Enhancing Crude Oil Flow Behaviour in pipelines of North-East India using Bacteria derived from Fish Waste and Sugarcane Bagasse

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Abstract

In this study, methane-producing bacteria were cultivated from fish waste and sugarcane bagasse and then introduced into crude oil samples to enhance their rheological characteristics. Crude oil's complex hydrocarbon composition poses challenges in extraction, transportation and refining due to unpredictable physical traits like high viscosity and pour point. This research pioneers an innovative, sustainable strategy, exploring the potential of methane-producing bacteria to improve crude oil flow. Tests conducted on crude oil samples from the North East India Oil Field involved growing bacteria under specific conditions and meticulously characterizing the oil's basic features. Methane-producing bacteria were then added to various crude oil samples incubated optimally to boost metabolic activity. Employing a rheometer and heat oven, the treated samples underwent thorough examination, measuring viscosity, shear stress, pour point and relevant parameters. Results demonstrated the bacteria's capability to reduce viscosity and shear stress while lowering the oil's pour point, enhancing flow properties, especially at lower temperatures.

This study uncovers promising applications in the oil sector, presenting bacteria as an eco-friendly remedy for crude oil viscosity, pour point and shear stress issues, thus offering a sustainable approach to improving crude oil handling and transportation.

Keywords: Bacteria, Fish waste, Flow Assurance, Pour Point Depressant, Shear Stress, Sugarcane Bagasse and Viscosity.

Introduction

Since crude oil has a wide range of physical qualities that are determined by its source and geological causes, it presents considerable challenges in the extraction, transportation and refining operations. Crude oil is a varied blend of hydrocarbons[8]. These characteristics have a big influence on how crude oil flows as it moves from reservoirs to processing plants. Crude oil is made up of a number of elements that contribute to its complex makeup and behaviour such as paraffins, aromatics, resins and asphaltenes. Paraffins are a significant fraction of crude oil that consist of both straight and branched-chain alkanes with molecular formulae between $C_{18}H_{38}$ to $C_{55}H_{112}$ [6]. When these heavier paraffins make up the majority of the crude oil, typically more than 50%, then it is referred as "waxy crude oil."

Aromatics including benzene rings and aromatic structures like xylene and toluene are naturally occurring hydrocarbons that can be found in crude oil. Resins are polar substances with a high molecular weight that are used as emulsifiers and can cause deposits in equipment and pipes. The heaviest and most polar components of crude oil, asphaltenes are known for their complex, high-molecular-weight structures and are in charge of causing solids to precipitate out of the oil. Wax deposition can result from the precipitation and crystallisation of waxes as crude oil moves from the high-temperature, high-pressure conditions of reservoirs to the lower-temperature, lower-pressure settings at the surface.

Reduced flow rates, increased pressure drops and the possibility of pipeline obstructions are just a few of the major flow assurance issues[1]. These issues will require expensive maintenance and repair work. Controlling temperature is a key tactic in the fight against wax deposition. Insulation, electric heating, or hot oil circulation systems are frequently used to keep crude oil above its wax appearance temperature (WAT) during storage and transit. Pour point depressants (PPDs) are chemical additives that can be used in certain situations to reduce the WAT of crude oil[2]. These traditional approaches do, however, come with a high price tag and negative environmental effects.

In recent years, numerous researchers have extensively explored biological methods for wax removal. Findings from Rana et al[10] demonstrated that engineered bacterial systems, incorporating paraffin-degrading bacteria along with nutrient supplements and growth enhancers, exhibited the capability to eliminate the necessity for recurrent wax scrapings over an extended period, spanning several months. The successful application of these approaches could offer the advantage of maintaining continuous control over wax deposition by ensuring ongoing biodegradation, as opposed to merely offering a short-term remedy. Sifour et al[13] extracted biosurfactants from *Pseudomonas aeruginosa* RB 28, identified as rhamnolipids and demonstrated efficient emulsification of sunflower oil, heptadecane and paraffin enhancing flow properties. Etoumi et al[5] used Pseudomonas

species which exhibited accelerated growth and high biodegradation efficiency at 1% (v/v) concentration of crude oil, demonstrating significant potential for the effective biodegradation of heavy paraffinic hydrocarbons. Gas chromatography analysis revealed increased iso-alkanes in the C_{15}–C_{20} range while liquid chromatographic results indicated the preferential degradation of saturated alkanes over aromatic and asphaltene compounds, suggesting the promising application of Pseudomonas species in enhancing crude oil properties. Studies done by Xiao et al[17] explored the impact of bio-treatment on paraffin deposits on stainless steel surfaces, revealing that biosurfactant-producing species altered stainless steel wettability to water-wet, reducing water adhesion and preventing paraffin deposition with a removal efficiency of up to 79.0%.

