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Biodegradation of asphalt by *Garciaella petrolearia* TERIG02 for viscosity reduction of heavy oil

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Abstract Petroleum hydrocarbon is an important energy resource, but it is difficult to exploit due to the presence of dominated heavy constituents such as asphaltenes. In this study, viscosity reduction of Jodhpur heavy oil (2,637 cP at 50°C) has been carried out by the biodegradation of asphalt using a bacterial strain TERIG02. TERIG02 was isolated from sea buried oil pipeline known as Mumbai Uran trunk line (MUT) located on western coast of India and identified as *Garciaella petrolearia* by 16S rRNA full gene sequencing. TERIG02 showed 42% viscosity reduction when asphalt along with molasses was used as a sole carbon source compared to only asphalt (37%). The viscosity reduction by asphaltene degradation has been structurally characterized by Fourier transform infrared spectroscopy (FTIR). This strain also shows an additional preference to degrade toxic asphalt and aromatics compounds first unlike the other known strains. All these characteristics makes TERIG02 a potential candidate for enhanced oil recovery and a solution to degrading toxic aromatic compounds.

Keywords Asphaltene degradation · Viscosity reduction · Fourier transform infrared spectroscopy · Gases and volatile fatty acids

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Introduction

Due to the depleting resources of conventional light oils, the primary focus is now shifting towards utilizing heavy oil reserves. Worldwide deposits of heavy hydrocarbons are estimated to total almost 5.4 trillion barrels (bbl). However, only about 500 billion to 1,000 billion are considered recoverable with conventional technology (Herron 2000). Heavy oil is the largest known potentially recoverable petroleum energy resource but is characterized by high viscosity and high density (Luo and Gu 2007). In crude oils, asphaltenes, resins and petroleum alkanes compose a dynamic stable system, in which the petroleum alkanes act as solvents, the asphaltenes as micelles and the resins as stabilizers (Mansoori 1997; Wiehe and Kennedy 2000). Asphaltenes have drawn considerable attention due to problems caused by their detrimental effects in the extraction, transportation and processing of crude oils because of their viscous and flocculating nature (Creek 2005; Sirota 2005). They can make oil production more arduous and expensive due to their partial plugging in oil wells and pipelines.

To overcome the shortcomings of conventional methods, microbial degradation of asphaltene has been accepted worldwide as the most environmentally sound technology for enhanced heavy oil recovery efficiency by gas generation and solvent production (Donaldson and Clark 1982; Bryant and Bruchfield 1989). These gaseous and liquid metabolites reduce surface and interfacial tensions in both

aqueous solutions and hydrocarbon mixtures, which make them potential candidate for emulsification processes, dissolving into the oil resulting in reduced viscosity (Donaldson and Clark 1982). Increased cell mass of *Xanthamonas*, *Bacillus* and *Leuconostoc* strains has maximized the fluid flow by increasing the sweep efficiency as shown in a previous report (Yakimov et al. 1997).

In the past, biodegradation of asphaltenes through the use of a microbial consortium or mixed cultures isolated from soil samples, sediments contaminated with hydrocarbons and oil wells have taken place but in low proportions of 0.55–3.5% (Venkateswaran et al. 1995; Thouand et al. 1999). This is most likely due to the complex molecular structure of asphaltene which makes it resistant to biodegradation thereby causing their accumulation in ecosystem where petroleum and its refining byproducts are spilled in either accidental or purposeful ways (Guiliano et al. 2000). The focus of many studies has generally been bioremediation of total petroleum hydrocarbon (TPH) contaminated sites (Iturbe et al. 2007; Machackova et al. 2008) and not much detailed work has been done on asphaltene biodegradation. This technique of microbial degradation of asphaltene to recover heavy oils is of great value and needs to be explored and studied deeply.

The present study was undertaken as the initial phase of the program to investigate the viscosity reduction of heavy oil by strain TERIG02. An attempt was also made to evaluate the metabolic factors and structural characterization of oil as well as asphalt fraction by Fourier transform infrared spectroscopy (FTIR). To the best of our knowledge, no detailed scientific studies have so far been reported on the viscosity reduction of heavy oil by asphalt biodegradation under anaerobic conditions.

