Oil pollution prevention: crude oil biodegradation by isolated bacterium of the Persian Gulf

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Abstract

The Persian Gulf is the most strategic waterways in the world due to its importance in the global oil transportation. Due to the war and high rate of water evaporation, extended drilling and oil extraction, land-based sources, dumping from ships and other human activities, water pollution has increased alarmingly. Many sea birds and other species of marine life have perished because of millions of tonnes of crude oil entering into the Gulf. The ecosystem is vulnerable and conservation of its marine environment is highly recommended.

The potential biodegradation of crude oil was assessed based on the development of a fermentative process with a marine bacterium which has been isolated from the Persian Gulf. This gram negative, spherical shape bacterium could degrade 50% of crude oil content and the cell growth curve of it was drawn on the basis of biomass high amount and its maximum production rate was determined which was diminished from 60 (before optimization) to 27 hours. It can grow on different carbon sources including crude oil and n-alkanes and produce biosurfactant which was revealed to be rhamnolipid. The biosurfactant extracted by special method from M3 medium and pursued by Thin Layer Chromatography. The outcomes of biomass production by crude oil and n-alkanes were so much different and showed that the hydrocarbon complexity and configuration plays a significant role in the biodegradation procedure. These bacteria can be used in environmental risk management by focusing on strategies to prevent this human made disaster.

Keywords: environmental risk management, human made disaster, oil biodegradation, marine bacterium, Persian Gulf.

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1 Introduction

The Persian Gulf is now one of the sensitive marine ecosystems. The Persian Gulf with the presence of coral colonies and plant species needs clear protection because of its crucial role in the earth's life supporting phenomena. The effect of industrial pollution, oil spills on the marine environment is forcing us to focus on the problem and the need for coastal conservation. The plants living on the seabed near the shore are supporting marine life such as dugongs and turtles. The extraction of oil from coastal areas is causing the most important problem, namely, pollution.

We experienced serious environmental damage in the 1991 Gulf war; the world largest oil spill, an estimated 8 million barrels. The gulf water in coastal areas of Iran, Kuwait, and Saudi Arabia were fouled. In general, the major sources of pollution are from ships, land-based sources, dumping from ships and aircrafts, exploration and exploitation of the bed of the territorial sea, the continental shelf and the sub-soil thereof, and from other human activities (human made disasters).

Oil spills, oily wastewater, especially from oil fields, have posed a great hazard for terrestrial and marine ecosystems [1]. Under certain conditions, living microorganisms (primarily bacteria, but also yeasts, molds, and filamentous fungi) can alter and/or metabolize various classes of compounds present in oil, a set of processes collectively called oil biodegradation. Spilled petroleum into the sea spreads over the surface of the water. It is subjected to many modifications, and the composition of the petroleum changes with time. This process is called weathering, and is mainly due to evaporation of the low molecular weight fractions, dissolution of the water-soluble components, mixing of the oil droplets with seawater, photochemical oxidation and biodegradation [2]. Microorganisms that grow rapidly on hydrophobic substrates often produce small molecular weight biosurfactants or polymeric bioemulsifiers that emulsify the substrate and thereby increase its bioavailability. Biosurfactants has been found to enhance degradation of crude oil [1, 3, 4] and other hydrocarbons [5]. Among various surfactants, rhamnolipids are considered to be the most in degrading hydrocarbons [6]. For example, the biodegradation of long-chain alkanes was stimulated by addition of rhamnolipid [7]. It is claimed that treatment by a dispersant enhances the biodegradation of petroleum [2]. This facilitated biodegradation is probably due to the increase of cell surface hydrophobicity after extraction of lipopolysaccharides from the cellular envelope by rhamnolipids, which subsequently stimulates uptake via direct contact between cells and hydrocarbon droplets. Thus, the interaction between addition of rhamnolipid and biodegradation of hydrocarbons seems to be highly specific[1]. The objective of this research was to evaluate the biodegradation of crude oil by isolated marine bacterium from the Persian Gulf in laboratory. It can utilize crude oil and n-alkanes as the sole carbon source. We investigated the feasibility of crude oil degradation by microbial process.

2 Materials and methods

2.1 Growth study

The bacterium used in this study is a gram negative coccus which has been isolated from the Persian Gulf. The bacterium was maintained on a plate of M1 culture medium at 4^0C and sub cultured each month .M1 medium is composed (per liter): 1.25g yeast extract; 0.75g peptone; 15g agar. We inoculated the organism from M1 to M2 medium. The M2 medium is composed of (per liter): 4.4g $Na_2HPO_4.2H_2O$, 2.4g KH_2PO_4, 2.5g $(NH_4)_2SO_4$, 1g $CaCl_2.2H_2O$, 0.6g $MgSO_4.7H_2O$, 30 ml raw petroleum – from Tehran oil refinery – (and n-alkanes including decane, tetradecane, pentadecane, and hexadecane) plus 1ml trace elements consists (per liter) of 40g $FeSO_4.7H_2O$, 5g $MnSO_4.H_2O$, 400g Citrate monohydrate, 1.2g $(NH_4)_6Mo_7O_{24}.4H_2O$. The cultivations were conducted in 1 L shaking (140 r/min) flasks containing 200 ml medium. The optimum temperature was controlled at 35 °C and pH was regulated on 9 (optimized). In the next stage the biomass from M2 was inoculated to M3 medium. M3 medium is composed (per liter) of 0.37g $CaCl_2.2H_2O$, 2.5g NaCl, and 10ml carbon source to produce biosurfactant and the biosurfactant was extracted after 48 hours.