The study suggests that further research should delve into the hydrophobic characteristics of bacterial cell surfaces for enhanced understanding and application in preventing paraffin deposition. Sood et al[14] studied the thermophilic bacterial strain *G. kaustophilus* (TERI NSM) for its proficiency in egrading paraffin wax efficiently under low-nutrient conditions, exhibiting growth at high temperatures and selectively degrading long carbon chain alkanes, making it a promising solution for oil wells facing paraffin deposition issues.

This study presents a novel approach within the field of microbiology, specifically focusing on the use of methane-producing bacteria. These microorganisms have demonstrated the capability to convert components of crude oil into both methane gas and biomass. While these bacteria show promise in various aspects of the oil industry, their potential effects on oil viscosity and pour point have not been thoroughly explored [9]. In a time where sustainable and economically viable solutions are of utmost importance, this research aims to investigate the feasibility of employing biological treatments involving methane-producing bacteria as an alternative to traditional methods, which often come with environmental and economic constraints. A critical aspect of this investigation is the relationship between shear stress, temperature and fluid viscosity.

Typically, as temperature increases, the viscosity of a fluid decreases due to increased molecular movement, resulting in smoother flow. In this context, our project delves into the potential of methane-producing bacteria to improve the flow properties of crude oil. This study investigates methane-producing bacteria derived from fish waste and sugarcane bagasse, aiming to offer valuable insights into their potential applications for addressing challenges related to the viscosity and pour point of crude oil.

Material and Methods

Materials: The waxy crude oil sample used for analysis was sourced from an oilfield in Northeast India. Fish waste was collected from a local fish shop and sugarcane was collected from a fruit juice vendor. Various chemicals including hydrochloric acid (HCl), iron (II) chloride tetrahydrate ($FeCl_2$), zinc chloride ($ZnCl_2$), manganese (II) chloride tetrahydrate ($MnC_2.4H_2O$), dihydrogen borate (H_2BO_3), calcium chloride ($CbCl_2$), nickel (II) chloride hexahydrate ($NiCl_2.6H_2O$), distilled water, copper (II) chloride ($CuCl_2.2H_2O$), sodium chloride (NaCl), magnesium chloride hexahydrate ($MgCl_2.6H_2O$), potassium dihydrogen phosphate (KH_2PO_4), potassium chloride (KCl), calcium chloride ($CaCl_2.2H_2O$), L-cystine, beef extract, vitamin B capsules and methyl blue solution were procured from a local vendor.

Crude Oil Characterization: Several characterization techniques were utilized to evaluate the properties of the crude oil in this investigation, with the pour point of the untreated crude oil determined using the established ASTM D97-17b method[11]. The water content of the crude oil was determined by the centrifuge method (ASTM D 96-58 T), with toluene serving as the carrier liquid[4]. Rheological tests on untreated crude oil were performed at various temperatures (10, 20, 30 and 40 °C) using Anton Paar Rheometer MCR-72 [7]. Density measurements were carried out utilizing a Bingham pycnometer, adhering to the ASTM D1480-15 standard. This procedure facilitated the determination of the API gravity of the crude oil, using distilled water as the benchmark liquid[3].

The wax content in the crude oil was assessed employing the modified Universal Oil Products (UOP) 46-64 method, involving the utilization of solvents such as n-pentane and acetone, with hexane employed as a washing solvent[15]. The determination of the saturates, aromatics, resins and asphaltenes (SARA) composition in the crude oil was conducted using column chromatography methods in accordance with the procedures outlined by Sara et al[11]. Ultimately, the wax appearance temperature (WAT) was deduced from rheological data wherein variations in the crude oil sample's viscosity in relation to temperature were observed and analyzed.

Preparation of Microorganisms: In a controlled environment devoid of oxygen, a setup was arranged to foster the growth of microorganisms, specifically methanogens. The sample was formulated by combining 50% sugarcane and 50% fish waste. To create an oxygen-free, anaerobic atmosphere, a glass bottle was employed with pipes connected to both ends. The openings were sealed in a manner that permitted the release of gas only when the cap was opened, ensuring that no external air could enter the bottle. Subsequent to placing the sample into the glass bottle and securely sealing the cap, an anaerobic condition was established by igniting a candle inside the bottle to illustrate the absence of oxygen within the container.

