ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 12, pp. 14828-14830, December, 2016

International Journal of Recent Scientific Research

Research Article

EFFECT OF MICROFUNGI ON BIOREMEDIATION OF HYDROCARBON FROM POLLUTED SOIL

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ARTICLE INFO

Article History:

Received 05th September, 2016
Received in revised form 08th October, 2016
Accepted 10th November, 2016
Published online 28st December, 2016

Key Words:

Bioremediation, Microfungi, screening, Hydrocarbon

ABSTRACT

In the current investigations on the effect of microfungi on hydrocarbon polluted soil sample from Salem district. The screening of microfungi from three different hydrocarbon molecules of benzene, toluene and xylene were involved for bioremediation potential by native fungi. Some of the microfungi were isolated and screening for degradation process were analysed. The maximum potential degradation of *Aspergillus flavus* on BH medium with 1% concentration of benzene, toluene and xylene were manipulated with other ingredients. The most potential fungi such as *A.flavus*, *A.fumigatus*, *A.niger*, *A.terreus*, *Fusarium solani*, *Penicillium citrinum* and *Trichoderma viride* were analysed and screened. The minimum zone of inhibition of *A.fumigatus* (2mm) with xylene and *Fusarium solani* (2mm) was determined. The aim of the study in attention on the survey of fungi and the potential ability to bring out degradation of hydrocarbon.

INTRODUCTION

Biodegradation of such contaminated soils is required by convention method that is affordable and eco friendly (Alexander, 1994). Many fungi are capable of utilizing and degrading the oil constituents in a better way (Gimsing *et al*., 2009). Petroleum hydrocarbons are not easily degradable. Microorganisms have the capacity to degrade majority of hydrocarbon components, the saturated and unsaturated alkanes, monoaromatic and low molecular weight polycyclic aromatic hydrocarbons (PAHs) were analysed. The organism must be in contact with their substrate to utilize and degrade the hydrocarbon. Individual microorganisms can metabolize only a limited quantity of hydrocarbon substrates. So the mixed cultures of microorganisms are required to increase the rate of petroleum biodegradation (Thenmozhi *et al*., 2011). When the organism grows in the contaminated soil, they utilize these constituent hydrocarbons as substrates. Such hydrocarbon utilizing microorganism shows emulsifying activity (Aparna *et al*., 2011 and Atlas, 1981). These microbial consortia use the hydrocarbon as substrates and degraded by fungi. These organisms have high capability of degrading the hydrocarbons. The majority of hydrocarbons are found in Diesel, Petrol, and Kerosene. The degradation rate is affected by several physico-chemical and biological parameters such as pH, temperature, nutrient content and quantity of hydrocarbon (Santhini *et al*., 2009).

The biological solution has become more familiar to remove hazardous substances from the environment. Bioremediation is an alternative that has been used to eliminate or minimise the effects of pollutants by using microorganisms which have biodegradation potential from micro fungi were analysed (Atlas, 1995). The biodegradation by filamentous fungi for bioremediation purpose has not been fully investigated. The uses of filamentous fungi isolated from contaminated soil may be attributed from the site area.

MATERIALS AND METHODS

Isolation and Identification of fungal Isolates

One gram of each soil sample was weighed into ten test tubes containing 9ml of sterile distilled water, and this was agitated for one minute using a magnetic shaker. Serial dilutions of each of the soil sample were made up to 10^{-3} dilution. The soil suspensions from 10^{-3} dilution were inoculated respectively by using spread plate method. The media used for the isolation of fungi by Potato Dextrose Agar (PDA) added with streptomycin (20mg/L) to prevent bacterial growth. The plates were incubated at 30°C for 4 days, after incubation the observations were recorded daily for the growth of filamentous fungi according to the methods as recommended by Nester *et al.* (2004).

Identification of soil fungi

Fungal morphology was studied macroscopically by observing colony features (colour and texture) and microscopically by

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staining with lactophenol cotton blue and observed under compound. The fungi were identified with the help of standard manual of soil fungi (Gillman, 1957). Hyphomycetes (Subramaniyan, 1971) A manual of penicillia (Raper and Thom 1949), the genus *Aspergillus* (Raper and Fennell, 1965).

Screening of potential isolates

The confirmed isolates were inoculated into Bushnell Hass (BH) liquid media and incubated at 25°C for 7 days. Prepare BH Agar plates and spread 1ml of oil (Benzene, Toluene and Xylene), then prepare wells (8mm) and load 50 µl of broth culture and incubate plate at 37°C for 48hrs. The zone of clearance around the wells and measured the zone of diameter from the petriplate.

Secondary screening by gravimetric analysis (Chrzanowski et al., 2007)

Three agar plugs (1 cm^2) of the 24 hrs pure cultures of each of the two best potential strains *(Aspergillus nige*r and *Fusarium* sp) were inoculated into the Bushnell Hass broth (80 ml/100 ml Erlenmeyer flask) containing benzene, xylene and toluene 0.5, 1.0 and 1.5 ml of crude oil. Inoculation of each organism was carried out in triplicate prior to incubation as stated above. Control tubes without the organism were prepared accordingly. After seven days the flask observes and takes results in growth biomass and crude oil absorbency. Absorbance was assayed spectrophotometrically for the residual hydrocarbon (both aliphatic and aromatic polymers). The mean results were then obtained.