Materials and methods

Oil collection

The oil used in this study was collected from an oil collecting station (OCS) of Oil India Limited (OIL) Jodhpur (Rajasthan), India in January 2010. The bottom hole temperature of the well was 45–50°C, salinity of formation was 0.5% with 1% of water cut. The geographical location of sampling site is 26° 18′ North 73° 04′ East. Jodhpur has a typical desert climate. The average temperature during summer months is 42°C while in winter is 24°C. It has medium rainfall of 32 cm during monsoons.

This site was selected for the study of viscosity reduction as this is known for having high oil viscosity. The viscosity of the oil is 2,637 cP at 50°C with 0.3 rpm and 62% torque. The oil density is 1.01 g/m^3. The compositional analysis including heavy metal analysis is presented in Table 1. The asphaltene content of the crude heavy oil is found to be 49.4 wt% (*n*-heptane insoluble fraction).

Table 1 Physical and chemical properties of the heavy oil

Heavy oil properties	
Physical	
Density	1.01
API gravity	10
Viscosity cp @ 50°C	2637
Pour point	37
Composition	
Aliphatic	20.35%
Aromatic	30.25%
Asphaltene	49.4%
Heavy metal	
Nickel (ppm)	42
Vanadium (ppm)	152
Sulfur (wt%)	1.7

TPH fractionation

TPH was extracted from oil as described by Mishra et al. (2001a). Extracted TPH (0.2–0.5 g) was dissolved in n-heptane and separated into soluble and insoluble fractions by SARA methodology IP 143/84 (1989). The soluble fraction was loaded onto a silica gel column. Alkane fraction was eluted with hexane, aromatic fraction was eluted with benzene, and finally, asphalt fraction was eluted with a mixture of chloroform and methanol. After evaporation of the solvents, each fraction (including asphaltenes) was determined gravimetrically (Mishra et al. 2001b). Alkanes and aromatic fractions were further analyzed by gas chromatography (GC, Hewlett Packard 5890 Series II).

Biodegradation studies with TERIG02

TERIG02 strain used in this study was isolated from sea buried oil pipeline (MUT) of Oil and Natural Gas Corporation (ONGC) at Bombay high offshore in western India. Its ability to degrade each eluted fractions viz., aliphatic, aromatic and asphalt was determined in separate serum bottles. The serum bottles contained 200 ml minimal salt medium (MSM) with 1% of each of the hydrocarbon fraction (total capacity of 1,350 ml, head space of 1,150 ml) under anaerobic conditions. MSM medium with the following composition in grams per litre: $MgSO_4 \cdot 7H_2O$, 0.5; KNO_3, 0.5; $(NH_4)_2HPO_4$, 0.05; KH_2PO_4, 1.0; K_2HPO_4, 1.0; yeast extract, 0.5; 10 ml trace element solution and 1.0 ml vitamin solution (Sood and Lal 2009), with asphalt fraction and molasses (1%) as a carbon source was prepared by boiling under a stream of O_2-free N_2 gas and dispensed into serum bottles. The bottles were then sealed and autoclaved for 20 min at 120°C. Prior to inoculation, 0.1 ml of 2% filter sterilized $Na_2S \cdot 9H_2O$ and 0.1 ml of 10% filter sterilized Na_2CO_3 were injected separately by syringe. Culture was maintained at 50°C under anaerobic conditions in anaerobic chamber (N_2:H_2:CO_2; 5:5:90).

To account for no biologically mediated losses, controls with no bacterial inoculation were prepared in the same manner and run in parallel. Culture bottles were sampled at different times to monitor the depletion of hydrocarbon fractions over the course of the experiment. As asphalt fraction plays a major role in raising oil viscosity, this fraction was used as a sole carbon source for all the subsequent experiments.

Identification and characterization of TERIG02

To identify the strain, the 16S rRNA full gene sequence was subjected to a BLAST search with the NCBI database (Altschul et al. 1997). The 16S rRNA gene sequence of the strain TERIG02 has been submitted to the NCBI GenBank Database with accession number as EU851973. Morphology of TERIG02 was studied by scanning electron microscopy.

To study the viscosity reduction by asphalt biodegradation

Asphalt biodegradation by strain TERIG02 was evaluated for viscosity reduction under anaerobic conditions. Experiments were conducted in order to determine the viscosity reduction by biodegradation of asphalt. Following treatments were conducted:

SET 1 MSM medium with asphalt fraction (1%) as a sole carbon source, *SET 2* MSM medium with asphalt fraction (1%) supplemented with molasses (1%) as carbon sources at 50°C. Observations were recorded for viscosity reduction and bacterial metabolites (gases H_2, CO_2 and CH_4, final pH, volatile fatty acids (VFA)) production for a period of 0–30 days with regular interval of 10 days.