After a period of two days, gas was emitted from the vessel containing the sample. When this gas came into contact with an open flame, it ignited, indicating the presence of methane.

Subsequent daily examinations consistently confirmed the ongoing production of methane.

Media Preparation for Microorganisms: Three solutions (Trace, Vitamin, Mineral Salt) were created by blending chemicals in specified quantities for microorganism survival media.

Table 1
For Trace Solution[16]

Chemicals Name	Volume
25%(w/v) HCl	10ml
$FeCl_2.4H_2O$	1.50g
$ZnCl_2$	0.070g
$MnCl_2.4H_2O$	0.100g
H_2BO_3	0.006g
$CaCl_2.2H_2O$	0.190g
$NiCl_2.6H_2O$	0.024g
Distilled water	990ml
$CuCl_2.2H_2O$	0.002g

Table 2
For Mineral Solution[16]

Chemicals Name	Volume
NaCl	1.0g
$MgCl_2.6H_2O$	0.4g
KH_2PO_4	0.2g
KCl	0.5g
$CaCl_2.2H_2O$	0.15g
L-Cystine	0.5g
Beef extract	1.0g
Methyl blue solution	1ml
Vitamin solution	1ml
Trace element solution	1ml
Distilled water	1000ml

The vitamin solution was prepared by mixing 10 vitamin B capsules in 100ml water by maintaining required proportion. To create the ultimate medium totalling 100ml, a conical flask was employed, wherein the three solutions, distilled water and bacteriological agar were meticulously blended in required proportions. The process of media preparation involved subjecting it to autoclaving at a temperature of 124°C at a pressure of 0.8 psi, followed by a cooling period of 15 minutes until the pressure reached 0 psi. Subsequently, the prepared medium was poured into four Petri dishes within a controlled laminar airflow enclosure to ensure the prevention of contamination.

Sequential distillation of original samples: The method of sequential distillation is employed to reduce the concentration of microorganisms. In this procedure, the initial bacterial sample, typically 1 ml, is mixed with a specific volume of distilled water, resulting in 1:10 dilution. This process is then repeated by taking 1 ml from the 1:10 dilution and combining it with 9 ml of diluent, creating a 1:100 dilution. This sequence continues to produce further dilutions, ultimately reaching a 1:1,000 ratio. Subsequently, known quantities of each dilution are spread onto agar plates using spread plating techniques. These agar plates are then placed in an incubator set at the appropriate temperature for bacterial growth. Following incubation, the number of bacterial colonies on each plate is tallied.

Figure 1: First generation of microorganisms after three days

Identification of Gram-positive Bacteria: The process involves several key steps. Firstly, a pure culture of the target bacteria is obtained. Next, a thin smear of this culture is delicately spread onto a glass slide, allowing it to air dry. Heat fixation is then performed by passing the slide through an open flame to ensure the bacteria firmly adhere to the slide. The Gram staining procedure consists of several sub-steps: (a) the application of crystal violet as the primary stain for one minute, (b) rinsing the slide with water to remove excess stain, (c) flooding the slide with an iodine solution (mordant) for one minute and allowing 45 seconds to 1 minute for crystal formation around the bacterial cell walls. A crucial decolorization step follows using 70% ethanol until the runoff is clear. Finally, safranin is applied as the secondary stain for a duration of 2 minutes, completing the Gram staining process for bacterial identification.

Microscopic Examination: Following the staining process, the next steps involve microscopic examination. When examining the stained slide under a microscope, Gram-positive bacteria will retain the primary stain and exhibit hues like purple, violet, or pink, while Gram-negative bacteria, having lost the primary stain, will appear either colorless or pink. Subsequently, to ensure clarity and precision, any excess moisture is removed by gently blotting the slide with tissue paper. To enhance the refractive index and improve microscopic observation, cedarwood oil is applied to the slide, enabling a more detailed examination of the bacterial specimens.

Results and Discussion

Properties of the crude oil were examined and the following results were obtained.

