Journal of Environmental Chemical Engineering 4 (2016) 4323–4329

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Kerosene biodegradation ability and characterization of bacteria isolated from oil-polluted soil and water

Mohammad Mojarad[a], Abbas Alemzadeh[b,*], Golafarin Ghoreishi[a], Mohammad Javaheri[c]

[a] Biotechnology Center, College of Agriculture, Shiraz University, Shiraz 7144165186, Iran
[b] Department of Crop Production and Plant Breeding, College of Agriculture, Shiraz University, Shiraz 7144165186, Iran
[c] Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz 7144165186, Iran

ARTICLE INFO

Article history:
Received 13 March 2016
Received in revised form 13 September 2016
Accepted 29 September 2016
Available online 30 September 2016

Keyword:
Bioremediation
Oil contamination
Enterobacter cloacae
Enterobacter hormaechei
Pseudomonas stutzeri

ABSTRACT

With increasing production and consumption of oil, the inevitable spillage of oil presents a significant challenge to protecting the environment and human health. Bioremediation is an effective approach to address this challenge. In this research, three soil and two wastewater samples contaminated by petroleum and a sample of crude oil were collected from Bandar Abbas Refinery, Iran. These samples were used for isolation and identification of bacteria, which can be used for cleaning polluted lands. Twelve strains were isolated and cultured at 28 °C standard succinate medium from which carbon or sulfur resources were eliminated and kerosene was added. Three isolates were selected for identification because of their high growth rate in kerosene tests. The biodegradation activity of these bacteria was analyzed by gas chromatography. Using biochemical tests, 16S rDNA sequence, and API 20 E kit, it was revealed that these bacteria belong to *Enterobacter cloacae*, *Enterobacter hormaechei*, and *Pseudomonas stutzeri*. They were able to degrade 67.43%, 48.48%, and 65.48% of 5% kerosene as carbon source in seven days, respectively. *Pseudomonas stutzeri* and *Enterobacter hormaechei* could respectively degrade 54.14% and 12.98% of 10% kerosene as sulfur source in seven days. Hence, these bacteria can be considered as excellent candidates for petroleum biodegradation.

1. Introduction

According to a British Petroleum (BP) statistical review of the world energy production, global oil production has reached 88673 million tonnes per annum in 2014, an increase of 2.3% compared to the previous year [1]. The same report states that the global oil consumption has also increased and reached 92086 t per annum in 2014. These figures indicate the ever-growing exploitation of this natural resource in today's world. Extraction, refining, processing, and transportation of oil have caused several unwelcomed consequences including leaks and accidental spills [2]. During the 2010 Deepwater Horizon accident in Gulf of Mexico it was estimated that at least 4.9 million barrels of crude oil had spilled into the environment [3]. These crises are more common in oil-producing countries such as Iran, which has produced an average of 1.82 million barrels a day of crude oil in the last five years. Currently, Iran holds the second place in oil production among OPEC members behind Saudi Arabia [4]. Crude oil extraction takes place in numerous sites in Iran and covers a fairly large geographical area of the country [5]. Naturally, oil contamination is a common problem in these areas. These accidents have caused contaminations to permeate into ground water aquifers and damaged the farmlands around the area. Crude oil and its derivatives contain highly toxic chemical compounds such as benzene, toluene, ethylbenzene, xylenes (BTEX), and naphthalene. Many of these compounds such as benzene are potentially mutagenic and carcinogenic for humans [6–8]. Contamination of soil, surface water, and ground water with oil hydrocarbons causes extensive damage to the affected ecosystems and can be hazardous to the health of plants, animals, and humans [6,9].

Currently, oil contamination is removed by three methods: Physical, Chemical, and Biological [6]. Biological method or bioremediationuses bacteria, fungi, and some algae to degrade and remove environmental pollutants. Compared to the other two methods, bioremediation is more economical, more efficient, and safer for the environment and human's health. Another benefit of bioremediation is that it converts toxic wastes to non-toxic compounds [6,9,10].

Bacteria are one of the best candidates for bioremediation because they have biodiversity, variety of catabolic genes and

* Corresponding author.
E-mail address: alemzadeh@shirazu.ac.ir (A. Alemzadeh).

http://dx.doi.org/10.1016/j.jece.2016.09.035
2213-3437/

enzymes, and vast potential for degradation of harmful contamination [11]. In addition, bacteria can adapt to the environment through their capability to reclaim cellular membrane in order to sustain necessary biological functions, release bio-products that lower the surface tension such as rhamnolipid, and decline concentration of toxic compounds inside the cells by use of efflux pumps [5,11,12]. The best bioremediation technique is intrinsic bioremediation because it has minimum intervention in the environment. In this technique, natural microflor as reduce contamination by themselves and human involvement is limited to isolating the biodegradation rate of bacteria [10].

Head et al. [13] reviewed several articles and reported that 79 bacterial genera are capable of using hydrocarbons as carbon and energy sources. The efficiency of different bacterial genera in biodegradation of oil hydrocarbons varies. Various researchers have shown that particular bacteria isolated from marine environments have a biodegrading capacity from 0.003% to 100% [13,14]. The biodegradation capacity of bacteria isolated from soil ranges from 0.13% to 50% [14]. The common features of bacteria ideal for bioremediation of contaminants in all environments are hydrophobicity, floating behavior, and oil degrading capability [15].

