum was prepared by transferring culture into Luria-Bertani medium for 24 h at 37°C at 120 rpm. Screening of potential paraffin wax–degrading bacteria was monitored in 100-ml Erlenmeyer flasks (in triplicate) containing 22 ml MSM, 2 ml DCPIP (0.01%) dye, 0.05 g of paraffin wax as the sole carbon source, and 1 ml of culture inoculum (10^7-10^8 cells/ml). The samples were incubated on a rotary shaker (120 rpm) at 37°C for 6 days. The absorbance of all the assays

was measured at 600 nm at regular time intervals of 24 h for 6 days. The percentage of dye reduction was calculated using the formula given below. The bacterial strains, which showed high percentage of DCPIP decolorization, were chosen as the best paraffin wax degraders.

$$\% \text{ Dye reduction } = C - T/C \times 100$$

where C is the control and T is the test culture.

Characterization and identification of the most efficient paraffin wax–degrading strain

The efficient paraffin wax–degrading bacterial strain was grown on Luria-Bertani agar at 37°C and characterized by morphological studies. The strain was also characterized by Gram's staining and biochemical tests. The strain was identified by 16S rRNA gene sequencing at GSBTM (Gujarat State Bio-Technology Mission). The sequence was deposited in the NCBI (National Center for Biotechnology Information) database and was assigned the accession number KJ210575.

Growth pattern of the selected organism

Growth pattern of the selected organism was measured by spectrophotometric method. Twenty-four milliliter MSM and 0.05 g of paraffin wax as the sole carbon source were inoculated with 1 ml culture (10^8 cells/ml). The flask was incubated on a rotary shaker (120 rpm) at 37°C for 6 days. The absorbance was measured at 600 nm at regular time intervals of 24 h till 6 days.

Extraction of undegraded paraffin and its analysis by gas chromatography

Degradation potential of paraffin wax by the selected isolate was monitored in 250-ml Erlenmeyer flasks (in triplicate) containing 100 ml MSM with 0.1% (*w*/*v*) of paraffin wax as the sole carbon source, and samples were incubated on a rotary shaker (150 rpm) at 37°C for 8 days. The isolates were grown overnight in Luria-Bertani broth, and 5% (*v*/*v*) of this inoculum (10^8 cells/ml) was used to inoculate MSM with paraffin wax. Uninoculated controls were maintained to monitor abiotic loss of paraffin wax. The residual undegraded paraffin wax was extracted thrice with equal volumes of hexane. For quantitative analysis, 1 μl of the paraffinic wax (dissolved in 10 ml hexane) was analyzed by gas chromatography (GC; PerkinElmer [Waltham, MA, USA]; auto-system XL model with flame ionization detectors [FID]) on a silica-based column using the method described by Mishra et al. (2001). The GC profile of the paraffinic fraction, extracted from inoculated flasks, was compared with that of obtained from uninoculated control flask.

Evaluation of the ability of the selected organism to degrade various alkanes present in paraffin wax

Degradation of alkanes (eicosane, heneicosane, octacosane, triacontane) that represent the paraffin wax was monitored in 100-ml Erlenmeyer flasks (in triplicate) containing 22 ml MSM, 2 ml DCPIP (0.01%) dye, 0.05 g of alkanes as the sole carbon source, and 1 ml culture inoculum (10^8 cells/ml). The samples were incubated on a rotary shaker (120 rpm) at 37°C for 6 days. The absorbance was measured at 600 nm at regular time intervals of 24 h till 6 days. Percentage of dye reduction was obtained using the formula mentioned in Screening of Paraffin Wax–Degrading Bacteria.

Degradation of paraffin crude oil by the selected strain

Degradation of paraffin crude oil by the selected strain was monitored in 250-ml Erlenmeyer flasks (in triplicate) containing 100 ml MSM with 2% (*w*/*v*) of paraffin crude oil collected from problematic wells of Limbodara oil wells of Gujarat State, India. The medium was inoculated with 5% overnight grown culture (10^8 cells/ml) of the selected paraffin wax–degrading strain in Luria-Bertani medium and incubated in shaking conditions (150 rpm) at 37°C for 10 days. At the end of the incubation period, the residual paraffin crude oil was extracted thrice with equal volumes of hexane using a liquid-liquid extraction technique. Hexane layer was separated and was analyzed by GC (PerkinElmer; Auto-system XL model, silica-based column with flame ionization detectors [FID]).