Analytical methods

Viscosity of the extracted microbially degraded asphalt fraction was determined by using a cone-plate viscometer (Brookfield-Programmable DV-II viscometer). The system was calibrated with standard oil (polydimethylsiloxane) having known viscosity of 498 cP at 25°C. About 18 ml of the extracted asphalt fraction was placed between the sample cup and a coned spindle. This assembly was rotated at a preset rate of 0.3–2.0 rpm. When the torque exerted on the spindle by the heavy oil was 50–100% of the full scale the viscosity value displayed on the digital LCD panel was recorded. The rate of rotation and the torque exerted are proportional to each other. Constant temperature of 50°C during viscosity reading was maintained by circulating water bath (Heto CBN 18-50/HMT 200) with the accuracy of ±0.1°C. The measurements were carried out in triplicates and the average values of the three were finally taken.

Fourier transform infrared spectroscopy (FTIR) spectra of the total heavy oil, extracted asphalt fraction and microbial degraded asphalt fractions were recorded. Briefly, 2.0 wt% spectroscopy-grade KBr was dried at 150°C for 5 h in an oven and stored over silica gel in dessicator till use. Following which 15-mm diameter pellets of this dried KBr were prepared. Each of the samples was then smeared on the KBr pellets. The FTIR spectra of the sample pellets was recorded in a Perkin Elmer BX FTIR spectrometer (Perkin Elmer Life and Analytical Sciences, Inc., Wellesley, MA, USA) with IRDM 3.3 software by coadding eight scans at 4 cm^{-1} resolution in the 4,000–400 cm^{-1} region. All spectra were recorded in a linear transmittance scale and the resulting spectra were normalized to an equivalent of

1 mg of the sample spread uniformly over the surface of a 15-mm diameter pellet.

The concentrations of C_2–C_6 VFAs in liquid phase were analyzed with GC 6890N (Agilent, USA) equipped with flame ionization detector and DB-WAXetr column (30 m × 530 μm × 1 μm). The oven temperature was 140°C with ramping of 1°C per min up to 158°C. The injector and detector temperatures were 220 and 230°C. Helium was used as the carrier gas. To analyze the gas composition generated another 6890 Plus GC (Agilent) with an HP PLOTQ column (15 m × 530 μm × 40 μm) and a thermal conductivity detector was used. The oven, injector and detector temperatures in this case were 40, 60 and 250°C respectively (Jayasinghearachchi et al. 2009). Nitrogen was used as a carrier gas for determination of H_2 and CO_2. Since the concentration gradient of H_2 gas can be formed in the head space, gas samples (0.5 ml) was taken out after mixing of the head space gas by sparging several times with gas tight syringe.

The microbial biomass in the culture bottles was determined by measuring the optical density at 600 nm (UV1100 Spectrophotometer, Shimadzu USA).

Results

Heavy oil properties

Heavy oil differs from light oils by their high viscosity and density. About 66% of the heavy oil produced annually is lighter than 15° API gravity, but about 50% of the estimated technically recoverable heavy oil is denser (less than 15° API gravity) (USGS 2003). In the present study the heavy oil selected represents a viscosity range of 10° API. This oil was characterized by heavy metal contaminants predominantly vanadium and nickel (Table 1). The gravimetric analysis showed that TPH composition for heavy oil used in this study was as follows: 20.35 ± 2.93% aliphatic, 30.25 ± 1.72% aromatics and 49.4 ± 1.83% asphaltenes (Table 1). Aliphatic and aromatic fractions were identified using the retention times of standards for each of the fractions given in ASTMD877. The degradation ability of the strain TERIG02 for each of the hydrocarbon fractions (aliphatic, aromatic and asphalt) was determined over a period of 30 days. In each case an anaerobic control was also included. Figure 1 depicts the concentration of hydrocarbon fraction with respect to the incubation time. The maximum degradation after 30 days of incubation under anaerobic conditions was observed in asphalt (55%) and aromatic (45%) fractions however 25% of hydrocarbon was degraded in the aliphatic fraction. In addition over the full time span of 30 days there was a significant decline in the residual hydrocarbon concentration from the 20th to the 30th day of the experiment as compared to the anaerobic control set. This clearly indicates that TERIG02 shows metabolic preference towards