Because of the influence of environmental factors such as temperature, humidity, and pH, in the flora of each region [14,16], the diversity of crude oil and its derivatives, and the variety of pollution caused by them, examination and identification of native species or strains in each area is essential.

This study had two goals. The first goal was to isolate and characterize oil-degrading bacteria from polluted soil and water from Bandar Abbas oil refinery, Iran. The second goal was to identify those bacteria which could grow in high concentrations of kerosene in medium without carbon or sulfur sources.

2. Materials and methods

2.1. Collected samples and the composition of culture mediums

Three types of samples were selected for this study: Soil, waste water and crude oil. Soils and waste water samples were collected from Bandar Abbas Oil Refinery, Bandar Abbas, Iran (27°11′N 56°16′E) and crude oil sample was collected from Kharg Oil Well in 2011 (Table 1).

In this research four types of media were used: Nutrient Agar (NA), Luria Bertani (LB), Yeast Extract Tryptone (2xYT) and Standard Succinate Medium (SSM). The compositions of these media are as follows:

- NA: 20 g of nutrient agar powder (Merck, Germany) suspended in 1 l of distilled water
- LB: Tryptone (10 gl^{-1}), yeast extract (5 gl^{-1}), NaCl (5 gl^{-1}) (pH 7)
- 2xYT: Tryptone (16 gl^{-1}), yeast extract (10gl^{-1}), NaCl (5 gl^{-1}) (pH 7)
- SSM: $K_2H_PO_4$ (6 gl^{-1}), $KH_{2P}O_4$ (3 gl^{-1}), succinic acid (4 gl^{-1}), $(NH_4)_2SO_4$ (1 gl^{-1}), Mg_SO_4 (0.2 gl^{-1}) (pH 7)

All media were sterilized by autoclaving at 120 °C for 20 min. In this study, SSM medium was modified in order to create a carbon-free medium (SSM^{-c}). This was done by removing succinic acid from the medium. To minimize changes in the sulfur-free SSM (SSM^{-s}), $(NH_4)_2SO_4$ and $MgSO_4$ were replaced by $(NH_4)_2PO_4$ and $MgCl_2$, respectively.

2.2. Isolation of bacteria and culture conditions

To isolate bacteria from soil, wastewater, and crude oil, two different approaches were utilized.

In the first approach, 1 g of each soil sample or 1 ml of each wastewater sample was added to 10 ml of LB medium. Then, the combination was transferred into a 50 ml tube and incubated for one day at 170 rpm in an orbital shaker incubator. After incubation, the establishment of consortium of bacteria was determined by the turbidness of the medium. Using $MgSO_4$ (100 mM), a serial dilution (10^{-3}–10^{-7}) was prepared from the consortium of bacteria. Then, 50 μl of each serial dilution was spread on NA plates and incubated for one day. Finally, the predominant bacteria with different colony morphologies were selected.

In the second approach, 2.5 ml of each crude oil sample was added to 25 ml of 2xYT medium and incubated for thirty days at 170 rpm on a rotary shaker incubator. Then, 50 μl of each serial dilution (10^{-3}–10^{-7}), which was prepared from grown bacteria, was spread on NA plates. Individual colonies were selected after one day. All selected isolates were stored at −80 °C in 20% (v/v) glycerol. Isolation of all samples was done in 28 °C.

2.3. Evaluation of isolates growth in kerosene

The isolates were grown in two concentration of kerosene as follows:

2.3.1. Concentration of 2.5% v/v kerosene

The ability of the isolates to grow was tested. Five ml of each over night culture which was grown in liquid SSM in 28 °Cat 170 rpm was used. Each sample was centrifuged for 5 min at 2600g rpm and then, the bacteria pellets were resuspended in SSM^{-c} and transferred to the SSM^{-c} plus 2.5% v/v kerosene as the sole carbon source. The initial optical densities (ODs) of the bacterial suspensions at 600 nm were from 0.47 to 0.53. The growth rate of isolate after incubation in 28 °C at 170 rpm was monitored by UV–vis spectrophotometer SP-3000 Plus (Optima Inc., Tokyo, Japan) every 24 h for 2 days.

2.3.2. Concentration of 20% v/v kerosene

The behavior of bacteria in high concentrations of petroleum compounds was surveyed. The isolates were cultured in 10 ml liquid SSM and incubated in 28 °C at 170 rpm in an orbital shaker incubator for overnight. Then, each fresh liquid culture was inoculated in the modified SSM medium from which carbon or sulfur source was removed and 20% v/v kerosene was added so that

Table 1
Characteristics of collected samples.

No.	Samples type	Visible Intensity of pollution
1	The mixture of oily sludge and soil, on which bioremediation process was conducted (BS)	None
2	Surface soils contaminated by heavy crude oil (CSs)	Intense
3	Soil in 5 cm depth contaminated by heavy crude oil (CSd)	Little
4	Wastewater input to the Bandar Abbas refinery water treatment facility (Wi)	Intense
5	Wastewater output from the Bandar Abbas refinery water treatment facility (Wo)	None
6	Heavy crude oil (CO)	–