Study on the role of Nocardia farcinica in enhancing the flow rate by changing the physical characteristics of crude oil (surface tension, viscosity, pour point, and flow rate)

Degradation of paraffin crude oil by the selected strain was monitored in 250-ml Erlenmeyer flasks containing

100 ml MSM with 2% (*w*/*v*) of paraffin crude oil. After degradation, extraction of crude oil from medium was done using hexane in a separating chamber. This extracted oil was used for the analysis of surface tension, viscosity, pour point, and flow rate of crude oil.

Viscosity of extracted oil was measured automatically at 40°C by viscometer and compared with the control crude oil (Symon 1971). Surface tension of distilled water and extracted oil was measured at 40°C by Du Noüy tensiometer. The instrument was set to zero before the commencement of the study. A 20–50 ml of distilled water was taken in a clear beaker. Platinum ring of the instrument was first immersed completely inside the water level. Later slowly, the level of the platinum was adjusted in such a way that the surface of the ring sits above the surface of liquid (in this case it is distilled water). The surface tension was measured by slowly raising the platinum ring until the liquid film between the liquid and ring breaks. The same procedure was followed for extracted oil. The surface tension was calculated as follows:

$$r = r_0 \times \theta/\theta_o$$

where r is the surface tension of the sample (dyne/cm); r_0 is the surface tension of distilled water (standard) (dyne/cm); Θ is the Verniar reading of the sample; and Θ_o is the Verniar reading of distilled water (standard).

Pour point was measured by pour point analyzer. The extracted oil was cooled inside a cooling bath to allow the formation of paraffin wax crystals. At about 9°C above the expected pour point, and for every subsequent 3°C, the test jar was removed and tilted to check for surface movement for 5 s. The temperature at which the oil flow stops was noted, and 3°C was added to the corresponding temperature. This temperature was considered as pour point.

The flow efficiency of oil was detected by the flow meter. The extracted oil sample was poured in to the funnel that was situated above the flow meter. From the funnel, it was passed through a spiral coil present inside the flow meter (pipe). The time was measured using a stopwatch when the oil was flowing in spiral coil. The flow efficiency of the extracted oil was compared with control (crude oil). It gave the indication whether the flow efficiency of oil was improved or not.

Statistical analysis

To compare the effect of various treatments employed in the study, the experimental data were analyzed statistically using analysis of variance (ANOVA). $p < .05$ was considered to be significant.

Results

Enrichment and isolation of paraffin wax–degrading bacteria

For the enrichment of paraffin wax–degrading bacteria, the minimal salts medium (MSM) was supplied with paraffin crude oil as the sole carbon source and incubated at 37°C for 7 days. After three repeated subculturing, a stabilized wax-degrading consortium was obtained. When this consortium was plated on MSM agar plates containing wax, 12 morphologically distinct bacterial colonies were yield.

Screening of efficient paraffin wax–degrading bacteria by spectrophotometric method

Following enrichment and isolation, all the 12 isolates were screened for their efficiency of paraffin wax degradation (as a sole carbon source) using 2,6-dichlorophenol indophenol (DCPIP). Color change of DCPIP was observed, which was measured at 600 nm at regular time intervals of 24 h till 6 days. Out of the 12 isolates, the isolate that showed 86% dye reduction within 6 days was selected.

Characterization and identification of the most efficient paraffin wax–degrading strain

The colonies of the strain appeared rough, convex with irregular margins, and white-orange in color. The selected strain was a gram-positive rod, nonmotile, spore forming, and aerobic. It was able to grow at temperatures from 25 to 50°C and could utilize glucose (trehalose). It was catalase, citrate, urease, and nitrate reductase positive and malonate, Ortho-Nitrophenyl β-galactoside test (ONPG), and oxidase negative. The light microscopy photographs and scanning electron micrographs of these cells are shown in Figure 1.

The sequence, when analyzed by comparison for 16S rRNA gene sequences of GenBank, revealed a 99% homology (Table 1) to the 16S sequence of the *Nocardia farcinica* strains IFM 0221, IFM 0228, and IFM 10152 (Figure 2). So the selected strain was identical to *Nocardia farcinica*.