2.2 Biosurfactant extraction from M3 medium

The optimized biomass which has been produced by M2 medium was centrifuged in 17000×g rotation speed for 15 minutes and then transferred to M3 medium. The pH of medium was lowered to 2.5-3 by sulfuric acid 10% and then poured into a decanter. The medium was mixed with equal volume of ethyl acetate twice (Merck) to split it to two layers. The upper layer includes Biosurfactant. The Biosurfactant soluble was put in rotavapor (RE 120) with 80^0C warm water bath. The precipitant was collected for further purification by thin layer chromatography (TLC) [8].

2.3 Analysis of partially purified biosurfactant by TLC

The Biosurfactant was separated and detected by TLC (Silica gel 60 F_{256} - 1/05735 Merck) with solvent system including: chloroform/methanol/glacial acetic acid (65:15:2 v/v/v). The components were observed under UV light. Then put the biosurfactant on TLC along with other commercial biosurfactants to determine its class.

2.4 The utilized crude oil by bacterium

We inoculated bacterium to the M2 medium and after 27 hours, 50ml CCl_4 was added to medium and the residual oil was separated by decanter and hence the consumed crude oil was measured [9].

2.5 Effect of different hydrocarbon sources on biomass and Biosurfactant production

We compare biomass and biosurfactant production in M2 and M3 media by different hydrocarbons including crude oil, n-decane, n-tetradecane, n-pentadecane, and n-hexane.

3 Results

Bacterial strain is a gram negative, motile, spherical-shaped bacterium. It can't degrade lactose or sucrose. The strain is catalase and oxidase positive and produced indole and H_2S. It's MR (Methyl Red) and VP (Vogues proskauer) tests are positive and negative, respectively. It didn't produce CO_2 and its TSI is Alk/A. the maximum amount of biomass production had been achieved after 60(fig 1) hours but after optimization this period declined to 27 hours (fig 2).

The biosurfactant which has been extracted in this study is exogenous. It was compared on TLC with various other biosurfactants and classified in rhamnolipids group. The bacterium produced most and least biomass amount in the presence of tetradecane and hexadecane respectively as carbon source but produce most biosurfactant by hexadecane (fig. 3); but this result is vice versa in the presence of crude oil. It means, in the presence of crude oil as carbon source, the more biomass increases the more biosurfactant enhances. By the way, it can degrade the crude oil by 50%., the maximum biomass and biosurfactant was achieved at the same time (after 27 hours) that shows the more biomass produces, the more biosurfactant generates in the presence of petroleum as carbon source (table 1).

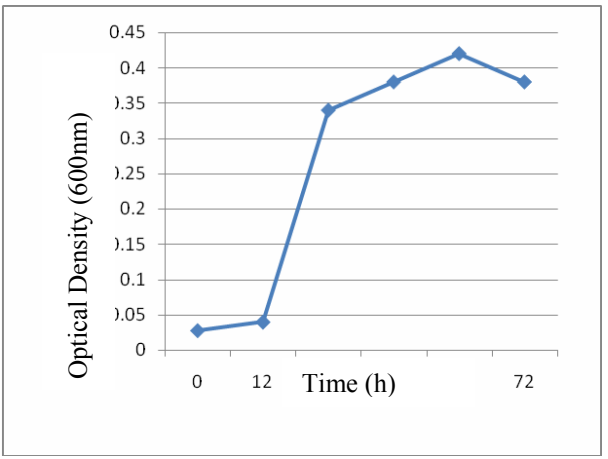

Figure 1: Biomass production measurement by spectrophotometer before M2 optimization.

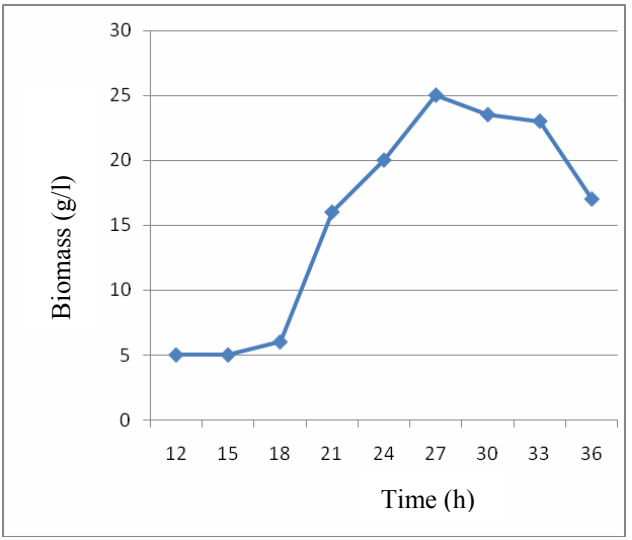

Figure 2: Biomass production after M2 optimization.