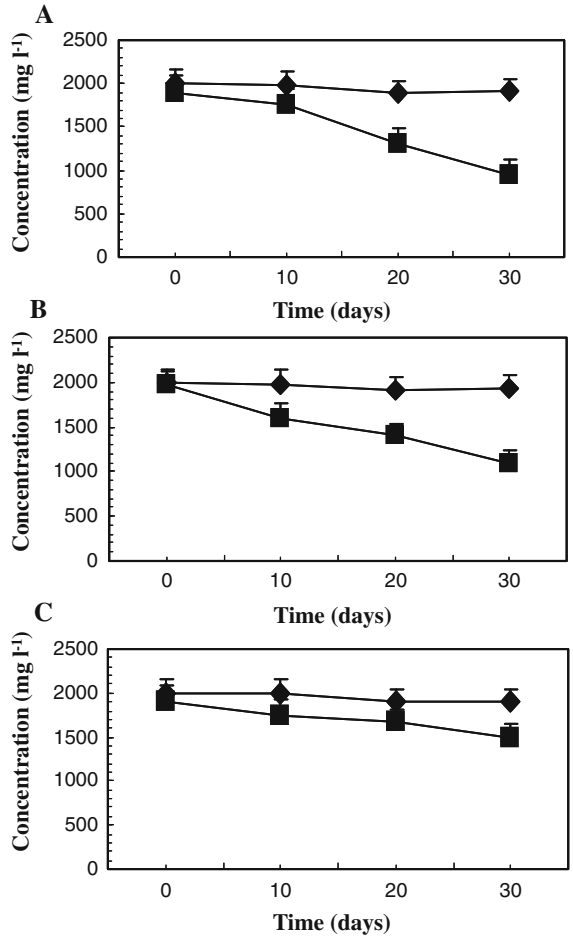

Fig. 1 Time–concentration study of aliphatic, aromatic and asphalt fractions in inoculated bacterial strain TERIG02 and un-inoculated controls. **A** Asphalt degradation (mg l^{-1}): (*filled diamonds*) control (*filled squares*) treated; **B** Aromatic degradation (mg l^{-1}): (*filled diamonds*) control (*filled squares*) treated; **C** Aliphatic degradation (mg l^{-1}): (*filled diamonds*) control (*filled squares*) treated

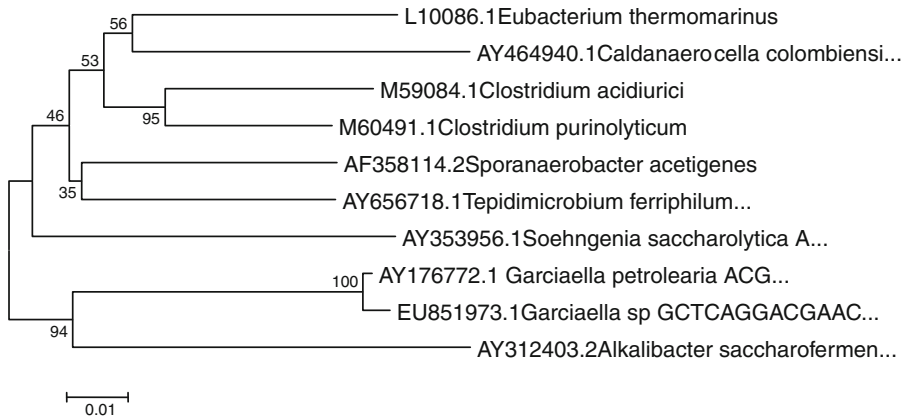

Fig. 2 Phylogenetic tree based on 16S rRNA full gene indicating the position of strain TERIG02 among closely related members. The topology shown is an unrooted tree obtained with a neighbor joining algorithm (Jukes–Cantor corrections) with bootstrap value expressed as percentage of 1,000 replications. Phylogenetic analyses were conducted in MEGA 4.0. Accession number obtained was EU851973 from NCBI