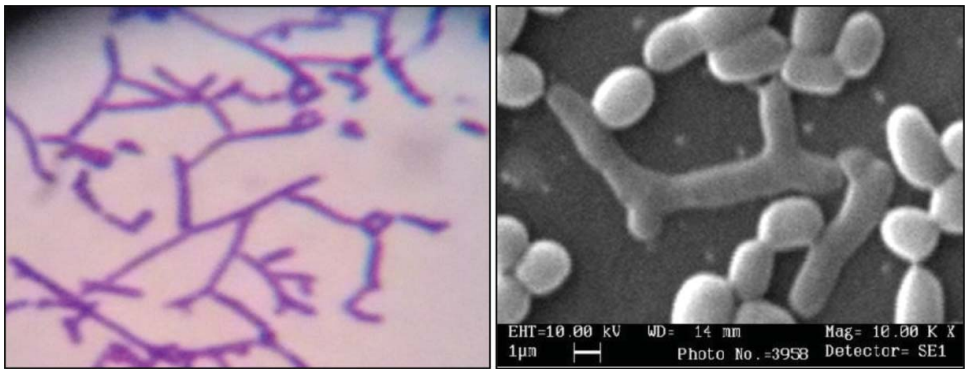

Figure 1. Light micrograph (100× magnification) of Gram-stained cells of *Nocardia farcinica* (left) and scanning electron micrograph of *Nocardia farcinica* (right).

Table 1. Study on the role of *Nocardia farcinica* in changing the physical characteristics of crude oil.

Parameter	Control	Test
Viscosity at 40°C by viscometer	210 cps	188 cps
Surface tension at 40°C by Du Noüy tensiometer	80.43 d/cm^2	74.13 d/cm^2
Pour point by pour point analyzer	32°C	30°C
Flow rate by flow meter	106 min/2 ml	95 min/2 ml

Growth pattern of Nocardia farcinica

Growth pattern of *Nocardia farcinica* was analyzed by spectrophotometric method. The organism showed a lag phase for the first day. It required 24 h for adapting to the paraffin-containing medium. After 24 h, the number of cells increased exponentially (log phase) till the fourth day of inoculation. After the fourth day of inoculation, the stagnant phase was observed (Figure 3).

Degradation potential of paraffin wax by the selected bacterial strain

Degradation potential of paraffin wax by the selected strain was measured by gas chromatographic method (Figure 4). Gas chromatogram of paraffin wax showed various paraffin components, from heneicosane (C21) to pentatriacontane (C35). Gas chromatogram of paraffin wax treated with the selected strain showed 100% utilization of heneicosane, 65.99% utilization of docosane, and 50.59% degradation of tricosane, the components of paraffin wax after incubation of 8 days.

Degradation of alkanes representing the paraffin wax by the selected bacterial strain

The selected strain efficiently degraded alkanes (eicosane, heneicosane, octacosane, triacontane) that represent the paraffin wax, but there was a difference in the rate of utilization of the four alkanes. The percentage of dye reduction in eicosane-, heneicosane-, octacosane-, and triacontane-containing MSM was found to be 86%, 80%, 61%, and 58%, respectively. The other hydrocarbons were degraded less by the organism, as the percentage of dye reduction was found to be less when compared with eicosane and heneicosane (Figure 5).

Degradation of paraffin crude oil by the selected bacterial strain

Degradation efficiency of paraffin crude oil by the selected strain was measured by GC (Figure 6). Gas chromatogram of paraffin crude oil showed various

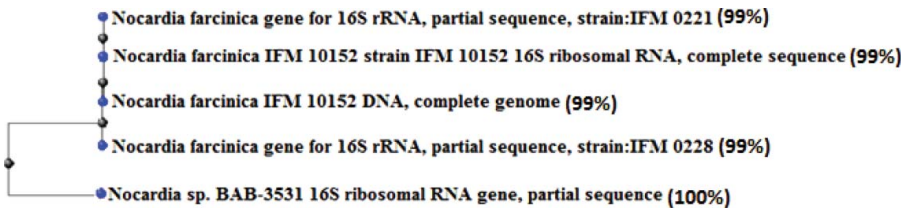

Figure 2. Phylogenetic tree constructed by neighbor-joining method.

