Petroleum Biodegradation by Two Mycobacterium Isolates from Persian Gulf

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Extended Abstract

A vast amount of oily wastes is produced during activities related to exploration, production, refinement and transportation of oil and gas products, which cause serious damages especially to marine and estuarine environments. Persian Gulf region which contains about 57-66% of world oil reservoirs is exposed to enormous oil pollutions. Although the physical and chemical approaches for removing these pollutions are effective, but still unable to completely remediate the environment. Recently bioremediation is known as an applicable and cost effective technique for treatment of oil polluted environments.

Success of oil spill bioremediation depends on our ability to optimize various physical, chemical, and biological conditions in the contaminated environment, including nitrogen and phosphorus sources, pH and temperature. The most important requirement is the presence of microorganisms with the appropriate metabolic capabilities.

If these microorganisms are present, then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH and temperature is suitable for bacterial growth.

Isolation of two petroleum biodegrading bacterial strains PG01 and PG02 from Persian Gulf and study of pH effect on oil mineralization have been previously reported. In this paper, the effect of temperature and nitrogen and phosphorus concentration on oil degradation by these bacteria was studied.

Furthermore, the ability of the strains to degrade crude oil fraction was gravimetrically evaluated. All experiments were statistically repeated. Finally, the two strains were subjected to identification of using morphological and biochemical methods and determination of guano sine plus cytosine content of the DNA.

The growth curves of strains PG01 and PG02 on crude oil as sole carbon source using different concentration of ammonium chloride as nitrogen source have been shown in Figure 1. In all experiments, both strain entered to exponential phase at second day of cultivation, but the amount of produced total protein and consequently the rate of degradation of oil varied in different concentrations of nitrogen source.

Strain PG01 had similar growth curves in the presence of 0.146, 0.195 and 0.244 gram ammonium chloride per gram crude oil, but in the other amounts of nitrogen source degradation of oil was decreased ($p<0.05$). Therefore the minimum amount of nitrogen source per gram oil for this strain was 0.146 gram. For strain PG02 maximum growth was observed in the presence of 0.195 gram ammonium chloride per gram crude oil ($p<0.05$). The optimum concentration of phosphorus source for biodegradation of petroleum hydrocarbons for both strains was equal to 0.024 gram disodium hydrogen phosphate per gram crude oil (fig. 2).

Results of biodegradation experiments for evaluating the effect of incubation temperature on crude oil degradation by strains PG01 and PG02 showed that both strains were mesophile capable to grow at 25-37 with best results at 35-37 degree centigrade. No growth was observed at 41 degree centigrade (fig. 3).

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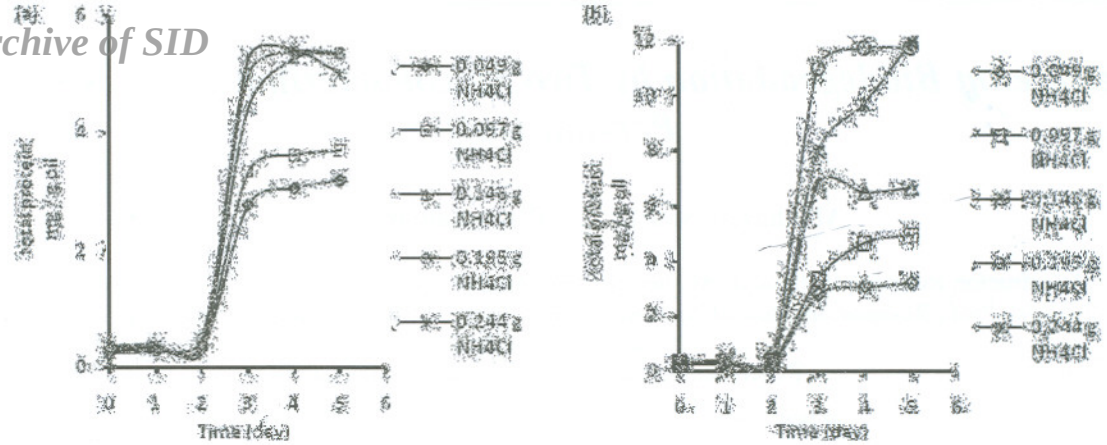

Fig. 1: Optimization of nitrogen source for biodegradation of crude oil by strains PG01 (a) and PG02 (b).

