RESEARCH ARTICLE

Epicoccum nigrum link. as a potential source of Mycoremediation against oil spill

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ABSTRACT

Incident of 2,00,000 barrels of crude oil spill in Alaska fetched attention of the world. The problems caused due to oil spill are environmental pollution along with risk to the marine life and human beings. The global use of crude oil, mainly derived from various fossils throughout the world, is estimated to be average 5.5 metric tons per year. Biodegradation of such complex hydrocarbons is very challenging task. Use of microorganisms viz. bacteria and fungi to degrade these hydrocarbons is a primary mechanism to eliminate the pollutants from the environment. Effect of environmental parameters on the microbial activities in terms of metabolic pathway and its impact on the genetic bases was always been an area of intense curiosity and reviews. In case of fungi, spp. of *Aureobasidium, Candida, Rhodotorula*, *Sporobolomyces, Trichoderma* and *Mortierella* are commonly used as source of mycoremediation for degradation of hydrocarbons. In the present studies, we have tested *Epicoccum nigrum*, as a potential source of mycoremediation against oil spill. The effective degradation of hydrocarbons from the oil and petrol was measured in terms of titrimetric estimation of phytosterols from the samples.

Keywords: Crude Oil, Biodegradation, Mycoremediation, Primary Mechanism

INTRODUCTION

Petroleum hydrocarbons are usually divided into four groups such as saturated, aromatic, asphaltenes and resins (Colwell and Walker, 1977). Depending upon their structures, the hydrocarbons are susceptible to microbial attack. It has been studied that n-alkenes are more prone to microbial degradation that the cyclic alkenes (Perry, 1984). Biodegradation rates are highest for the saturated hydrocarbons, followed by the light aromatics. The high-molecular weight aromatics and polar compounds exhibiting extremely low

rates of degradation (Jobson *et. al*., 1972; Walker *et. al*., 1976; Fusey and Oudot, 1984). Cooney *et. al.* (1985) reported greater degradation losses of naphthalene than hexadecane in case of freshwater lake while Jones *et. al.* (1983) observed extensive biodegradation of alkylaromatics of the crude oil in marine sediments. Fedorak and Westlake (1981) also reported rapid degradation of aromatic hydrocarbons during the degradation of crude oil by marine microbial populations. Formation of emulsions through the microbial production and release of biosurfactants is an important process in the uptake of hydrocarbons by bacteria and fungi (Singer and Finnerty, 1984).

Fungi have been reported to be important inhabitants of specialized niches such as sub-merged wood (Kirk and Gordon, 1988). They are also components of the surface film of water, decomposing algae, and the surface of tarballs (Ahearn and Crow, 1986). Few studies have directly compared the degrees of hydrocarbon degradation accomplished by bacteria and fungi in the marine environment. Hydrocarbon utilizing fungi are readily isolated from soil (Llanos and Kjoller, 1976; Pinholt *et. al.*, 1979; Atlas *et. al.*, 1980) and the application of oil or oily wastes to soil results in increased numbers of fungi (Jensen, 1975; Llanos and Kjoller, 1976; Pinholt *et. al.*, 1979). Hydrocarbon degradation by microbial communities depends on the composition of the community and its adaptive response to the presence of hydrocarbons (Song *et. al*., 1986). The factors mainly responsible for process of degradation are pH, temperature, light intensity, pressure, surface area, salinity and availability of nutrients (Atlas, 1981).

Epicoccum nigrum Link. is a common pathogenic fungal organism isolated from the soil. Morphological, cultural and metabolic characters of *Epicoccum nigrum* are similar to the spp. of *Aureobasidium, Candida, Rhodotorula*, *Sporobolomyces, Trichoderma* and *Mortierella*, which are commonly used as source of mycoremediation for degradation of hydrocarbons in oil spill (Kirk and Gordon, 1988). Hence, in the present studies, the authors have tested *Epicoccum nigrum* Link. as a potential source of mycoremediation against oil spill.

MATERIAL AND MATERIALS

1. Isolation of *Epicoccum nigrum*:

Epicoccum nigrum was isolated from the soil in the College campus by using **Agar plate method:**

a) Preparation of Potato Dextrose Agar medium (PDA) –

Peeled potatoes - 200 gms.
Dextrose - 20 gms.
Agar agar - 20 gms.
Distilled water - 1000 ml.

200 gms of peeled potatoes were boiled in 500 ml of distilled water till the solution becomes sticky by dissolution of the potato pieces. The solution was filtered and 20 gms of dextrose was added in it. 500 ml of distilled water was taken in the other beaker, it was boiled and 20 gms of agar agar powder was dissolved in it. Both the solutions were mixed and final volume was made to 1000 ml.

b) Sterilization of the glass ware

Required glass wares i.e. Petri plates, conical flasks; beakers etc. with PDA Medium were sterilized in an autoclave at 120º C at pressure of 15 lbs (pounds) for about 30 minutes. The glass ware and medium were preserved for the further use.

c) Incubation

The plates were incubated at 28º C (± 2) in the incubator.