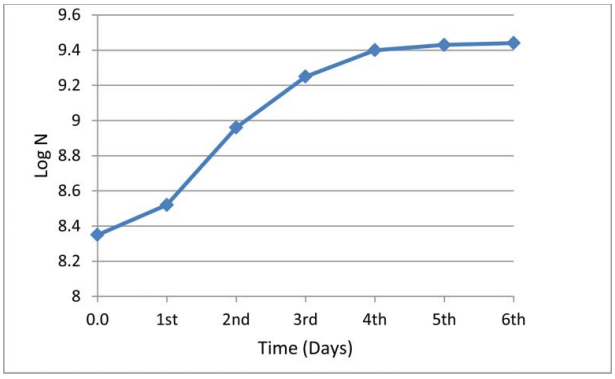

Figure 3. Growth pattern of *Nocardia farcinica* in MSM with paraffin wax.

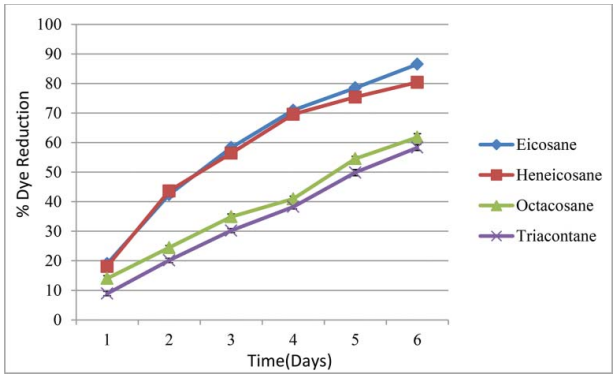

Figure 5. Degradation of different alkanes representing the paraffin wax by *Nocardia farcinica* at 37°C. Values are mean ± *SD*. $n = 3$.

paraffin components with a carbon chain length of C5 to C29 (Figure 7). The selected strain could effectively degrade all the carbon fractions of crude oil. Gas chromatogram of paraffin crude oil treated with the selected strain showed 50% degradation of this paraffin crude oil fraction after incubation of 10 days (Figure 8).

Evaluation of various physical parameters to test the role of Nocardia farcinica *in enhancing the flow rate of crude oil*

Parameters such as viscosity, surface tension, pour point, and flow rate were determined to investigate the role of *Nocardia farcinica* in enhancing the flow rate. The results showed that the viscosity and surface tension of crude oil were reduced by 22 and 6.30 points, respectively, after the addition of *Nocardia farcinica*. Pour point and flow rate were reduced by 2 and 11 points, respectively, when compared with control (Table 1).

Discussion

Crude oil is a complex mixture mainly containing insoluble compounds, including long-chain alkanes (up to C40 and above), that are not easily dispersed in

