Bioremediation Potential of *Bacillus licheniformis* in Heavy Crude Oil Polluted Soil

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***Abstract*—Bioremediation is an eco- friendly approach for environmental crude oil cleans up. In this study, 20 different Bacillus species were isolated from heavy crude oil contaminated soil. The strain which showed higher growth at high concentration of crude oil (3%) was selected for the study. The isolate was identified as *Bacillus licheniformis* by 16S rDNA sequencing and the genetic sequence was submitted to NCBI GenBank under accession no. KP119115. Under aerobic condition, it was found that the isolate degraded heavy crude oil to 63% compared to that of the control by GC-MS analysis. The ability of the isolate to grow at 3% heavy crude oil indicated the capability of *Bacillus licheniformis* to degrade heavy oil.**

***Keywords*—Bioremediation, *Bacillus licheniformis*, heavy crude oil.**

I. Introduction

Increase in energy demands and extensive exploration of new oil fields will result in environmental pollution with crude oil [1]. There are inhabitant microbes present in the soil capable of biodegrading crude oil. Using microbes for bioremediation is the preferred method since the alternative methods like incineration leads to toxic compound [2]. There are numerous microbes capable of biodegrading crude oil, but researches done earlier proved the efficiency of microbes adapted to a similar condition are more efficient [3]. The different strategies that can be used for bioremediation include introducing nutrients and oxygen into the soil (biostimulation), or through inoculation of an enriched mixed microbial consortium into the soil (bioaugmentation) [4]. In the present study, the potency of *Bacillus licheniformis* to be used in bioremediation was determined.

II. Materials and Methods

A. Collection of soil samples

Soil samples contaminated with heavy crude oil was collected aseptically from Oman oil fields and stored at room temperature until use.

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B. Isolation of Bacillus spp.

Spore forming Bacillus spp. were isolated by boiling the soil samples in distilled water for 30 min, culturing the microbes in LB media aerobically at 40ºC, 160 rpm and spread plating the supernatant on Bushnell Haas (BH) agar media plated with heavy crude oil. The isolate which showed maximum growth in BH agar media was selected for the study.

C. Identification of the isolates

The isolates are identified by 16S rDNA sequencing using universal primers, 27 F and 1492R. The amplification reaction was performed on a total volumeof 25 µL containing: 12.5 µL master mix (Taq DNA polymerase, dNTP mix and $MgCl_2$), 9.5 µL doubledistilled H2O, 1µL extracted DNA and 1 µL of each primer. PCR amplification was performed with initial denaturation step at 94°C for 3 min followed by 35 cycles of 1min denaturation step at 94°C, 2 min annealing step at 53°C and 2 min elongation step at 72°C, and a final extension step at 72°C for 7 min using a thermal cycler (Applied Biosystem). The PCR products were detected in 1.5% agarose gel electrophoresis. The PCR products were purified using QIAquick PCR purification kit (QIAGen) and sequenced using 3130 XL Genetic Analyzer (Applied Biosystem, Hitachi). The genetic sequence was submitted to NCBI GenBank.

D. Effect of concentration of heavy crude oil on growth of the isolate

The effect of heavy crude oil concentration on the growth of the isolate BS10 was studied by inoculating in BH media with 1-3% heavy crude oil and incubating at 40ºC and 160 rpm aerobically. The growth of the isolate was measured spectrophotometrically at an optical density (OD) of 660nm for a period of seven days and the pH variation in the media was studied.

E. Heavy crude oil biodegradation study

The biodegradation of heavy crude oil by the isolate BS10 was measured using GC-MS. The isolate was incubated in 1% heavy crude oil in BH media for a period of 12 days. The biodegraded oil was extracted with hexane : dichloromethane (1:1).

III. Results And Discussion

Twenty different isolates belonging to genus *Bacillus* were isolated from heavy crude oil contaminated soil collected from Oman oil fields. Five of the isolates showed considerable

growth in BH agar medium. The isolate which showed maximum growth was selected for the study. Many different *Bacillus* sp. Have already been reported to degrade crude oil (Fig.1) [5-11].

The isolate was identified by 16S rDNA sequencing and submitted under accession number KP119115 as *Bacillus licheniformis* strain BS10.

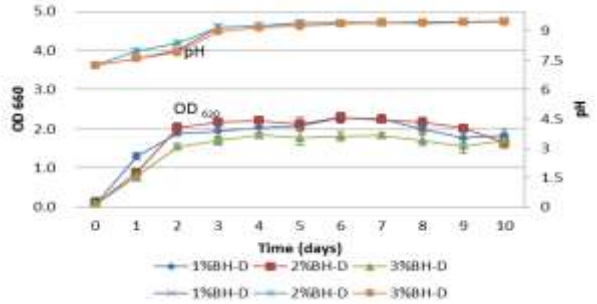

Fig.1. The growth characteristics of the strain BS10 in 3 different concentrations of heavy crude oil

There was no significant effect of different concentrations of heavy crude oil on the growth of the isolate. Statistical analysis was performed using MINITAB 14. It was observed that F= 3.11; p>0.05.

The GC- MS analysis of the biodegraded heavy crude showed a gradual decrease in the total fraction of the crude oil along the time, day 3, 6, 9 and 12 [5, 6, 12-16].

It was shown that the total decrease in HC fractions was 63% compared to that of a control (BH media with heavy crude oil) (Fig. 2).

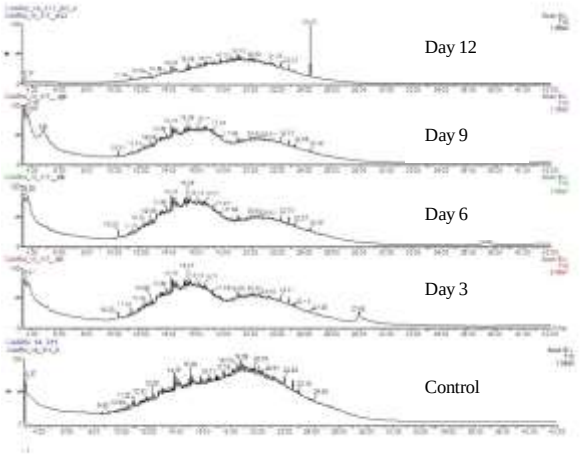

Fig. 2. GC-MS analysis of the biodegraded heavy crude oils

IV. Conclusion

The potential of the isolated *Bacillus* strain BS10 in degrading heavy crude oil is evident from the study and can be recommended to use for bioremediation and elimination of crude oil pollutant from the environment.

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