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Relationship between cell surface hydrophobicity and degradation of hexadecane

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ABSTRACT

Some properties of compounds in degrading bacteria are required for biodegradation of contaminants to higher performance. Those strains which have a high percentage of these features are more effective at biodegradation. The present experiments were designed to measure these parameters. In this study, measurement of cell surface hydrophobic-degrading bacteria was designed which oil was separated from contaminated soils. Hydrophobic cell surface helps to binding bacteria to hydrocarbons which most of them are hydrophobic. In this experiment, the optical density was measured before and after addition of hexadecane at 600 nm. The highest percentage of Cell surface hydrophobicity (BATH%) of strains were 27,32,24 and 29 by *Rhodococcus*, *Achromobacter*, *Stenotrophomonas* and *Tsukamurella*, respectively and the growths of hydrocarbon degrading bacteria in hexadecane (1%) contain 1.9, 1.85, 1.4 and 1.12, respectively. It shows that whit increasing the amount of hydrophobic cell levels; consequently, the amount of hexadecane degradation will increase.

Keywords: Bacteria, Hexadecane, Cell hydrophobicity.

INTRODUCTION

Petroleum hydrocarbons are the most widespread contaminants in the environment(Santas et al. 1999; Yakimov et al. 2007c) entering into aquatic environments because of catastrophic accidents (shipping disasters or pipeline failures), chronic pollution (ships, ports, oil terminals, freshwater runoff, rivers and sewage systems), natural oil seepages and natural sources(Floodgate 1972; Cappello et al. 2007a; Hassanshahian et al. 2012a). Many physical, chemical and biological technologies have been developed to remove hydrocarbon pollutants from soils and marine environments. However, these techniques often are not able to fully remove pollutants from environments. Bioremediation, including biostimulation and bioaugmentation, has proven to be an effective method for cleaning up residual oil in a variety of environments(Van Hamme et al. 2003) and has been proposed as the only viable management option that can be implemented on a large scale in marine environments(Snape et al. 2001; Hassanshahian et al. 2012b). At sea, petroleum hydrocarbon degradation is mainly performed by microorganisms, and the

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microbial communities of marine ecosystems involved in this process have been extensively studied(Harayama et al. 1999; Yakimov et al. 2004). Biodegradation by natural populations of microorganisms is the most basic and the most reliable mechanism by which thousands of xenobiotic pollutants, including crude oil, are eliminated from the environment(Cappello et al. 2007a). The effects of environmental conditions on the microbial degradation of hydrocarbons and the effects of hydrocarbon contamination on microbial communities are areas of great interest(Rahman et al. 2004). Bioremediation is a strategy to utilize biological activities to the greatest extent possible for the rapid elimination of environmental pollutants. Stimulation of the growth of indigenous microorganisms, biostimulation, and inoculation with foreign oil-degrading bacteria are promising means of accelerating the detoxification of a polluted site with minimal impact on the ecological systems(Cappello et al. 2006). The growth of microorganisms on hydrocarbons presents particular problems because hydrocarbons are immiscible in water. Many bacteria are able to emulsify hydrocarbons in solution by producing surface active agents such as biosurfactants that increase the adhesion of cells to the substrate. Biosurfactants reduce the surface tension by accumulating at the interface of immiscible fluids, increasing the surface area of insoluble compounds, which leads to increased bioavailability and subsequent biodegradation of the hydrocarbons(Batista et al. 2006). The aims of the present work were to study bacterial strains isolated from Hydrocarbon contaminated soils. We evaluated the abilities of these strains to biodegrade hexadecane (1%) and analyzed the relationship between cell surface hydrophobicity and degradation of hexadecane.

MATERIALS AND METHODS

Sampling

To isolate of alkane hydrocarbon degrading bacteria from contaminated soils, samples were collected from 16 different sites in Tehran petroleum reservoirs regions. Sixteen samples had been collected into sampling which they were contaminated to hydrocarbons. The soil samples were collected from 1 to 12 cm below the surface of soil using a sterile knife and had been transported on ice to the laboratory for isolation(Hassanshahian et al. 2012b).

Isolation and selection of alkane degrading bacteria

A synthetic Bushnell Haas Mineral Salts medium (BHMS) was used for the isolation of alkane degrading bacteria (Kohno et al., 2002). BHMS medium contain (g l^{-1}) KH_2PO_4, 1; K_2HPO_4, 0.2; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.02; NH_4NO_3, 1; and 2 droplet of $FeCl_3$ 60%. The pH adjusted to 7(Cappello et al. 2012b). BHMS medium was supplemented with 1% (v/v) hexadecane as sole carbon source and energy. Portion of soil (1 g) or waste water (1 ml) samples were added to 250 ml Erlenmeyer flasks containing 100 ml BHMS medium and the flask incubated for 10 days at 30 ^oC on rotary shaker (INFORS AG, Switzerland) operation at 180 rpm. Then 5 ml aliquots were removed to a fresh BHMS medium. After a series of four further subcultures, inoculums from the flask were streaked out and phenotypically different colonies were purified on BHMS agar medium. Phenotypically different colonies obtained from the plates were transferred to a fresh BHMS medium with and without hexadecane to eliminate autotrophs and agar-utilizing bacteria. The procedure was repeated and isolated, only exhibiting pronounced growth on hexadecane were stored for further characterization(Hassanshahian et al. 2013).

Identification of isolates (Biochemical characterization)

To identify and characterize the isolated bacteria, biochemical tests such as gram staining, oxidation/fermentation, production of acid from carbohydrates, hydrolysis of gelatin and citrate were carried out according to the Bergey's manual of Systematic Bacteriology(Holt et al. 1998).