Table 3
Crude oil parameters

Parameters	**Observed**
Specific Gravity	1.0073 gm/ml^2
API Gravity	28.88
Pour Point	33^{o}C
Water Content	0.8%
WAX	7.45%
Saturates	64.66%
Aromatics	21.4%
Resins	10.12%
Asphaltene	2.72%

The study involved measuring the viscosity of the three samples (S1, S2, S3) and the control sample in a rheological machine to assess their flow properties after few days of mixing the bacteria samples with the crude oil. In this context, the researchers compared the shear stress and viscosity of the original crude oil sample with the shear rate and viscosity of the crude oil after mixing it with samples S1, S2 and S3 as well as with just the media (the control sample) at various temperatures.

The flow behavior of the raw crude oil and crude oil treated with different samples of bacteria is studied over a broad temperature range 10-45 °C as observed in rheology plots depicted in figures 2 and 3. A non-Newtonian shear thinning behavior is observed for both the virgin as well as treated crude oil samples over the examined temperatures. Viscosity of crude oil reduces with increase in temperature. As observed from the rheology plots, maximum reduction in viscosity occurs in crude oil treated with bacteria sample S1 at temperatures 20, 25, 30 °C indicating the best flow improvement among all other used samples.

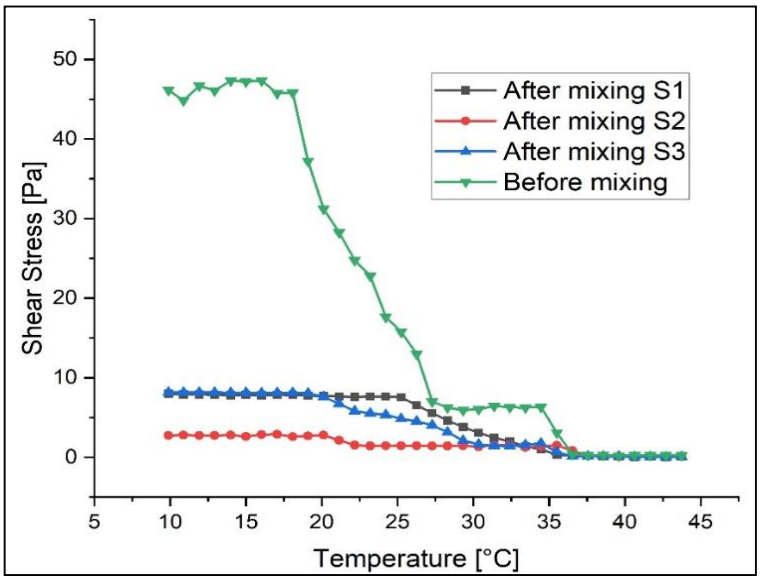


Figure 2: Shear Stress vs Temperature graph for Crude Oil before and after addition of bacteria culture

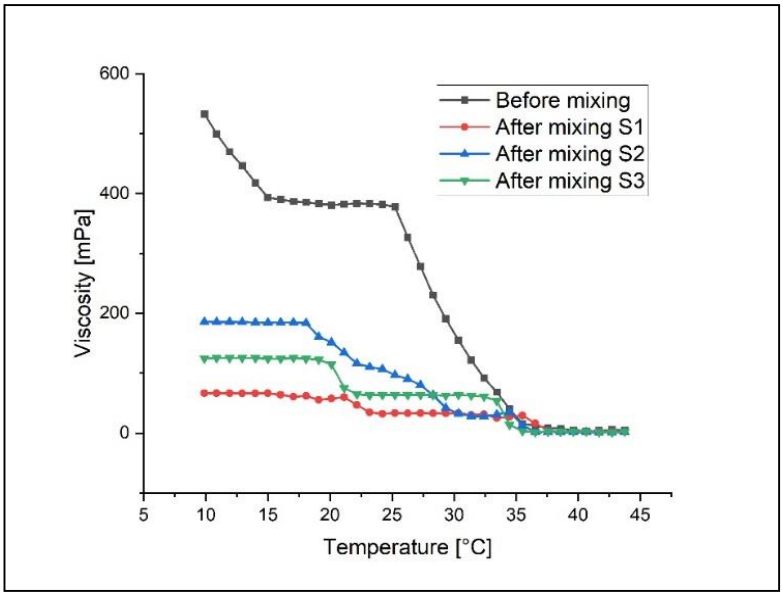


Figure 3: Viscosity vs Temperature graph for Crude Oil before and after addition of bacteria culture

Viscosity of crude oil at 25 °C reduced significantly from 400 cP of virgin crude oil up to 10 cP in case of crude oil treated with S1, 23 cP (in case of S2) and 35 cP (S3). The rheology plots suggest a substantial enhancement in flow, as there is a notable decrease in viscosity observed in crude oil treated with bacterial samples, indicating significant improvement in fluidity.

