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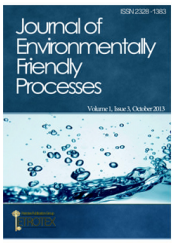

Application of Organic Surfactants to Recover Hydrocarbons from Oil Sludges

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Abstract:

Biosurfactant can be produced from various substrates, mainly renewable resources such as vegetable oils, distillery and dairy wastes. The Biosurfactant was a rhamnolipid-type and had a good foaming, emulsifying and antimicrobial activities. Biosurfactants can also be involved in microbial enhanced oil recovery (MEOR). MEOR methods are used to recover oil remaining in reservoirs after primary (mechanical) and secondary (physical) recovery procedures. The objective of this processes is oil separation of hydrocarbons from oil sludge by biosurfactant. The first step is about isolation pseudomonas bacteria from polluted soil and sludge, was capable producing biosurfactant to emulsify oil. In the second part investigate the effect of various experimental conditions, such as solution pH, biosurfactant concentration and salinity in separation process.

Keywords: Biosurfactant, Pseudomonas, bacteria, Oil Sludge

1. Production of Biosurfactant

Abouseoud et al. Studied on Production of Biosurfactant from olive oil by Pseudomonas fluorescens. They obtained the best result when using olive oil and ammonium nitrate as carbon and nitrogen sources respectively with a C: N ratio of 10. The surface tension dropped rapidly after inoculation, reaching its lowest value (30 dyne/cm) during exponential phase after about 40 hours of growth [1].

Sifour et al. investigated on the production of Biosurfactant from two bacterial isolates, Bacillus licheniformis(B5) and B.subtilis (B6). They obtained Maximum production of biosurfactants from both strains when using glucose and glutamic acid as carbon and nitrogen sources respectively [2].

Batista etal.studied on Biosurfactant-producing bacteria were isolated from terrestrial and marine samples collected in areas contaminated with crudeoil or its byproducts. Isolates were screened for biosurfactant/bioemulsifiers production in different carbon sources (glucose, fructose, sucrose and kerosene) using the qualitative drop-collapse test. Glucose was a better carbon source.Eight of the 17 isolates reduced the growth medium surface tension below 40 mN m^{-1}[3].

In another study, by ManojKumaret et al. isolated strain DHT2 from oil-contaminated Soil. The organism grew and produced biosurfactant when cultured in variety of substrates at salinities up to 6 g l^{-1} and temperatures up to 45C°. It was capable of utilizing crude oil, fuels, alkanes and PAHs as carbon source across the wide range of temperature (30–45C°) and salinity (0–6%) [4].

Ebrahimpour et al. isolated Pars Q2, an extreme halophilicarchea bacterium from the Namakdan salt lake on Qeshm Island, it was capable producing biosurfactants to emulsify and degrade crude oil. Studied the effects of salinity, pH, temperature, aeration (shaker speed) and the minimum optimized concentration of nitrogen and phosphate sources on the bioremediation of crude oil. The results showed that 15- 21% NaCl, pH 8.2, 35° C and 140 rpm (shaker speed), 0.2 g of $(NH4)_2SO4$ and 0.1 g. of KH2PO4 provided optimal conditions for oil biodegradation. Under such optimal conditions, the bacterium degraded 100% of the available crude oil after seven days of incubation [5].

In another study by Safary et al. in the initial screen, hemolysis in blood agar and visual observation of the oil emulsion in the culture medium are the qualitative methods used to prove the production of biosurfactant. In the secondary screen, surface tension reduction is the one quantity method determined by surface tension measutment device [6].

AkhavanSepahy et.al investigated on the Production of biosurfactant from Iranian oil fields by isolated Bacilli. The ability of surfactant producing bacteria indicated by reduction of surface tension (ST) and interfacial tension (IFT) of the supernatant .Eight strains obtained the IFT reduction in crude oil, hexadecane, sucrose, glucose, fructose and mannose medium as a sole source of carbon and energy at 40 0C by 15-30 mN/m. [7].

Anandarajet et.al investigated isolation and production of biosurfactant producing organism from oil spilled soil. The five organisms were screened for the purpose of biosurfactant production. From the screening tests i., (oil spreading test and blood haemolysis test) one of the culture Pseudomonas sps showed the capacity to producer biosurfactant [8].