Growth Measurement (Biomass assay)

After incubation, the mycelial mat was harvested and weighed using an electronic balance to find out the wet weight of the mycelium. One wet biomass of the fungal mycelium is accounted as the growth of parameter of the fungi. From the duplicate values of mean value were derived and presented.

$$\text{The calculation of mycelial biomass} = \frac{W1 - W2}{C} X\ 100$$

Where, W1- initial value of mycelial biomass
W2- final value of mycelial biomass
C- Control of mycelial biomass

RESULTS AND DISCUSSION

In the current study, the effect of microfungi of *Aspergillus flavus* was maximum growth formation of 20, 18, and 16mm recorded with 1% of benzene, toluene and xylene on the BH medium respectively. The *Penicillium citrinum* fungi was second moderate growth on the 1% hydrocarbon treated with BH medium with benzene, toluene and xylene individually and the fungi was 16, 12 and 12 mm growth observed respectively and followed by *Trichoderma viride* was 9, 13 and 10 mm growth with treated with benzene, toluene and xylene on the BH medium. The minimum growth of the fungi *Aspergillus fumigatus* and *Fusarium solani* in each 2 mm growth was observed and treated with hydrocarbon respectively (Table 1). Burghal *et al*. (2016) reported that the four fungal species in this study isolated from contaminated soil that showed potentials for hydrocarbon degradation. Moustafa (2016) reported that the seventeen fungal isolates recovered from oil-contaminated soils were screened for crude oil biodegradation activity in a shakeflask culture.

Table 1 Screening of potential fungal isolates from hydrocarbon with BH medium

Name of the fungi	Growth measurement (mm)		
	Benzene	Toluene	Xylene
A. flavus	**20**	**18**	**16**
A.fumigatus	**-**	**-**	**2**
Aspergillus niger	**5**	**36**	**-**
A. terreus	**5**	**-**	**-**
Fusarium solani	**2**	**-**	**-**
Penicillium citrinum	**16**	**12**	**11**
Trichoderma viride	**9**	**13**	**10**

Among the seventeen fungal isolates, only seven showed potentials for biodegradation. Of these seven isolates, two of them identified as *Aspergillus niger* and *Lichtheimia ramosa* which showed the highest ability of crude oil biodegradation. To optimize the nutrient components for a greater yield of fungal biomass and higher total petroleum hydrocarbon (TPH) degradation, preliminary experiments are to be conducted. The selection process of nutrients is based on fungal growth response towards the different nutrient concentration, whereas the ranking determines which combination of yields the greater biomass. The numeric score for each nutrient is weighted by a factor, followed by summing all scores. The highest graded combination implies the best combination (Chu, 1993 and Willis and Huston, 1990).

Maximum up taking of TPH from oil sludge was elucidated using by the *Aspergillus versicolor*. Three sets of experiment were conducted to study the growth of fungal biomass and TPH degradation. Each set of flask were examined for the *Aspergillus versicolor* growth and oil sludge degradation at a regular interval of 10 days.

The maximum growth of the fungi with biomass test on the BH medium supplemented with 1, 2 and 3% concentration of hydrocarbon treated. The weight of biomass was neutrally high treated with 1% concentration was 32.8, 26.0, 29.0 and 21.9 gm with hydrocarbon of benzene, toluene, xylene and combined with all components treated with BH broth with fungi weight was calculated respectively. The microfungi *Penicillium citrinum* was maximum growth and development with 1% concentration of hydrocarbon treated 28.1, 26.2, 24.9 and 21.6 gm weight increased respectively whereas *Trichoderma viride* also minimize growth was observed. In the findings of 25.7, 24.7, 25.5 and 20.1 gm weight recorded on BH medium respectively.

Table 2 studies on the growth and biomass of potential micro fungi on BH broth supplemented with hydrocarbon

Name of the fungi	Concentration of hydrocarbon (%)	Biomass (g)			
		Benzene	Toluene	Xylene	Benzene + toluene + xylene
Aspergillus flavus	1	23.8	26.0	29.0	21.9
	2	17.8	23.1	27.9	18.4
	3	12.5	18.9	19.6	15.2
Penicilium citrinum	1	28.1	26.6	24.9	21.6
	2	24.0	29.2	20.6	17.2
	3	22.7	30.1	23.0	16.5
Trichoderma viride	1	23.7	24.7	25.5	20.1
	2	20.6	22.6	18.3	15.7
	3	19.2	14.8	12.0	9.6

Among the three fungi *Penicillium citrinum* was high growth of biomss weight when compared other fungi of *Aspergillus flavus* and *Trichoderma viride* due their chemical constituents may tolerate on the growth of fungi (Table 2). Hence the fungi are high potential degradation of hydrocarbon and tolerance the growth of *invitro* condition and reduce the pollution from the environment.

Tony and Sanro (2009) and Joseph and. Rita (1990) at the end of thirty days fungal biomass exhibited maximum growth (27.1 g/L) in the flask containing oil sludge as a carbon source. The decreasing trend with increase in *Aspergillus versicolor* life cycle. However their abilities to degrade a specific hydrocarbon as a source of energy and/or biomass may differ. The chemical composition of a crude oil may also be a factor in determining the type of fungi, which may grow on it Maximum growth of *Aspergillus versicolor* biomass was found till twenty days and later observed a declination stage in the *Aspergillus versicolor* growth cycle which results in the 82.4% degradation of oil sludge for first twenty days and later 4.7% degradation till 13th day of study. It may be due to the limiting of nutrients in the culture medium. Nutrients play a key role in the metabolic activates, such as production of many intracellular enzymes which metabolizes the hydrocarbons and helps in the biomass production and in cell growth. It might be the reason for the decrease in the growth of *A. versicolor* biomass.

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How to cite this article:

Meera B *et al*.2016, Effect of Microfungi on Bioremediation of Hydrocarbon From Polluted Soil. *Int J Recent Sci Res.* 7(12), pp. 14828-14830.