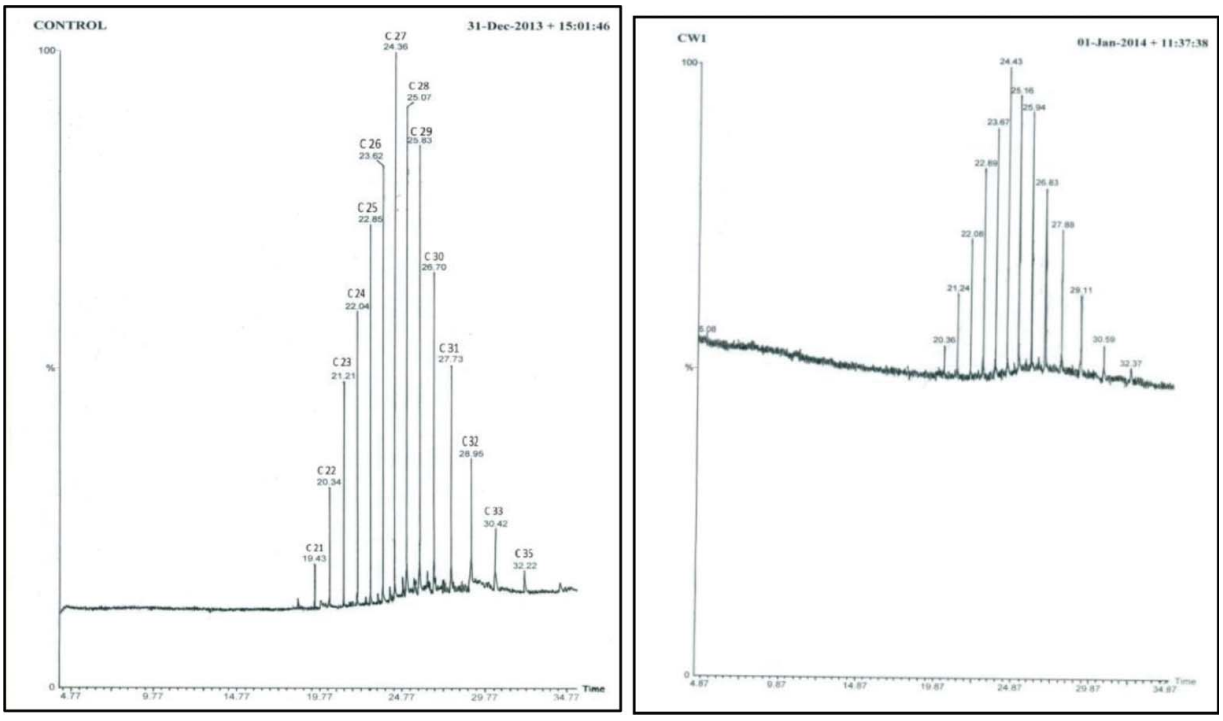

Figure 4. Degradation of different carbon fractions of the paraffin wax by *Nocardia farcinica* at 37°C in 8 days.

Figure 6. Dispersion of degraded crude oil due to degradation by *Nocardia farcinica* after incubation of 10 days at 37°C.

water. Paraffinic oils with high proportions of long-chain *n*-alkanes of C18 and longer (paraffin waxes) cause problems during extraction, transportation, and handling due to the formation of wax deposits, which lead to clogging of, e.g., transportation pipes (Burger, Perkins, and Striegler 1981; Singh et al. 2001; Azevedo and Teixeira 2003; Correra et al. 2007). Oil industry loses millions of dollars due to lowered productivity of oil from oil wells and in remedial measures. To address the problem of paraffin deposition in oil well tubing and pipelines, crude oil samples were collected from Gujarat State of western India. These oil samples were then enriched using paraffin wax as the sole carbon source in minimal salts medium. The use of minimal salts medium with wax as the carbon source was to make the enriched bacteria become acclimatized to the nutrition that would be available to it in the harsh reservoir conditions. The strains thus obtained were purified.

Screening for potential hydrocarbon-utilizing bacteria by DCPIP assay has been reported (Afuwale and Modi 2012; Mariano et al. 2008; Varjani et al. 2013). DCPIP dye is an enzyme-catalyzed oxidation electron acceptor and pH-dependant redox indicator. It is blue when oxidized and colorless when reduced. Its loss of color is monitored spectrophotometrically at 600 nm. As microorganisms utilize hydrocarbons present in crude oil, DCPIP is reduced; due to this, as time passes after inoculation, DCPIP become colorless. All the isolates were screened for their efficiency to utilize paraffin wax (as a sole carbon source) using DCPIP indicator. Color change of DCPIP was observed periodically at intervals of 24 h till 6 days visually, and optical density was measured. Based on the percentage of DCPIP decolorization, the isolate that showed 86% dye reduction within 6 days was selected. All isolates were not able to decolorize DCPIP completely in 6 days.

The strain was characterized on the basis of its morphological and biochemical characteristics. The selected organism was identified as *Nocardia farcinica* by 16S rRNA sequencing. The ability of organism to degrade paraffin was confirmed by gas