2. Application of Biosurfactant Formulas for Recovering Hydrocarbons from Oil Sludge.

Chenggang Zheng.et. al studied on Oil extraction from oil sludge with biosurfactant formulas was optimized by a Taguchi orthogonal array design of L16 (45) with five main factors, including biosurfactant type (surfactin, lichenysin, rhamnolipidandemulsan), biosurfactant concentration, pH, salinity and solvent. Oil recoveries obtained with the sixteenbatch washing experiments with the selected levels of each factor were processed with DesignExpert/SPSS .The predicted optimal biosurfactant formula of 2.0 g/L rhamnolipid, pH 12.0, 10 g/L NaCl, and 5.0 g/L nbutanol were validated by a washing experiment that yielded an oil recovery of 74.55%, which was27.28% higher than the grand average oil recovery of the whole experiment design. Based on the optimum biosurfactant formula, the oil extraction process followed first-order kinetics as the washing rate constant and final oil recovery increased with temperature. The effect of the individual factors at different levels is representing in Figure 1 [9].

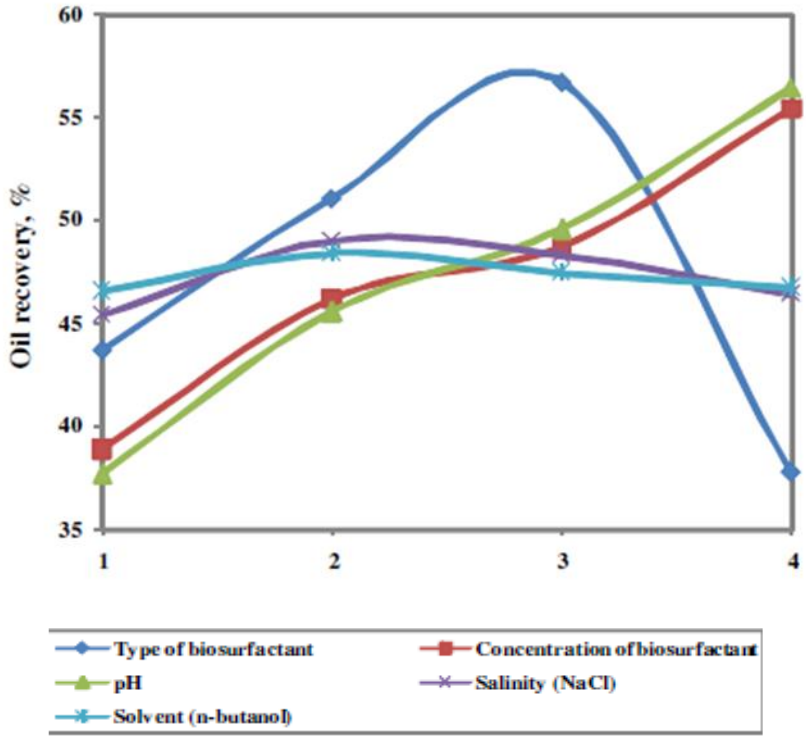

Figure 1. Performance of individual factors at different levels (average value of obtained experimental results [10].

In another study by P.J, Joseph et al. separated the oil from the petroleum sludge by induced biosurfactant production by bacteria. The sludge used for the investigation contained TPH in the concentration range of 850 ± 150 g·kg−1. The efficiency of removal of the various isolates ranged from 91.67% to 97.46%. Therefore, it has been observed that the biosurfactant produced by the primary inoculum remained in the supernatant and it was enough to continue the reaction. The biosurfactant displayed the property to reduce surface and interfacial tensions in both aqueous and hydrocarbon mixtures and hence had potential for oil recovery [11].

WuxingLiuet.al isolated from oily sludge and petroleum-contaminated soil from Shengli oil field in north China. These bacteria were used to treat oily sludge and the recovery efficiencies of oil from oily sludge were determined. The oil recovery efficiencies of different isolates ranged from 39% to 88%.Bacterial isolateBZ-6 was found to be the most efficient strain and the three phases (oil, water and sediment) were separated automatically after the sludge was treated with the culture medium of BZ-6.Based on morphological, physiological characteristics and molecular identification, isolate BZ-6 was identified as Bacillus amyloliquefaciens[12].