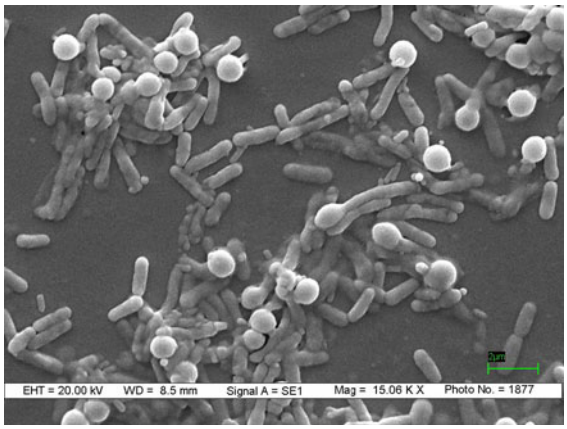

Fig. 3 Scanning electron micrograph of TERIG02

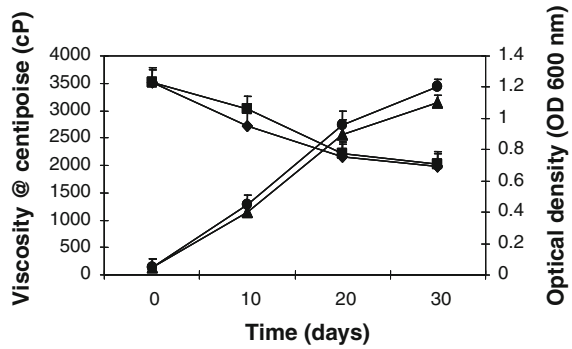

Fig. 4 Time course study of TERIG02 with respect to viscosity and cell growth. Viscosity reduction of asphalt (cP): (*filled diamonds*) asphalt as carbon source (1%), (*filled squares*) asphalt (1%) along with molasses (1%); optical density (OD 600 nm): (*filled circles*) asphalt as carbon source, (*filled triangles*) asphalt (1%) along with molasses (1%)

degradation of aromatic and asphalt fractions. The experiments were repeated in three sets, which showed same trends of degradation.

Identification and characterization of TERIG02

TERIG02 was identified as *Garciaella petrolearia* (99% identity) by 16S rRNA full gene sequence analysis. The sequence has been submitted to the NCBI GenBank database with an accession number EU851973. A phylogenetic tree was mapped with closely related matches obtained from BLAST search (Fig. 2). Microscopic examination revealed the shape of TERIG02 as rods with terminal spores (Fig. 3).

Biodegradation of asphalt for viscosity reduction

Viscosity reduction

At the end of 30 days it was observed that when asphalt along with molasses was used as a sole carbon source the viscosity decreased from 3,520 to 2,029 cP, a total reduction of 42% (Fig. 4). While in case when only asphalt is used, 37% reduction was observed. From 0 to 10 days the reduction in viscosity is quiet sharp but after 10, 20 and 30 days the trend is quiet similar.

Viscosity reduction was further confirmed by FTIR analysis. FTIR analysis provides a fingerprint

image of the gas product generated during any degradation process (asphalt in our case). While comparing the FTIR spectrum of total heavy oil and asphalt fraction (Fig. 5A, B), it was observed that the IR bands at 3,458 and 1,637 cm^{-1} were exactly matching in both the cases. These IR bands corresponds to OH and NH (stretching vibration) in carbazole type compounds and conjugated carbonyl and/or amide functionalities respectively as described by Sharma et al. (2007). However in case of asphalt fraction some of the bands disappeared or became negligible indicative of the extraction of asphalt from heavy oil. Some of the prominent bands were positioned at 2925.11, 2855.93, 1458.31 cm^{-1} in heavy oil are visibly reduced in area in case of asphalt. While band at 1377.91 cm^{-1} seems to have completely disappeared. Usually the characteristic bands for volatiles like CO_2, SO_2 and H_2O were observed at 2360, 1374 and 3500–4000 cm^{-1} respectively (Braun et al. 2007; Baker et al. 2005; Marques et al. 2006) For heavy oil and asphalt CO_2 was the main volatile product with the characteristic band at 2365.02 and 2364.95 cm^{-1} respectively. Other substances though present in small amounts such as H_2O were positioned between 3,500 and 4,000 cm^{-1} for both heavy oil and asphalt. The band for SO_2 was detected at 1377.91 cm^{-1} in case of heavy oil only. The slight shifting of the values could be attributed due to temperature fluctuation which instrument dependant.