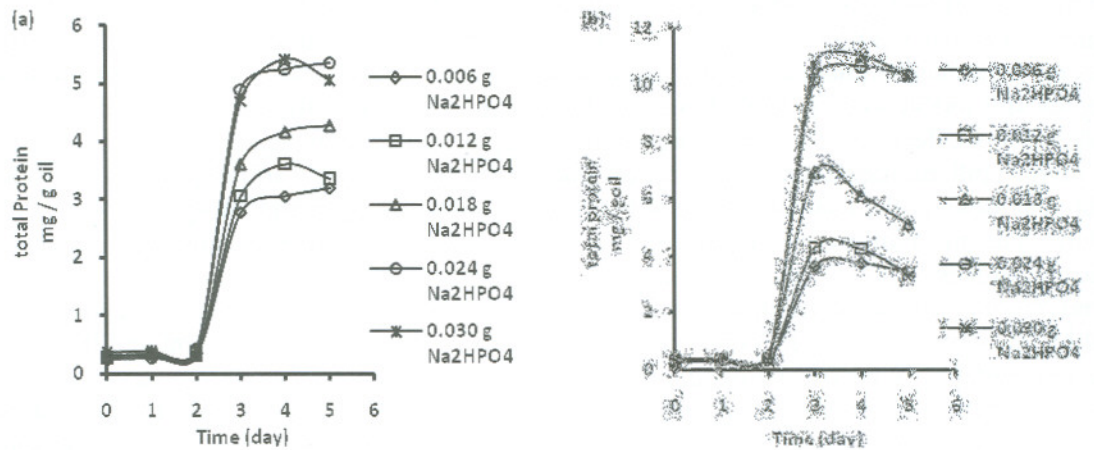

Fig. 2: Optimization of phosphorus source for biodegradation of crude oil by strains PG01 (a) and PG02 (b).

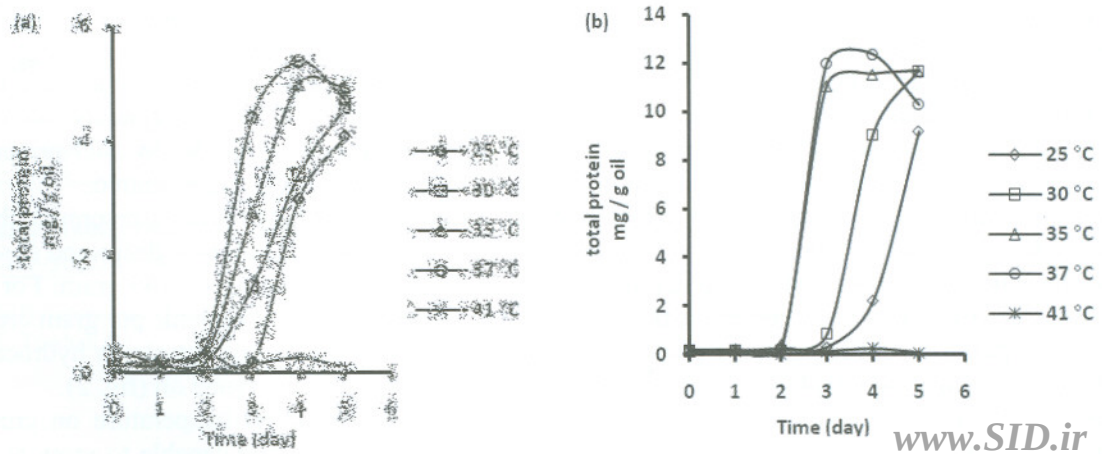

Fig. 3: Optimization of temperature for biodegradation of crude oil by strains PG01 (a) and PG02 (b).

In order to assay the biodegradation of different fractions of crude oil by strains PG01, PG02 and the mixture of them, gravimetric determination of crude oil fractions was performed. Crude oil was supplied by National Iranian Oil Company and was composed of 70.90% saturated hydrocarbons, 22.31% aromatic hydrocarbons, 3.99% resins and 2.80% asphaltenes. After a 5 day biodegradation period these fractions were reduced to 12.56%, 10.71%, 3.03% and 2.89% for strain PG01, 4.70%, 6.32%, 2.09% and 2.95% for strain PG02, and 2.32%, 4.97%, 1.32% and 2.97%, respectively.

Overall ability of Strains PG01 and PG02 and the mixture of two strains to degrade crude oil was 70.81%, 83.94% and 87.61%, respectively.

Identification of strains was performed using morphological, physiological and biochemical methods. For determination of guanosine plus cytosine content of bacterial DNA, the HPLC method was used. DNA was extracted and purified by cold isopropanol precipitation.

Purified DNA and salmon DNA (standard DNA) were hydrolyzed by nuclease P1 enzyme. The hydrolysates and a mixture of deoxy-nucleotide mono phosphate (dCMP, dTMP, dGMP and dAMP) were injected to high performance liquid chromatography. The HPLC conditions were as follow:

Column:	C18, MCH 10
Mobile phase:	10 mM phosphate buffer (pH 7)
Flow rate:	0.5 ml/min
Detector:	UV 260 nm

The GC% of bacterial DNA was calculated using the resulted peak area of each base, which was 69.685 for strain PG01 and 67.817 for strain PG02.

Regarding results from morphological, physiological and biochemical characteristics of strains PG01 and PG02, it seems that both strains are members of the genus *Mycobacterium*. These characteristics are: curved rod cell shape; weakly gram positive; Acid fast in fresh culture; 2-3 day's growth duration; resistant to lysis by lysozyme; non-motile; aerobic and catalase positive; G+C content of DNA; round smooth colonies with orange and reddish color, respectively for PG01 and PG02; and resistant to penicillin.

In specious level also both strains have similarities to *M. obuense*, including growth in 5% NaCl concentration, degradation of polycyclic aromatic substrates, formation of colored colonies, unable to grow at 42 degree centigrade, nitrate reduction negative, and did not produce acid from arabinose and xylose.

Key words
Crude oil, Biodegradation, Persian Gulf. Olamosein