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Enhanced Biodegradation of Heavy Hydrocarbons by Aspergillus Pseudodeflectus F13 in the Presence of Rhamnolipid

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Introduction

Aromatic and aliphatic hydrocarbons are the major contaminant of the petroleum polluted sites, and among these compounds, the most of them are representative of low molecular weight molecules. Moreover, the hydrocarbons have been shown to be readily degraded by many microorganisms. However, long-chain alkanes, with the range of equivalent carbon number ($C > 18$) and polycyclic aromatic hydrocarbons (PAHs) especially high molecular weight PAHs (HMW PAHs), consisting of two or more benzene rings were considered to be only slightly biodegradable [1, 2].

Many reports have indicated that bacteria play important roles in hydrocarbon mineralization despite the fact that fungi may also mineralize hydrocarbons. Many aliphatic hydrocarbons degrading organisms have been identified, and some of them have shown a broad substrate range, including those long chain aliphatic hydrocarbons that are solid at ambient temperature [1].

This study was designed to isolation of an efficient fungal strain in degradation of recalcitrant hydrocarbons based on the soil of aged polluted sites.

Experimental Procedure

Isolation

One gram of the soil sample was added to 10 ml of the Ringer's saline solution, and shaken for 2 h at 120 rpm. Then, for enriching the potent microorganisms in the petroleum hydrocarbon degradation, 1 ml of the resulting mixture was added to 100 ml of the Bushnell Haas medium supplemented by 1 g of heavy crude oil (as the only carbon source), and incubated for about 72 h (at 120 rpm, and 28 °C). Each medium (100 µl) was transferred to different PDA plates, incubated at 28 °C for 7 days, and monitored every day [3].

Crude oil degradation of fungal strains

1 ml inoculum of each strain was added to the 50 ml flasks containing 9 ml Bushnell Haas medium with 1% crude oil, which acted as the sole carbon source. Afterward, all the cultures were incubated for 21 days on a rotary shaker under dark conditions (120 rpm at 28 °C). After 21 days, the samples were collected, and the remaining oil was extracted by adding 10 ml toluene, and measured by the gravimetric analysis method [4]

Pyrene And Tetracosane Degradation By Fungal Strains And Effect Of Surfactants

1 ml inoculum of selected strain was added to the two different 50 ml flasks one contained 9 ml Bushnell Haas medium with 500 mg/l pyrene and the other contained 9 ml Bushnell Haas medium with 1% w/v tetracosane which acted as the sole carbon source. Afterward, all the cultures were incubated for 21 days on a rotary shaker under dark conditions (120 rpm at 28 °C). After 21 days, the samples were collected, and the remaining pyrene and tetracosane were extracted by adding 10 ml toluene, and measured by the GC method [5].

Also, surface-active compounds can increase the degradation yields of petroleum hydrocarbons by raising their bioavailability. Accordingly, in this study, the effect of a typical synthetic surfactants Tween-80 (0.2% w/v) and a biosurfactant, Rhamnolipid (0.001% w/v), were studied individually for degradation of

pyrene (500 mg/l) and tetracosane (1% w/v) by the mixed cultures [6].

Gas Chromatography

Samples analyzed using a gas chromatograph (Shimadzu GC 8AIT, Japan) were equipped with a split-splitless inlet, and a flame ionization detector (FID) was used for separation and determination of pyrene and tetracosane. The inlet was held at 250 °C. Furthermore, separations were carried out with a DB-5 (5% phenyl, 95% methylpolysiloxane) capillary column (30 m × 0.25 mm i.d., df: 0.25 µm) purchased from Shimadzu. Helium (99.999%) at a constant flow rate of 1 ml min-1 was used as the carrier gas. The oven temperature was programmed as follows: initial temperature of 100 °C (held for 1 min) and then increased at a rate of 20 °C min-1 to 320 °C. The FID temperature was maintained at 300 °C. All data were obtained by injection of 1 µl of the extracts to which an appropriate internal standard was added [7].

Results and Discussion

Isolation

43 fungal isolates were isolated from the soil samples, and between them only 8 isolates showed an appropriate growth in minimal broth medium with only crude oil as carbon source

Crude Oil Degradation and Best Isolate Selection

The amounts of crude oil degradation by 8 isolated selections were examined, and the best isolate was selected for furthermore researches. Based on these results Strain F13 with 52.97% crude oil degradation in 21 days had the highest amount of degradation yield.

Identification

The results of sequence alignment of ITS segments indicated that F13 strains had 100% similarity to Aspergillus pseudodeflectus.

Pyrene and Tetracosane Degradation

The strain Aspergillus pseudodeflectus F13 degraded 47.06 and 58.73% of pyrene and tetracosane, respectively, in 21 days. This result showed the higher tolerance of pyrene to degradation and better performance of isolated strain in degradation of aliphatic compounds.

The effect of Surfactants

Tween-80 and Rhamnolipid used as chemical and natural surfactants. Both of them increased the amount of degradation. Rhamnolipid had a much more positive effect than tween-80 in lower amounts (1:20). Tween 80 increased the degradation yield of crude oil, pyrene and tetracosane from initial values of 52.97, 47.06 and 58.77% to 64.89, 57.39 and 70.78% respectively. In fact, by using Tween-80, the efficiency of the biodegradation process by Aspergillus pseudodeflectus F13 increases about 20 to 30%. In contrast, Rhamnolipid increased the biodegradation of crude oil, pyrene and tetracosane about 25 to 35% and the measured values for crude oil, pyrene and tetracosane were 68.86%, 60.31% and 75.24% respectively.

Conclusions

According to this study, these results indicate the higher effect of Rhamnolipid on biodegradation in 20-time lower concentration than tween 80.

Nomenclatures

FID: Flame Ionization Detectori.d.: Inside Diameter
Isolation

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