Microscopic Observations: To analyze the microorganisms, the sample was placed under a microscope which scrutinizes their staining characteristics. Using the Gram staining technique, bacteria were identified as Gram-positive, displaying colors like purple, violet, or pink. Gram-positive bacteria, identified by their purple stain retention in the Gram staining process, displayed a thick peptidoglycan layer in their cell walls. This structural feature contributes to their robustness and resilience.

Observations revealed a uniform and dense appearance under magnification, reflecting the cohesive nature of the cell wall composition (Fig. 4). The microscopic examination allowed for the identification of various shapes such as cocci or rod-shaped formations, providing essential information about the morphology of the Gram-positive bacteria sample.

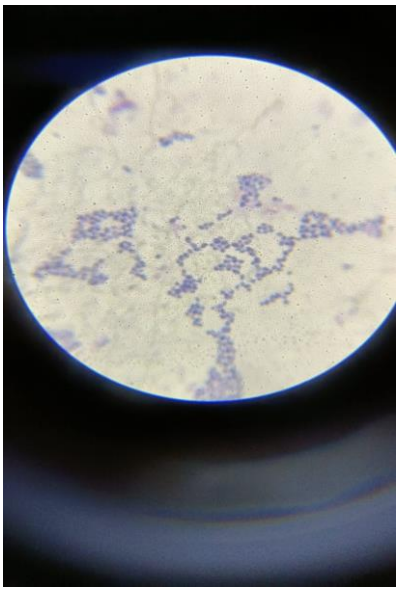


Figure 4: Microbes under Microscope

Storage and loss modulus: The outcomes of the frequency sweep measurements performed on the linear viscoelasticity region are illustrated in figure 5. The results reveal a continual increase in both the storage modulus (G′) and loss modulus (G″) with the ascending sweep frequency. Initially, at lower frequencies, G′ is lower than G″, indicating a predominance of viscosity. However, beyond a certain frequency range, this relationship shifts, indicating a prevalence of elasticity. Specifically, the viscoelastic behavior of heavy crude oil is characterized by G′ being less than G″ at lower sweep frequencies, transitioning to G′ being greater than G″ as the frequency exceeds a certain threshold. The Eigen value of the complex viscosity also increases with the ascending sweep frequency. The frequency at which G′ equals G″ in the sweep frequency measurement is denoted as the critical frequency point (fc) or crossover point. Below this critical frequency point, heavy crude oil demonstrates a viscous-dominant behavior, transitioning to elastic dominance as the sweep frequency surpasses this critical point. The critical frequency consistently decreases as temperature rises, indicating a consistent trend across all samples. In summary, higher system temperatures and frequencies expedite the shift of heavy crude oil from viscous dominance to elastic dominance.

Table 4
Comparison of pour point temperature (°C) of all the samples and raw crude

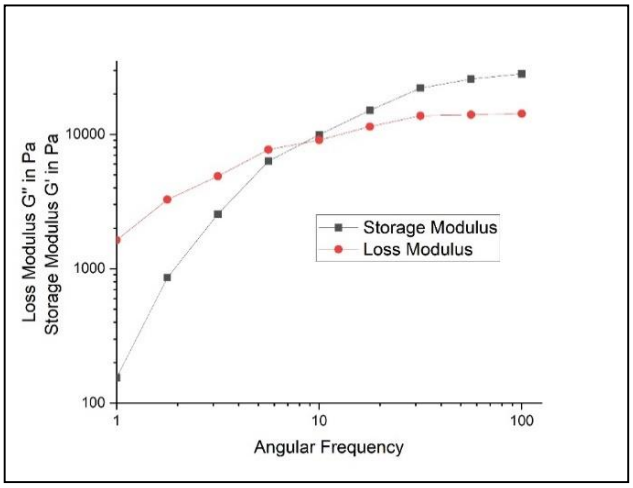
Day	Sample 1	Sample 2	Sample 3	Control	Raw sample
1	30	30.5	30.2	31	33
2	27	27	27.5	28	33
3	24	24.3	24.8	24.5	33

Pour Point Test: According to table 4, it was observed that the presence of microorganisms in all samples led to a decrease in the pour point value, reducing it from around 33°C to approximately 24°C on day 3. Therefore, the combined effect of the three sets of samples resulted in an approximate reduction of 9°C.