Vanessa S. Cerqueira isolated three bacteriafrom petrochemical oily sludge, identified as Stenotro phomonas acidaminiphila, Bacillus megateriumand Bacillus cibi, and two bacteria from a soil contaminated by petrochemical waste, identifiedas Pseudomonas aeruginosa and Bacillus cereus demonstrated efficiency in oily sludge degradationwhen cultivated during 40 days. The bacterial consortium demonstrated excellent oily sludge degradationcapacity, reducing 90.7% of the aliphatic fraction and 51.8% of the aromatic fraction [13].

Khalifehetal.was obtained the best results in 0.1% concentration of biosurfactant, PH=8.5 and salinity of 1.0%. The total pollution amount considered is 1000 ppm and oil separation species is 89% [13].

Rahman et al. examined the bioremediation of n-alkan in petroleum sludge containing an oil and grease content of 87.4%.Remarkably, 10% of the sludge continuing C8-C11 alkenes were degraded 100%; whilst C12-C21,83-89%;C22-C31 between 80-85% and finally C32-C40,57-73% after 56days with addition of a bacterial consoryum, nutrients and rhamnolipid[14].

3. Literature on Treatment of Contaminant Sludge

This section reviews some similar studies in two main fields of study around the world and also in Iran. One is about researches having been done on production of Biosurfactant and the other discusses about studies on the removal of hydrocarbon from waste sludge by use of Biosurfactant.

3.1. Biosurfactant activity assays

3.1.2. Hemolytic activity

Isolated strains were screened on blood agar plates containing 5% (v/v) human blood and incubated at room temperature for 24 h. Hemolytic activity was detected as the occurrence of a define clear zone around a colony[15].

3.1.3. Emulsification Measurement

Emulsification activity was measured according to the method of Cooper and Goldenberg (1987) with a slight modification. To 4 ml of culture supernatant or biosurfactant crude extract (0.5%, w/v), 4 ml of n-hexadecane were added and vortexes at high speed for 2 min. The mixture was allowed to stand for 10 min prior to measurement. The emulsification activity is defined as the height of the emulsion layer divided by the total height and expressed as percentage [16]. The emulsification indices after 24 h, 48 h, 72 h, etc. were represented as E24, E48, and E72 respectively[17].

E_{24} (%) $=\frac{hemulsion}{htotal} \times 100$

3.1.4. Oil Spreading Assay

The oil spreading assay was developed by Morikawaet al. For this assay, 10 μL of crude oil is added to the surface of 40 ml of distilled water in a petri dish to form a thin oil layer. Then, 10 μL of culture or culture supernatant is gently placed on the centre of the oil layer. If biosurfactant is present in the supernatant, the oil is displaced and a clearing zone is formed. The diameter of this clearing zone on the oil surface correlates to surfactant activity, also called oil displacement activity. For pure biosurfactant a linear correlation between quantity of surfactant and clearing zone diameter is given. The oil spreading method is rapid and easy to carry out, requires no specialized equipment and just a small volume of sample.36 It can be applied when the activity and quantity of biosurfactant is low [18].

3.1.5. Du-Nouy-Ring Method

The Du-Nouy-Ring method is based on measuring the force required to detach a ring or loop of wire from an interface or surface. The detachment force is proportional to the interfacial tension. It can be measured with an automated tensiometer which is available from many manufacturers. The ring must be free from contaminant, which is usually achieved by using a platinum ring that is flamed before use. Instead of a ring, a platinum plate, a so called Wilhelmy plate, can be applied in the same manner. The Du-Nouy-Ring assay is widely

applied for screening of biosurfactant producing microbe. The advantage of this method is the accuracy and the ease of use. However, it requires specialized equipment [18].

4. Extraction of the Biosurfactant

The recovery and purification of biosurfactants from complex fermentation broth is a major problem in the commercialization of biosurfactants [19].Bacterial cells were removed by centrifugation (12,000 x g, 4oC, and 30 min). Cultural supernatant was acidified with 6 N HCl to obtain the pH of 2.0. The extraction was performed twice with an equal volume of ethyl acetate. Biosurfactant yield was expressed as g/l was measured in the cell-free culture medium. Known amounts of crude precipitate were re-suspended in distilled water and used for the determination of the critical micelle concentration (CMC) [19].