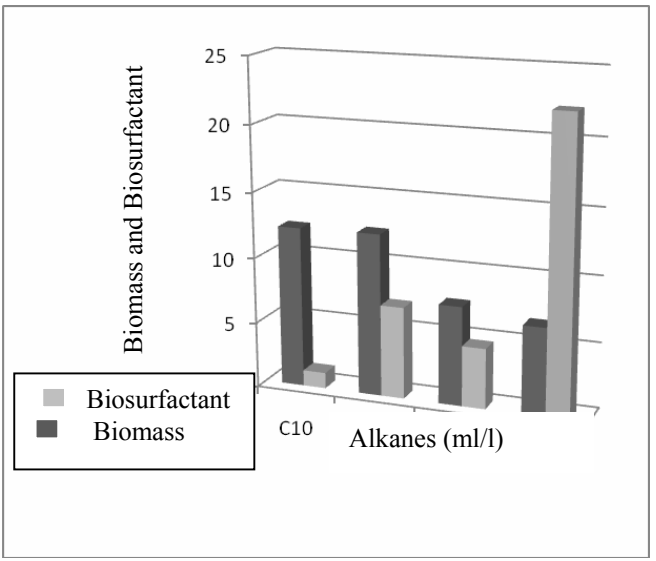

Figure 3: Biomass and biosurfactant production by different alkanes.

Table 1: Maximum biomass and biosurfactant production.

Time(h)	12	15	18	21	24	27	30	33	36
Biomass(g/l)	5	5	6	16	20	25	23.5	23	17
Biosurfactant (g/l)	-	-	6.2	7.2	8.3	8.6	8.2	7.8	6.3

4 Conclusion

Using microbes in environmental clean up activities is of significance. They are called as 'anti-polluters' that metabolize toxic substances and remove recalcitrant compounds present in the pollutant. Biodegradation is another process in which microorganisms or their enzymes breakdown the toxic chemical substances into smaller non-toxic substances. Through microbial biodegradation, petroleum hydrocarbons can be degraded in the oiled coastal ecosystems (e.g. Pseudomonas sp. and Raulstonia sp. Planococcus halophilus). Earlier studies indicate that microorganisms have the capacity to degrade 10 tons of oil per/day in the oil polluted area [10].

There are so many different bacteria which have been isolated from diverse geographical locations. New marine bacteria were isolated from sea water enrichment cultures. When the carbon source is crude oil; the more it produces biomass, more biosurfactant achieves. The greatest amount of biosurfactant is produced from the end of the exponential phase of growth curve. But in the presence of n-alkanes including tetradecane and hexadecane this phenomenon does not go true which shows the importance and role of carbon sources in biosurfactant production. It seems this phenomenon is due to the enhancement and inhibition role of biosurfactants to biodegrade organic compounds which its mechanisms behind these phenomena remain incomprehensible [11]. The bacterium can degrade oil by 50% and it can be seen by a dark colour and the droplet appearance of the culture medium. Most of the petroleum-degrading bacteria produce surfactants. Therefore, their cultures become dark brown and turbid as an oil slick is transformed to many small oil droplets [2].

Emphasis has to be made especially on microbial diversity, the largest untapped reservoir in the biosphere. Through biotechnological applications, microorganisms can be used for the discovery of new biotechnology products essential for pharmaceuticals, synthesis of new enzymes, chemicals and new organisms that carry out novel processes.Oil spills constitute a serious environmental and socio-economic problem [12]. Results obviously show that we can use this bacterium to remove oil pollutants in soils and waters, Microbial Enhanced Oil Recovery (MEOR), clean up of oil tanks [13] and petroleum extraction from oil sludge in the bottom of depository petroleum tanks [14]. Current oil production technologies recover only about one-third to one-half of the oil originally present in an oil reservoir [15]. The ability of degrading oil with average quality and biosurfactant production make this bacterium a good candidate to be used in these fields especially in MEOR and recovery of oil sludge that have economic justification. Biosurfactants are resistant against high

temperatures, therefore they can be autoclaved and used in various pharmaceutical, sanitary, polymer and food industries. Study of the biodiversity of microorganisms and its potential applications provide large scope for the development of new strains through genetically engineered microbes/recombinant DNA technology. These unique combinations may be able to degrade complicated pollutants prevalent in the environment. Further, creating awareness among general public and providing up-dated information on biodiversity of microorganisms and its various applications are essential to our society for a sustainable eco-friendly and pollution free environment [10].

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