Growth and hexadecane removal assay

Bacterial isolates were grown at 30 ^{o}C for 1 week on rotator shaker (180 rpm). The growth of the isolates was routinely assessed indirectly by measuring the turbidity (OD600 nm) using a UV– visible spectrophotometer (Shimadzu UV-160, Japan). The hexadecane removal assay was carried out by dissolving the residual hexadecane in the medium in dichloromethane (DCM) and reading the optical density of the hexadecane extract against a blank at a wavelength of 420 nm (Hassanshahian et al. 2012a).

Bacterial adherence to hydrocarbons (BATH) test

The surface tensions of different bacterial cultures were measured after 10 days of growth. The surface tension was measured by the Wilhelmy plate method using a digital tensiometer (Gibertini, Italy), in accordance with the manufacturer's instructions. The surface tension was expressed in units of mN m_1. (Hassanshahian et al. 2012b).

RESULTS

Isolation and identification of bacteria

Ten alkane-degrading bacterial strains were isolated from enrichment cultures that established at 30 ^{o}C for 2 weeks. Four isolated strains that show higher growth rate on hexadecane were selected between the ten isolated bacteria for further study. Strains isolated were identified by classical biochemical tests which they were *Rhodococcus, Achromobacter, Stenotrophomonas* and *Tsukamurella*.

Growth rate, hexadecane removal by strains and cell surface hydrophobicity(BATH%

All bacterial strains were grown in hexadecane (1%) for 1 week with shaking (160 rpm). During this week, ever day optical density was read for each strain until the end of week and O.D that was related to exponential phase was reported as growth rate. The results of the growth, degradation and BATH were presented in Table 1. As reported in this table, the strains *Achromobacter* has the highest percentage of hexadecane biodegradation and cell surface hydrophobicity (BATH%) but *Rhodococcus* has the lowest percentage of hexadecane biodegradation and cell surface hydrophobicity (BATH%).

Table (1) Growth, hexadecane removal by strains and measurement of cell surface hydrophobicity (BATH%) in isolated strain.

Strain	Growth rate (O.D600/h)	Percentage of hexadecane removal	Cell surface hydrophobicity(BATH%)
Achromobacter	1.85	84	32
Tsukamurella	1.12	76	29
Stenotrophomonas	1.4	68	24
Rhodococcus	1.51	51	22

DISCUSSION

Millions of liters of petroleum enter the environment from both natural and anthropogenic sources every year. Hydrocarbondegrading bacteria were first isolated almost a century ago, and a recent review lists 79 bacterial genera that can use hydrocarbons as the sole source of carbon and energy, as well as 9 cyanobacterial genera, 103 fungal genera and 14 algal genera that are known to degrade or transform hydrocarbons. Jurelevicius et al. (2012) described alkane degrading bacteria in soils taken from King George Island in Maritime Antarctic. They found that there is a high diversity of alkane degrading bacteria in this environment. Quatrini et al. (2008) isolated some gram positive n-alkane degraders from a hydrocarbon contaminated Mediterranean shoreline. One major difference between our study and other reports was the isolation of hexadecane degrading bacteria from petroleum reservoirs waste water in Iran. There are many reports about the isolation of hydrocarbon degrading bacteria from contaminated soil, sediment, sludge and water which are provided but no report on the isolation of hexadecane degrading bacteria from petroleum reservoirs waste water has been published. Roy et al. (2002) described petroleum-degrading bacteria from the Indian part of the Sunderbans as part of their evaluation of the distribution of the naturally occurring petroleum-degrading aerobic bacteria. They isolated some crude-oil-degrading bacteria that belong to the generaPseudomonas, Mycobacterium, Acinetobacter, Micrococcus and Nocardia. Radwan et al. (2005) isolated crude-oil-degrading bacteria from cyanobacterial mats in the Persian Gulf. These researchers describe crude-oil-degrading bacteria including *Pseudomonas*, *Bacillus*, and *Acinetobacter* that were associated with cyanobacterial mats such as *Synechocystis* and *Pleurocapsa*. Hassanshahian et al. (2012) isolated several bacteria from contaminated sites such as soil and water in Iran. (Hassanshahian et al. 2012a,2012b,2013). In this research, four strains were identified from contaminated soils in Tehran petroleum reservoirs regions which had been contaminated by curd oil (hydrocarbons). All researchers collected their samples from those sites which were contaminated by petroleum products and these sits were similar to our sites that were selected as sampling. In present study, *Achromobacter* and *Tsukamurella* have the highest percentage of degrading hexadecane and cell surface hydrophobicity (BATH%) among other strains. Pruthi and Cameotra (1997) screened biosurfactant producing bacteria using a cell surface hydrophobicity assay. They found a relationship between the cell surface hydrophobicity and the level of biosurfactant production(Pruthi et al. 1997). Hassanshahian et al. (2008) reported that a correlation between emulsification activity, cell adherence to hydrocarbon and growth rate of the crude oil degrading

bacteria in crude oil media (Hasanshahian and Emtiazi et al. 2008). In this study, it is confirmed that whit increasing the growth and percentage of hexadecane removal; consequently, the cell surface hydrophobicity (BATH%) will increase. As we showed in this report, with reducing the percentage of hexadecane removal, the BATH will reduce which this research emphasized and showed this direct relationship between cell surface hydrophobicity and degradation of hexadecane.

CONCLUSION

In this research, four strains were identified from Tehran petroleum reservoirs regions which they could degrade the amount of hexadecane(1%) and also showed that between cell surface hydrophobicity and degradation of hexadecane was a direct relationship.

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