5. Sludge Washing Kinetics

The prepared oil sludge was mixed with the optimized biosurfactant formula at elevated temperatures for the washing experiment with a constant shaking speed of 200 rpm. At time intervals, the test tubes containing oil sludge and biosurfactant solution were sampled and oil recoveries were calculated. The washing process continued until the oil recovery became almost invariant with time. During this investigation, the following first order kinetics for recovery of oil by sludge washing was used (Cilek, 2004; Polat and Chander, 2000):

$$\text{Ln}\ (R\infty / (R\infty - R)) = kt$$

where k is the washing rate constant, R is the oil recovery at any time and R1 is the final oil recovery at the end of washing (at 60 min), beyond which the oil recovery does not change appreciably. Based on the model above, the relation between washing time and oil recovery was concluded and the value of slope (washing rate constant, k) was determined [6].

6. Industrial and Environmental Applications of Biosurfactants

The main commercial use of biosurfactants is in pollution remediation because of their ability to stabilize emulsions. This enhances the solubility and availability of hydrophobic pollutants, thus increasing their potential for biodegradation [20].Biosurfactants (Microbial Surface Active Agents) have become recently an important product of biotechnology for industrial and medical applications [22,21].

6.1. Microbial De-emulsification of Oil Emulsions

Oilfield emulsions, both oil-in-water and water-in oil, are formed at various stages of exploration, production and oil recovery and processing, represent a major problem for the petroleum industry. A process of de-emulsification is required to recover oil from these emulsions. Since the presence of water and sediments in oil causes corrosion and scaling in tanks and pipelines, a basic sediment and water (BS & W) content of 0.5 to 2.0% has been specified as the maximum allowable in crude oil for transportation through the existing pipelines. Factors that influence the stability of emulsions include viscosity, droplet size, phase volume ratio, temperature, pH, and age of emulsion, type of emulsifying agent present, density difference and agitation. Traditional de-emulsification methods include centrifugation, heat treatment, and electrical treatment and chemicals containing soap, fatty acids and long-chain alcohol. Several microorganisms are known to possess demulsification properties (Table 1).

N. Nadarajah and et al, isolated a mixed bacterial culture from a petroleum-contaminated site, was evaluated for its de-emulsification capabilities using a surfactant-stabilized kerosene–water model system. The culture produced high de-emulsification activity with an initialde-emulsification rate of 44%/h (44% of emulsion separated in 1 h) and a maximum extent of de-emulsification of 96% in 24 h [23].
Gh.Mohebaliet.al. isolated a new de-emulsifying bacterium, O. anthropi strainRIPI5-1from the oil-polluted sandy bank of Siri Island, Iran [26] Initial rate (DeI1) of breaking of the multiple water–crude oil emulsion by whole culture and whole cells were calculated as 11% and 54%, respectively. However, overall demulsification (DeI8.5) for whole culture and whole cells was calculated as 63% and 72%, respectively.

Table 1. Microbial species with de-emulsifying capability [*23*].

Microbial species
Acinetobactercalcoaceticus
Acinetobacterradioresistens
Aeromonas sp.
Alcaligeneslatus
Alteromonas sp.
Bacillus subtilis
Corynebacteriumpetrophilum
Kingelladenitrificans
Micrococcus sp.
Nocardiaamarae
Pseudomonas aeruginosa
Pseudomonas carboxydohybrogena
Rhodococcusauranticus
Rhodococcusgloberulus
Rhodococcusrubropertinctus
Sphingobacteriumthalpophilum
Torulopsisbombicola

7. Conclusion

It is an important tertiary process where microorganisms or their metabolites, including biosurfactants, biopolymers, biomass, acids, solvents, gases and also enzymes, are used to increase recovery of oil from depleted reservoirs. Application of biosurfactants in enhanced oil recovery is one of the most promising advanced methods to recover a significant proportion of the residual oil. The remaining oil is often located in regions of the reservoir that are difficult to access and the oil is trapped in the pores by capillary pressure .Biosurfactants reduce interfacial tension between oil/water and oil/rock. Biosurfactants can also bind tightly to the oil-water interface and form emulsion. This stabilizes the desorbed oil in water and allows removal of oil along with the injection water.

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