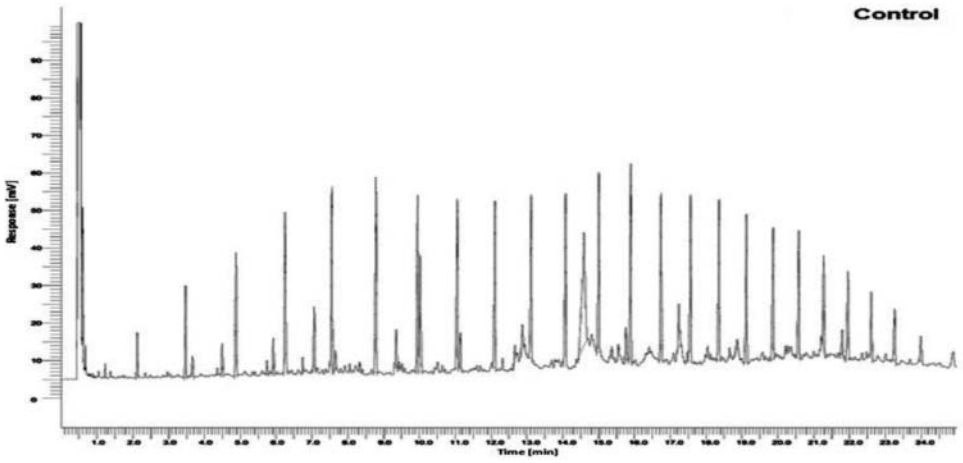

Figure 7. Standard gas chromatogram of paraffin crude oil.

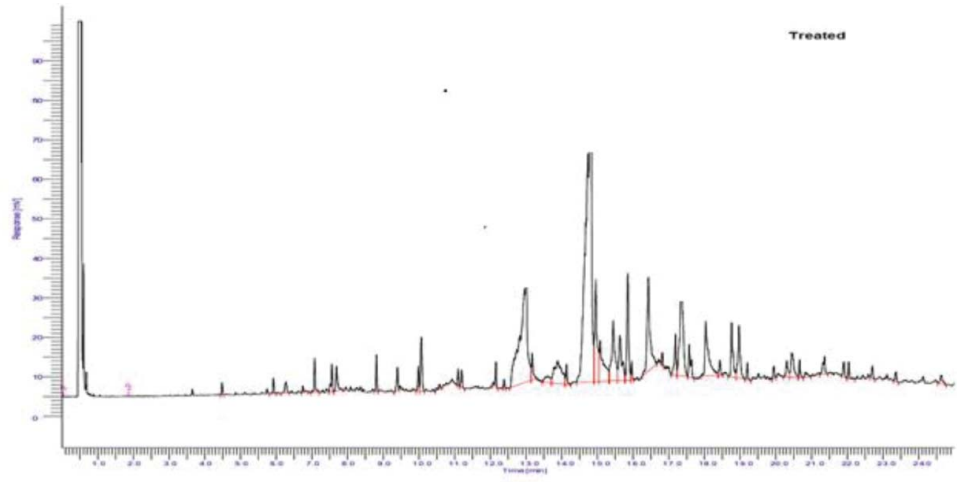

Figure 8. Gas chromatogram of paraffin crude oil treated with *Nocardia farcinica* at 37°C for 10 days.

chromatographic analysis. *Nocardia farcinica* showed 100% degradation of heneicosane, 65.99% degradation of docosane, and 50.59% degradation of tricosane, the major components of paraffin wax, in 8 days, which was observed by gas chromatography. Gas chromatographic analysis revealed that *Nocardia farcinica* degraded 50% of paraffin crude oil in 10 days.

Das and Mukherjee (2006) isolated *Bacillus subtilis* and *Pseudomonas aeruginosa* strains from a petroleum oil–contaminated soil from northeast India that were able to degrade crude petroleum oil. Few studies (Annweiller et al. 2000; Ijah and Antai 2003; Sorkhoh et al. 1993) have reported on the roles of *Bacillus* spp. in hydrocarbon bioremediation. Lazar et al. (1999) achieved 95.3% petroleum hydrocarbon degradation from paraffin crude oil at 28°C in 10–12 days by using a consortium of 15 bacterial isolates. Etoumi (2007) noticed the reduction in wax appearance temperature and heavy hydrocarbon fractions by biodegradation of paraffinic hydrocarbons using *Pseudomonas* and *Actinomyces* species. Mukherjee and Bordoloi (2011) found bacterial consortium consisting of *Bacillus subtilis* DM-04 and *Pseudomonas aeruginosa* M and NM strains that showed a significant reduction in total petroleum hydrocarbon level in contaminated soil (76% degradation) as compared with the control soil (3.6% degradation) 180 days post-inoculation. When compared with all these bacteria, *Nocardia farcinica* degraded hydrocarbons within 10 days, indicating its paraffin wax–degrading potential.