In case of microbial treated asphalt with molasses (Fig. 5C) there seems to be an increase in the number of bands between 700 and 1,500 cm^{-1} due to the degradation of asphalt leading to the presence of new products. Among these new bands the one that appear at 722.47–969.25 cm^{-1} represent the styrene part of the styrene–butadiene–styrene (SBS). A characteristic C–CH_3 band is positioned at 1462.55 cm^{-1} which form the small aromatic ring clusters having alkyl tails in aromatic substitution. Very low intensity bands between 1,300 and 1,400 cm^{-1} correspond to O–H and show the presence of alcohols and phenols. The band at 1610.61 cm^{-1} depicts the the heptane mobile fraction due to the stretching of aromatic C–C bond and the substitution of the aromatic rings. Molecular gaseous products such as CO_2, SO_2 and H_2O were also released. The band for SO_2 was observed at 1378.04 cm^{-1} instead of the characteristic position of 1,374 cm^{-1}. This is most likely because of the transmittance of stretching vibration of S=O which is emitted as SO_2 products. On the other hand it seems only trace amounts H_2O and CO_2 are released since the bands are quiet diffused. The band at 2935.84 cm^{-1} is the transmittance peak of methane. Bands between 2,675 and 3,115 cm^{-1} represent saturated aliphatic hydrocarbons. Bands lying in this range become more prominent in case of asphalt and molasses.

Production of gases by TERIG02

TERIG02 produced H_2: 38.22 mmol l^{-1}; CO_2: 4.28 mmol l^{-1} on the 30th day when asphalt fraction was used along with molasses. For enhanced viscosity reduction, we supplemented molasses in MSM medium along with asphalt as carbon sources. Significant increase was observed in gas production in presence of molasses as a secondary carbon source (Fig. 6). No methane production was found by TERIG02.

Production of VFA

Maximally, acetic acid and butyric acid production was observed by TERIG02 (AA: 789 mg l^{-1}; BA: 145 mg l^{-1}) in presence of asphalt as a carbon source along with molasses on the 30th day (Fig. 7). Production of VFAs were slightly lower when asphalt was used as a sole carbon source. Reduction in pH was also observed in TERIG02 (4.04) as compared to control (7.5). The lowering of pH was due to the production of VFAs.

Discussion

Microbial degradation of heavy oil for reducing the viscosity of heavy oils so as to make it more accessible and utilizable has been earlier investigated. Degradation is a sequential process in which *n*-alkanes are generally removed first, followed by the degradation of iso-alkanes, cycloalkanes, 1–3 ring aromatics, and finally polyaromatics (Greenwood et al. 2008). However, the typical pattern of degradation varies with different bacteria as well as type and composition of oil (Greenwood et al. 2008; Diaz-Ramirez et al. 2008; Zrafi-Nouira et al. 2009). It is the asphalt content which is responsible for