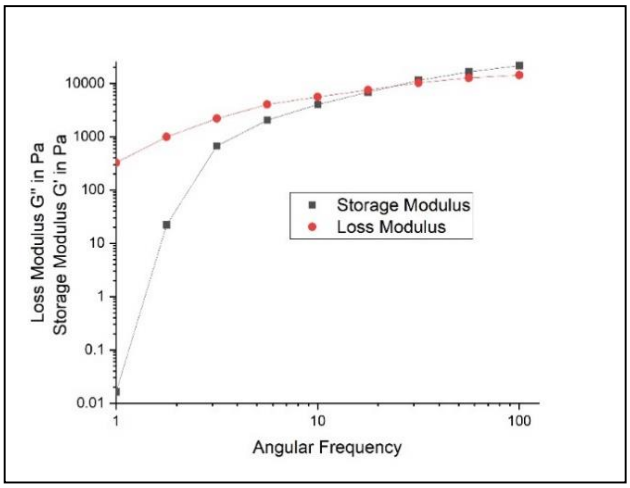
Conclusion

To improve the flow properties of crude oil, common methods involve the addition of surfactants, chemical additives, polymers and adjustments in temperature. In our study, we explored the effects of microorganisms derived from biodegradable sources, specifically fish waste and sugarcane bagasse, when mixed with crude oil in specific proportions. These microorganisms were cultivated by decomposing the two samples in an oxygen-deprived environment and suitable media were prepared to serve as their primary energy source. Using a rheometer, we conducted rheological tests to evaluate the flow characteristics of crude oil before and after introducing these microorganisms.

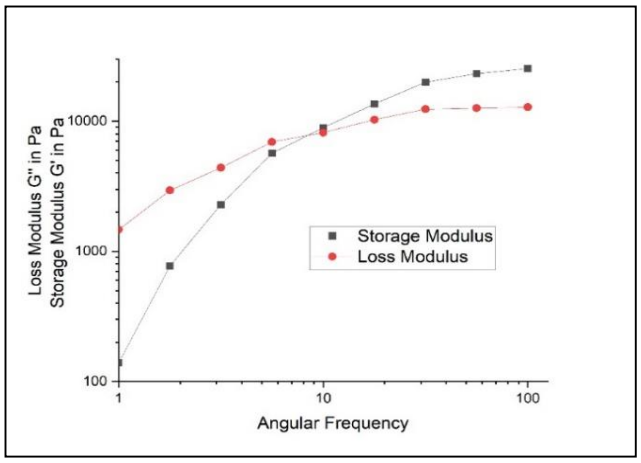
The results revealed a decrease in viscosity and shear stress for all samples, as shown in figure 2 and 3 with samples 1 and 2 showing good results. This suggests that samples 1 and 2 have the potential to enhance the flowability of crude oil by reducing its viscosity. Furthermore, we observed a decrease in the pour point across all samples, dropping from around 33 to 24 °C. If these microorganisms are non-biodegrading, implying that they do not utilize hydrocarbons as their energy source, they could be valuable for microbially enhanced oil recovery without impacting the hydrocarbon chains in the oil field.



(A)S1



(B)S2



(C)S3

Figure 5: Storage Modulus and Loss Modulus vs Angular Frequency after mixing with sample (A) S1, (B) S2 and (C) S3

Conversely, if they are found to be biodegrading, using hydrocarbons as their energy source in the absence of media, they could be beneficial for mitigating oil spills. In summary, our project highlights the potential of employing methane-producing bacteria to enhance the flow properties of crude oil. The reduction in shear stress, viscosity and pour point achieved through these bacteria's metabolic activities can contribute to more efficient processes in oil recovery, transportation and processing. Nonetheless, further research and practical implementation are crucial to validate and to optimize the utilization of methane-producing bacteria in the oil industry.

Acknowledgement
The authors would like to express their sincere gratitude to the Department of Petroleum Technology, Department of Petroleum Engineering and Centre for Biotechnology and Bioinformatics, Dibrugarh University for providing the excess of institutional facilities and resources to carry out the work without which this work would not have been possible.

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(Received 02nd February 2024, accepted 16th March 2024)