Parameters such as viscosity, surface tension, pour point, and flow rate were determined to investigate the role of *Nocardia farcinica* in enhancing the flow rate. The viscosity and surface tension of crude oil were reduced by 22 and 6.30 points, respectively, after the addition of *Nocardia farcinica*. Pour point and flow rate were reduced by 2 and 11 points, respectively, when compared with control.

Conclusion

Nocardia farcinica was isolated from crude oil samples obtained from oil wells of Gujarat. *Nocardia farcinica*'s ability to degrade paraffin was confirmed by the comparison of gas chromatogram of paraffin and the chromatogram obtained after inoculation of the organism. Gas chromatograms showed 100% utilization of heneicosane, 65.99% utilization of docosane, and 50.59% degradation of tricosane, the major components of paraffin wax. The selected organism degraded 50% paraffin crude oil in 10 days. The viscosity and surface tension of crude oil were reduced by 22 and 6.30 points, respectively, after the addition of *Nocardia farcinica*. Pour point and flow rate were reduced by 2 and 11 points, respectively, when compared with control.

References

Addison, G. E. 1984. Paraffin control more cost effective. *Soc. Petrol. Eng. J.* SPE-13391-MS.

Afuwale, C., and H. A. Modi. 2012. Study of bacterial diversity of crude oil degrading bacteria isolated from crude oil. *Life Sci. Leaflets* 6:13–23.

Aiyejina, A., D. P. Chakrabarti, A. Pilgrim, and M. K. S. Sastry. 2011. Wax formation in oil pipelines: A critical review. *Int. J. Multiphase Flow* 37:671–694.

Alghanduri, L. M., M. M. Elgarni, J. L. Daridon, and J. A. Coutinho. 2010. Characterization of Libyan waxy crude oils. *Energy Fuels* 24:3101–3107.

Al-Yaari, M. 2011. Paraffin wax deposition: Mitigation and removal techniques. In *Proceedings of the SPE Saudi Arabia Section Young Professionals Technical Symposium*, Dhahran, Saudi Arabia. SPE-155412-MS.

Annweiller, E., H. H. Richnow, G. Antranikian, S. Hebenbrock, C. Garms, S. Franke, W. Francke, and W. Michaelis. 2000. Naphthalene degradation and incorporation of naphthalene-derived carbon into biomass by the thermophile *Bacillus thermoleovorans*. *Appl. Environ. Microbiol.* 66:518–523.

Azevedo, L. F. A., and A. M. Teixeira. 2003. A critical review of the modeling of wax deposition mechanisms. *Petrol. Sci. Technol.* 21:393–408.

Bidoia, E. D., R. N. Montagnolli, and P. R. M. Lopes. 2010. Microbial biodegradation potential of hydrocarbons evaluated by colorimetric technique: A case study. In *Current research, technology and education topics in applied microbiology and microbial biotechnology*, ed. A. Mendez-Vilas, 1277–1288. Spain: Formatex Publishing.

Burger, E. D., T. K. Perkins, and J. H. Striegler. 1981. Studies of wax deposition in the trans Alaska pipeline. *J. Petrol. Technol.* 33:1075–1086.

Correra, S., A. Fasano, L. Fusi, and D. Merino-Garcia. 2007. Calculating deposit formation in the pipelining of waxy crude oils. *Meccanica* 42:149–165.

Das, K., and A. K. Mukherjee. 2006. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. *Bioresour. Technol.* 98:1339–1345.

Elsharkawy, A. M., T. A. Al-Sahhaf, and M. A. Fahim. 2000. Wax deposition from Middle East crudes. *Fuel* 79:1047–1055.

Etoumi, A. 2007. Microbial treatment of waxy crude oils for mitigation of wax precipitation. *J. Petrol. Sci. Eng.* 55:111–121.

Hanson, K. G., J. D. Desai, and A. J. Desai. 1993. A rapid and simple screening technique for potential crude oil degrading microorganisms. *Biotechnol. Technol.* 7:745–748.

Ijah, U. J. J., and S. P. Antai. 2003. Removal of Nigerian light crude oil in soil over a 12-month period. *Int. Biodeterior. Biodegrad.* 51:93–99.