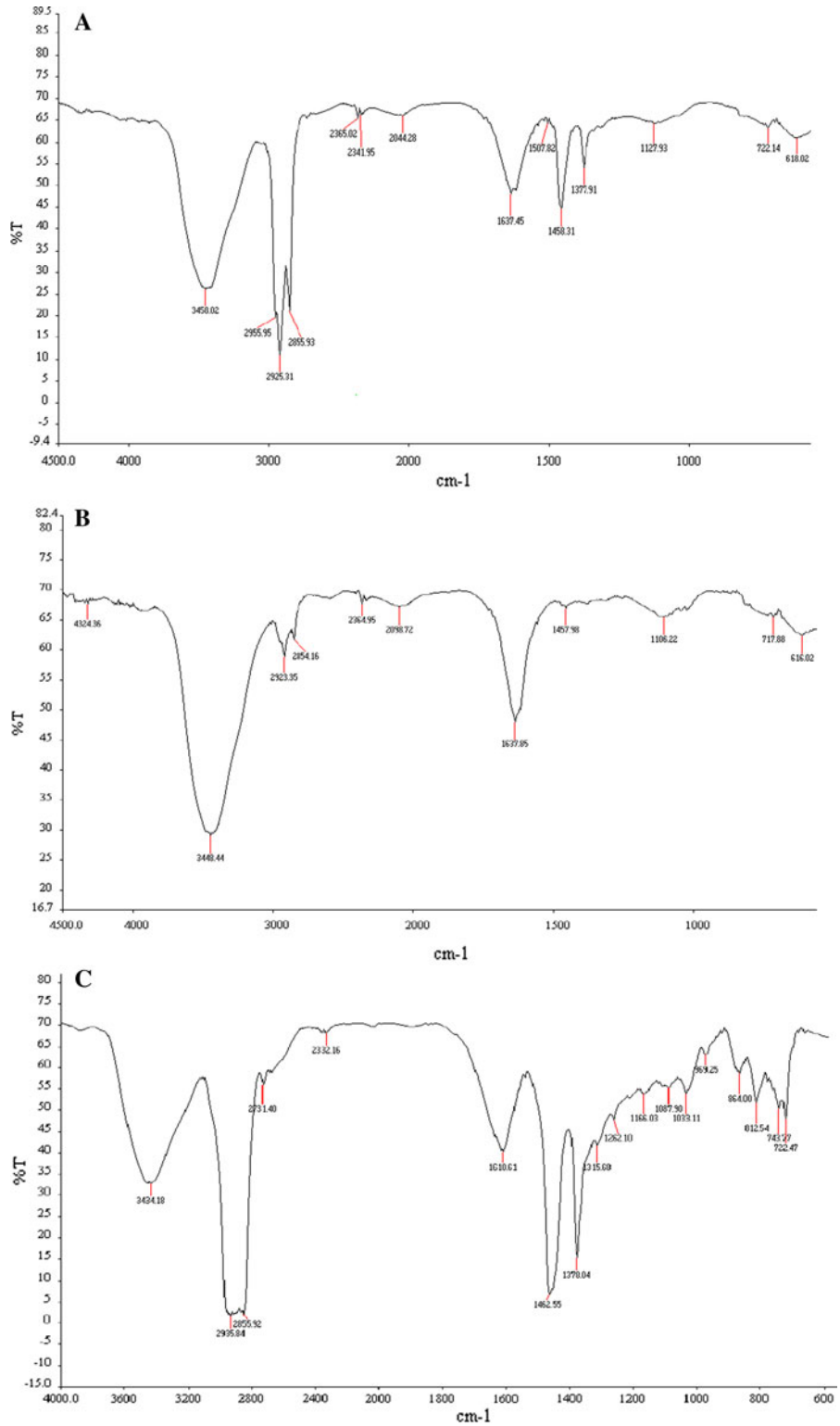

Fig. 5 FTIR spectra of heavy oil and its asphalt fraction. **A** Heavy oil. **B** Asphalt (untreated control) on 30th day. **C** Treated with TERIG02 (asphalt along with molasses) on 30th day

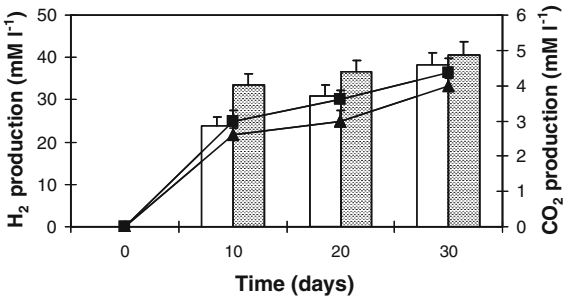

Fig. 6 Gas analysis (H_2 and CO_2) of strain TERIG02 over the course of experiment. H_2 production in mM l^{-1} asphalt: (*open squares*) asphalt, (*squared dots*) asphalt along with molasses; CO_2 production in mM l^{-1}: (*filled triangles*) asphalt, (*filled squares*) asphalt along with molasses

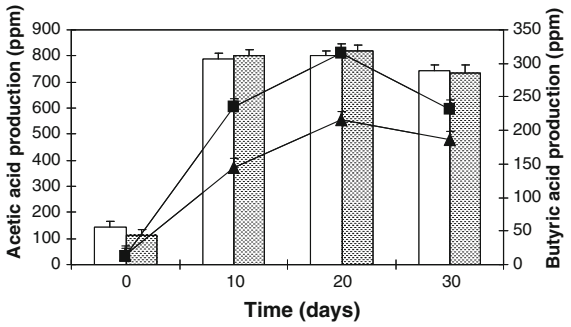

Fig. 7 VFA analysis of strain TERIG02. Acetic acid in ppm: (*open squares*) asphalt, (*squared dots*) asphalt along with molasses; butyric acid in ppm: (*filled triangles*) asphalt, (*filled squares*) asphalt along with molasses

determining the viscosity of heavy oils and also the least preferred component to be degraded by most bacteria. Therefore the present study made use of the bacterial strain TERIG02 to degrade the asphalt and aromatic hydrocarbon for viscosity reduction of heavy oil. TERIG02 has been identified as *G. petrolearia* according to 16S rRNA. Some *Garciaella* strains are known for nitrate and thiosulphate reduction mechanism (Shimoyama et al. 2009). However, there have been no studies on biodegradation of asphalt.

