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# Specialized Screening and Purification of *Thalassospira* and *Chromohalobacter* Bacteria from Oil Sludge using Chemotaxi to Petroleum

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## Abstract

Large amounts of waste from various stages in the process of refining crude oil are produced in the Persian Gulf in Iran. This waste is a viscous oil sludge containing high amounts of petroleum derived hydrocarbons. So oil sludge is environmental concerns and should be treated. Biological treatment (bioremediation) to deal with a wide range of organic pollutants and petroleum hydrocarbons is useful. In this study, the oil sludge has been obtained from the contaminated site from Bahregan area in the Persian Gulf of Iran. Some oil sludge containing bacterial consortium, Chemotaxis in synthetic Mineral semisolid agar medium was used for the isolation of alkane degrading bacteria from oil sludge biodegradation of octadecane, hexadecane, naphthalene, and anthracene were determined. Strains isolated in this study were identified by 16S rRNA gene sequencing as *Thalassospira* and *Chromohalobacter*. They are Gram negative and belonged to Rodospiracea which could hydrolyzed aromatic and linear oil compound.

**Keywords:** Purification, Oil Sludge, *Thalassospira*, *Chromohalobacter*, Chemotaxi.

## Introduction

Oil is an important source of energy in the world, and it has not yet been replaced by any suitable, accessible and reliable energy source [1]. A large amount of oil sludge is produced at various stages of the extraction and refining of crude oil [2]. Large amounts of waste from various stages in the process of refining crude oil are produced in the Persian Gulf in Iran. This waste is a viscous oil sludge containing high amounts of petroleum derived hydrocarbons. So oil sludge is environmental concerns and should be treated. Biological treatment (bioremediation) to deal with a wide range of organic pollutants and petroleum hydrocarbons is useful. In oil sludge and crude oil, there are microorganisms that are native to that location and adapted over time. Also, the presence of oil deposits in the soil causes the growth of different degrading microorganisms in the contaminated soil, [1, 3]. Therefore, in this study only isolation and analysis of oil sludge and soil contaminated with oil sludge in the region of Behrgan were investigated.

## Methodology

In this study, the oil sludge has been obtained from the contaminated site from Bahrgan area in the Persian Gulf of Iran. Some oil sludge containing bacterial consortium, Chemotaxis in synthetic Mineral semisolid agar medium was used for the isolation of alkane degrading bacteria from oil sludge biodegradation of octadecane, hexadecane, naphthalene, anthracene were determined. The microscopic characteristics of each bacterium were studied, then growth of bacteria in sea water and oil sludge and medium without agar investigated. Molecular identification of bacteria done by 27F and 1492R

primers.

## Discussion and Results

In chemotaxis, using the ability of bacteria to perform chemotaxis and increasing biodegradation, it is possible to isolate, in a shorter time, the best bacteria that have the greatest ability to move and compete with other bacteria to move toward carbon sources. In the present study, the results of the analysis of the Bahrgan oil sludge showed the highest volume of alkane. In some isolated strains, Three strains selected by microscopic studies. Strains isolated in this study were identified by 16S rRNA gene sequencing as *Thalassospira* and *Chromohalobacter*. They are Gram negative and belonged to *Rodospiracea* that could hydrolyzed aromatic and linear oil compound. Selected bacteria were cultured in presence of different percentages of seawater to determine the optimal concentration of seawater in which the bacteria had the best growth. It was found that as *Thalassospira* has the best growth rate in 10% of seawater, and *Chromohalobacter* is a salt-loving bacterium which has the ability to grow in various percentages of water. An alkaline decomposer bacterium called *Acinetobacter haemolyticus* AR-46 was isolated by Bihari et al in 2007 [4]. This alkaline decomposer bacterium is capable of decomposing long-chain alkanes; moreover, it is able to use hexadecane as the sole source of carbon [4]. The petroleum refineries of a species of *Acinetobacter baumannii* which could grow well in a culture medium containing hexadecane as a carbon source was isolated by Shiri and his colleagues in 2015; moreover, 62% hexadecane were eliminated by them in 6 days [4, 5].

## Conclusions

Alcan decomposing bacteria were isolated by chemotaxis. In addition to its ability to decompose petroleum compounds, these bacteria are more capable of using one or more hydrocarbons. By finding the best source of hydrocarbons for a bacterium, this bacterium can be used to clean up petrochemicals and contaminated areas with pure hydrocarbons in addition to cleaning up oil spills.

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