Jacques, R. J. S., B. C. Okeke, F. M. Bento, M. C. R. Peralba, and F. A. O. Camargo. 2007. Characterization of a polycyclic aromatic hydrocarbon degrading microbial consortium from petrochemical sludge land farming site. *Bioremediat. J.* 11:1–11.

Lal, B., and S. Khanna. 1996. Degradation of crude oil by *Acinetobacter calcoaceticus* and *Alcaligenes odorans*. *J. Appl. Bacteriol.* 81:355–362.

Lazar, I., A. Voicu, C. Nicolescu, D. Mucenica, S. Dobrota, I. G. Petrisor, M. Stefanscu, and L. Sandulescu. 1999. The use of naturally occurring selectively isolated bacteria for inhibiting paraffin deposition. *J. Petrol. Sci. Eng.* 22:161–169.

Mariano, A. P., D. M. Bonotto, D. F. De Angelis, M. P. S. Pirollo, and J. Contiero. 2008. Biodegradability of commercial and weathered diesel oil. *Braz. J. Microbiol.* 39:133–142.

Merino-Garcia, D., and S. Correra. 2008. Cold flow: A review of a technology to avoid wax deposition. *Petrol. Sci. Technol.* 26:446–459.

Mishra, S., J. Jyot, R. C. Kuhad, and B. Lal. 2001. In situ bioremediation potential of oily sludge degrading bacterial consortium. *Curr. Microbiol.* 43:328–333.

Mukherjee, A. K., and N. K. Bordoloi. 2011. Bioremediation and reclamation of soil contaminated with petroleum oil hydrocarbons by exogenously seeded bacterial consortium: a pilot-scale study. *Environ. Sci. Pollut. Res.* 18:471–478.

Sanjay, M., B. Simantha, and S. Kulwant. 1995. Paraffin problems in crude oil production and transportation. *SPE Prod. Facilit.* 10:50–54.

Satpute, S. K., I. M. Banat, P. K. Dhakephalkar, A. G. Banpurkar, and B. A. Chopade. 2010. Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. *Biotechnol. Adv.* 28:436–450.

Singh, C., and J. Lin. 2008. Isolation and characterization of diesel oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. *Afr. J. Biotechnol.* 7:1927–1932.

Singh, P., R. Venkatesan, H. Scott Fogler, and N. R. Nagarajan. 2001. Morphological evolution of thick wax deposits during aging. *AIChE J.* 47:6–18.

Sorkhoh, N. A., A. S. Ibrahim, M. A. Ghannoum, and S. S. Radwan. 1993. High temperature hydrocarbon degradation by *Bacillus stearothermophilus* from oil-polluted Kuwait desert. *Appl. Microbiol. Biotechnol.* 39:123–126.

Symon, K. R. 1971. *Mechanics*, 3rd ed. Boston, MA: Addison-Wesley Publishing Company, Inc.

Taraneh, J. B., G. Rahmatollah, A. Hassan, and D. Alireza. 2008. Effect of wax inhibitors on pour point and rheological properties of Iranian waxy crude oil. *Fuel Process. Technol.* 89:973–977.

Varjani, S. J., D. P. Rana, S. Bateja, M. C. Sharma, and V. N. Upasani. 2014. Screening and identification of biosurfactant (bioemulsifier) producing bacteria from crude oil contaminated sites of Gujarat, India. *IJIRSET*. 3:9205–9213.

Varjani, S. J., D. P. Rana, S. Bateja, V. N. Upasani. 2013. Isolation and screening for hydrocarbon utilizing bacteria (HUB) from petroleum samples. *Int. J. Curr. Microbiol. Appl.* 2:48–60.

Vasconcellos, S. P., B. M. Dellagnezze, A. Wieland, J. H. Klock, E. V. Santos Neto, A. J. Marsaioli, V. M. Oliveira, and W. Michaelis. 2011. The potential for hydrocarbon biodegradation and production of extracellular polymeric substances by aerobic bacteria isolated from a Brazilian petroleum reservoir. *World J. Microbiol. Biotechnol.* 27:1513–1518.

Zhu, T., J. A. Walker, and J. Liang. 2008. Evaluation of wax deposition and its control during production of Alaska north slope oils. Technical Report, University of Alaska. http://www.osti.gov/scitech/biblio/963363 (accessed December 1, 2015).