Biodegradation ability of TERIG02 was assessed by growing the strain with asphalt as carbon source over a period of 30 days. Initially only asphalt was used as the sole carbon source. However it was found that there was low microbial population of TERIG02 (data not shown). This could be probably because the strain needs to be supplemented with sugar for its initial growth. Therefore molasses was added along with asphalt as it is a mixture of sugars. It was observed that with molasses the biodegradation proceeded slowly in the first 10 days of the experiment but after 20 days of incubation the degradation became relatively high. A viscosity reduction of 37% was observed when only asphalt was provided. But addition of molasses enhanced the ability of the strain further and reduced the viscosity by 42%.

Microbes reduce the viscosity by degrading heavy oil into smaller components such as biological surface active substance, acid, gas etc. These secondary metabolite (gases) of microbes increase reservoir pressure leading to oil swelling resulting in easy production process (Kraemer and Bagley 2005). Kraemer and Bagley (2005) had reported that anaerobic fermentation leads to the production of acids, CO_2, H_2 and alcohols. Anaerobic bacteria produce acetate and butyrate during the initial growth phase (acidogenic phase) of the fermentation process. As the culture moves to the second phase of fermentation, the stationary growth phase, there is a shift in the metabolism of the cells to solvent production (solventogenic phase). These gaseous and liquid metabolites dissolve into the oil resulting in reduced viscosity (Bryant et al. 1998). Moreover, the reaction of asphalt degradation within an acidic background is preferable as the H^+ proton effectively interact with the polar positions of the asphalt molecules thereby efficiently reduce the polar interactions which results in breaking of agglomerates and finally one has reduced oil viscosity. Finnerty and Singer (1983) reported that acid producing microbes are able to reduce the viscosity of crude oil (8–150° API) with hexadecane from >25,000 cP to 275 cP.

It was observed that with TERIG02 the degradation products of asphalts are mainly composed of CO, CO_2, H_2O, hydrocarbon, formaldehyde, tetrahydrofuran, formic acid, acrylate fragments, aromatic compounds, naphthenic acids, heptanes, methanol, phenols etc. as shown by the FTIR spectra (Fig. 5). Some of these products were also seen in the reports published by Xu and Huang (2010) who concluded that the main gas products of asphalt are CO, CO_2, H_2O, hydrocarbon etc. The release of CO_2 is mainly attributed to the cracking and reforming of carbonyl and carbonyl groups. The amount of CO_2 is higher, which indicates that the asphalt is partly oxidized to CO_2 as expected (Takafumi et al. 2003). The release of methane is mainly because of the cracking of

methoxyl (–O–CH_3) and breaking of methylene also generates methane. Additionally, acids were also identified by spectral subtraction to remove the interfering bands. The appearance of bands lying in the range 2,675–3,115 cm^{-1} are indicative of the degradation of the asphalt into saturated aliphatic hydrocarbons. Thus from the FTIR data it became quiet clear that TERIG02 was able to reduce the viscosity of the heavy oil mainly due to the presence of the metabolites that were derived during the degradation process.

When each of the fractions namely aliphatic, aromatic and asphalt were treated with TERIG02 for 30 days it was found that maximum degradation was in the case of asphalt followed by aromatic fraction. This is quiet interesting and useful since there are very few strains reported so far which show a preference for asphalt. The solubility of hydrocarbon compounds is an important consideration when evaluating its toxicity to organisms. Aromatic hydrocarbons are more toxic and their harmful effects to organism are enhanced when the chemicals are more soluble in water, increasing the organisms exposure's to the pollutant. In this study, asphalt and aromatics degraded faster, suggesting that the TERIG02 could tolerate the toxicity of these compounds and was capable in utilizing them as a carbon and energy source.

Conclusion

This study was undertaken to analyze the heavy oil viscosity reduction through asphaltene degradation using the strain TERIG02. In particular, it highlights the fact that oil viscosity is directly proportional to the concentration of asphaltenes. This is the first time whereby *G. petrolearia* (TERIG02) has been reported to be responsible for reduction in viscosity by splitting of asphalt into smaller fragments. In addition, this strain prefers to degrade asphalt as well as aromatic compounds which are the most toxic. Thus it can play an essential role not only in bioremediation of complex petroleum hydrocarbon products but can also reduce the associated health risks involved. Nevertheless these findings warrant a need for further evaluation to study the factors affecting the viscosity reduction individually or in various combinations to identify the active principles involved in the microbial degradation of asphalt.

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