Egyptian Journal of Petroleum (2016) xxx, xxx–xxx

HOSTED BY

Egyptian Petroleum Research Institute

Egyptian Journal of Petroleum

www.elsevier.com/locate/egyjp
www.sciencedirect.com

FULL LENGTH ARTICLE

Bioremediation of petroleum based contaminants with biosurfactant produced by a newly isolated petroleum oil degrading bacterial strain

Debajit Borah [a,*], **R.N.S. Yadav** [b]

[a] *Centre for Studies in Biotechnology, Dibrugarh University, 786004 Dibrugarh, India*
[b] *Department of Life Sciences, Dibrugarh University, 786004 Dibrugarh, India*

Received 14 January 2016; revised 7 February 2016; accepted 14 February 2016

KEYWORDS

Bioremediation;
Biosurfactant;
Contact angle;
MOER;
Emulsification index

Abstract Petroleum based hydrocarbon degrading and biosurfactant producing bacterial strain was isolated from an automobile engine. The strain was identified as *Bacillus cereus* DRDU1 on the basis of 16S rDNA sequencing analysis. The strain was found to be efficiently degrading 96% of kerosene making it a potential tool for bioremediation of petroleum based contaminants. Production and optimization of the biosurfactant produced by the isolate were also carried out. Surface hydrophobicity trait of isolate was found to be 60.67 ± 1.53% and foaming percentage of the crude biosurfactant was found to be 31.33 ± 0.58%. The presence of amino acids and sugar moieties in the biosurfactant was confirmed by biochemical tests and were further validated by FTIR (the Fourier transform infrared) spectrometric analysis revealing the presence of υ_{OH}, $\upsilon_{CO=O}$, υ_{COOH}, υ_{CH} (stretching), υ_{NH}, υ_{CH_2}, υ_{CH_3}, and υ_{CH} (bending), and $\upsilon_{C=O}$ (ester) in the surfactant. The decrease in contact angle of hydrocarbon oil from (30.67 ± 1.15)° to (21.3 ± 1.53)° respectively after 3 and 6 days of incubation reveals its potential to emulsify petroleum oil. Further, emulsification index (E_{24}) of biosurfactant against kerosene, crude oil, and used engine oil were determined to be 55.33 ± 1.53%, 29.67 ± 1.53%, and 20 ± 1% respectively which attracts its future application in MEOR (microbial enhanced oil recovery) process.

1. Introduction

The usage of petroleum hydrocarbon products increases the chances of soil contamination with diesel and used engine oil that becomes one of the major environmental problems. Statistical analysis, carried out by the International Tanker Owners Pollution Federation Ltd. (ITOPF) since the year 1970–2013, shows three oil spills of 700 tons or more occurred in year 2013 itself [1]. Large-scale marine oil spills, and oil spill accidents, have received great attention worldwide, due to their cataclysmic effect on the environment. Numerous physical and chemical techniques are practiced worldwide but bioremediation provides the most cost effective and eco-friendly measure for the remediation of petroleum contaminated soil and water to bring back its native environment. Although extensive

* Corresponding author.
E-mail address: dborah89@gmail.com (D. Borah).
Peer review under responsibility of Egyptian Petroleum Research Institute.

http://dx.doi.org/10.1016/j.ejpe.2016.02.005

Please cite this article in press as: D. Borah, R.N.S. Yadav, Bioremediation of petroleum based contaminants with biosurfactant produced by a newly isolated petroleum oil degrading bacterial strain, Egypt. J. Petrol. (2016), http://dx.doi.org/10.1016/j.ejpe.2016.02.005

research has been conducted on microbial bioremediation of oil contaminants during the last decade the effectiveness of stress tolerant hydrocarbon degrading microorganisms are less studied with the evidence of scientific publications [2–10]. Stress tolerant microbes are of great interest for their ability to survive in a wide range of geographic and climatic conditions. As it is known hydrocarbon residues are retained in various parts of automobile engines run on petroleum fuel, and release more or less converted form of hydrocarbon residues after combustion [10]. The circulating flow of lubricating oil can carry away a lot of heat and have the supplementary advantage of reducing the thermal stress on the part of automobile engine. Therefore, it is assumed that automobile engine facilitates the growth of stress tolerant hydrocarbon degrading microorganisms, which are capable of tolerating physical and nutritional stress. Earlier it was reported that a stress tolerant hydrocarbon degrading *Bacillus cereus* strain DRDU1 isolated from an automobile engine could degrade 77%, 67%, and 16% of diesel, crude oil and used engine oil respectively in the absence of external nitrate (N) and phosphate (P) supplement and the degradation rates were significantly increased up to 99%, 84% and 29% in the presence of N and P supplement [11]. The important requirement for the success of bioremediation is the existence of microorganisms with the suitable metabolic activities for the degradation of a specific type of petroleum oil. One of the most important features of hydrocarbon degrading bacteria is the ability to produce biosurfactants comprising various chemical structures, such as polysaccharides, fatty acids, glycolipids, peptides, and proteins with hydrophobic and hydrophilic moieties that diminish surface and interfacial tension between individual molecules making them potential candidates in emulsification and enhancing oil recovery [12].

Therefore, the study was extended for the production and characterization of biosurfactant produced by the isolate *B. cereus* strain DRDU1, which attracts its further application in microbial enhanced oil recovery (MEOR).

2. Materials and methods

2.1. Isolation, screening, and identification of hydrocarbon degrading microorganisms

Hydrocarbon degrading microorganisms were screened by spreading hydrocarbon residues from various parts of automobile engine on Bushnell and Haas (BH) media (composition g/L: $MgSO_4$-0.2, $CaCl_2$-0.02, KH_2PO_4-1.0, K_2HPO_4-1.0, NH_4NO_3-1.0, $FeCl_3$-0.05, agar–agar-20.0, pH-7.0 at 25 °C) supplemented with 200 μL of used engine oil [11]. The best isolate was further screened on the basis of growth on BH broth supplemented with 2% (*v*/*v*) used engine oil as sole source of carbon. Best isolate was identified on the basis of 16S rDNA sequencing using:

Forward primer: 8F (5′-AGA-GTT-TGA-TCC-TGG-CTC-AG-3′) and

Reverse primer: 1492R (5′-GGT-TAC-CTT-GTT-ACG-ACT-T-3′)

BLAST was carried out with the consensus sequence hence generated and the phylogenetic analysis was carried out using MEGA-6 software by neighbor-joining method with 1000 bootstrap [13–15]. The evolutionary distance of the isolate with 10 most closely related strains on the basis of BLAST result was computed using the Kimura 2-parameter method by forcing *Bacillus amyloliquefaciens* ATCC-23350 as out group. The consensus sequence hence generated was deposited in NCBI GenBank database to receive the GenBank accession number [16].

2.2. Optimization of growth conditions

Hundred milliliter freshly prepared BH broth supplemented with 2% *v*/*v* petroleum oil (kerosene, crude oil, and used engine oil) was inoculated with 1 mL of overnight bacterial broth (O.D. $\geqslant$ 0.1) of the most potent isolate taken in separate air tight Erlenmeyer flasks. The optimum concentration of the salt components for the strain was determined by growing the isolate at different concentration of $MgSO_4$, $CaCl_2$, KH_2PO_4, K_2HPO_4, NH_4NO_3 and $FeCl_3$ individually, keeping the rest of the components constant as described earlier [17]. Bacterial growth in terms of cfu was monitored on the 7th day of incubation maintained at 37 ± 2 °C and 135 rpm. Optimum pH and temperature for the maximum growth of the isolate was determined by maintaining the media at a pH range from 1 to 14 and incubation temperatures at 25, 30, 35, 40 and 45 °C individually at 135 rpm for 7 days.

2.3. Biodegradation of kerosene by the isolate

Degradation of kerosene oil by the isolate was determined after 28 days of incubation at 135 rpm, 37 °C by the isolate in BH broth supplemented with 2% (*v*/*v*) kerosene oil by gravimetrically. Degradation was finally confirmed by gas liquid chromatographic (GLC) analysis [18].

2.4. Recovery of biosurfactant

The most potent isolate was inoculated in BH broth supplemented with 2% (*v*/*v*) of the most preferred hydrocarbon supplement *i.e.*, diesel oil as reported earlier under optimized condition for 7 days [11]. After incubation, the culture was centrifuged at 10,000*g* for 15 min at 4 °C to separate the biomass. Crude biosurfactant was precipitated by adding three volumes of chilled acetone to the cell free supernatant, maintained at 4 °C with vigorous stirring for 10 h on a magnetic stirrer [19]. The crude biosurfactant was recovered by separating the precipitate under 10,000*g* for 10 min followed by air drying.

2.5. Bacterial adhesion to hydrocarbon (BATH) test

Hydrophobicity assay of the isolate was carried out by the method described by Ramasamy et al. (2014) [18]. The isolate was grown in BH broth supplemented with 2% (*v*/*v*) diesel oil under optimized condition. The cell pellet was obtained after centrifugation at 8000*g* for 10 min and washed twice to remove the hydrocarbon and other biopolymer residues. The pellet was resuspended in buffer salt solution (composition g/L: K_2HPO_4-16.9, KH_2PO_4-7.3, urea-1.8, $MgSO_4{\cdot}7H_2O$-0.7, and pH-7.0) and adjusted the optical density (OD) at 1.0 at wavelength 600 nm [(A_0) = 1.0]. Four milliliter of cell suspension was mixed with 1.0 mL of hexadecane in a screw capped tube

Please cite this article in press as: D. Borah, R.N.S. Yadav, Bioremediation of petroleum based contaminants with biosurfactant produced by a newly isolated petroleum oil degrading bacterial strain, Egypt. J. Petrol. (2016), http://dx.doi.org/10.1016/j.ejpe.2016.02.005