2. Preparation of pure culture of *Epicoccum nigrum*:

Colonies of various fungal organisms obtained in Agar Plate Method were identified on the basis of study of colony characters, sporulation and spore specifications. Colonies of *Epicoccum nigrum* were selected and pure culture was obtained by serial purification method by using Agar Plate Method as mentioned above.

3. Treatment of various sources of hydrocarbons with *Epicoccum nigrum*:

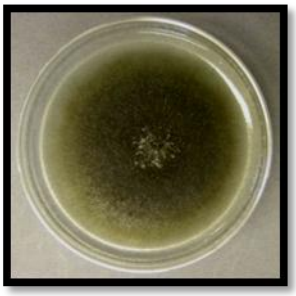
1 ml sample of Petrol, Til oil, Castor oil, Clove oil and Parachute oil (Coconut oil) each was treated with a loopful pure culture of *Epicoccum nigrum* and 4 sets were prepared. The sets were incubated for 7, 14, 21 and 28 days respectively at 28º C (± 2) in the incubator. At the end of decided period, each set was estimated to find out the amount of phytosterols present in the sample.

Estimation of phytosterols:

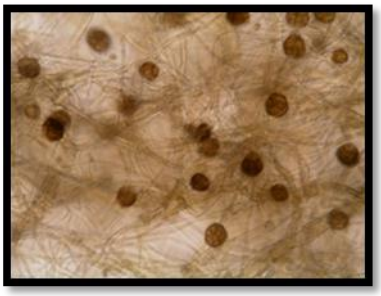
Lipids in the oils in the form of phytosterols were estimated by method given by Tomita *et. al*. (1970). According to this method, 1 gm oil was extracted with 10 ml 80% alcohol and the mixture was warmed slightly. The homogenate was allowed to cool for 10 minutes and filtered through Whatman No. 1 filter paper. The filtrate was collected and used for estimation of phytosterols. 0.5 ml of the extract was taken in a test tube and 2ml glacial acetic acid and 2 ml coloured reagent were added to it. The total volume was adjusted to 5 ml by adding 80% alcohol in it. The test tubes were incubated in an ice bath at 0 °C for 10 minutes and absorbance was read at 440 nm. The values were calculated by using 0.1 as the multiplication factor.

RESULTS AND DISCUSSION

Table 1of Estimation of Phytosterols clearly indicates decrease in the amount of phytosterols in various types of oils and petrol during incubation with *Epicoccum nigrum* indicating decrease in the amount of hydrocarbons. Clove oil showed presence of highest amount of phytosterols i.e. 0.096 mg in the control followed by 0.095, 0.094, 0.092 and 0.043 mg of phytosterols during successive incubation of 7, 14, 21 and 28 days. Parachute (Coconut) oil was in the second place with presence of 0.094 mg of phytosterols in control degrading to 0.086, 0.075, 0.069 and 0.023 mg during 7,14, 21 and 28 days of incubation respectively. Til oil showed presence of 0.069 mg of phytosterols in control; which degraded to 0.063, 0.026, 0.022 and 0.011 mg respectively during range of incubation from 7 to 28 days. Petrol showed presence of 0.068 mg of phytosterols in control decreasing to 0.051, 0.017, 0.013 and 0.006 mg during successive incubation for 7, 14, 21 and 28 days. The lowest amount of phytosterols (0.053 mg) were present in castor oil and degraded to 0.049, 0.042, 0.021and 0.008 mg of phytosterols in respective period of incubationfrom to 28 days.



Pure Colony of *Epicoccum nigrum*



Fruiting bodies of *Epicoccum nigrum*

Control Set

Experimental Set

Table 1: Estimation of Phytosterols (Milligrams per Gram)

Sr. No.	Category	Control	7 Days	14 Days	21 Days	28 Days	Consumption
1	Petrol	0.068	0.051	0.017	0.013	0.006	91.18%
2	Til Oil	0.069	0.063	0.026	0.022	0.011	84.06%
3	Castor Oil	0.053	0.049	0.042	0.021	0.008	84.91%
4	Clove Oil	0.096	0.095	0.094	0.092	0.043	55.21%
5	Parachute Oil	0.094	0.086	0.075	0.069	0.023	75.53%

The highest amount of consumption of hydrocarbons by *Epicoccum nigrum* was seen in case of petrol (91.18%) follwed by castor oil (84.91%), til oil (84.06%) and parachute oil (75.53%). The lowest amount of consumption was shown by clove oil which was 55.21 %. Hence, *Epicoccum nigrum* supposed to be very effective in degradation of hydrocarbons from petrol.

CONCLUSION

Biodegradation of petroleum and other hydrocarbons in the environment is a complex process, whereby quantitative and qualitative parameters are dependent upon nature and amount of hydrocarbons along with environmental conditions and type of the microbes. Microbial degradation of oil occurs due to attack on aliphatic or light aromatic components in the oil while high-molecular-weight compounds exhibit very low rates of biodegradation. The microbial degradation of hydrocarbons is limited due to various factors such as nutrient concentrations, pressure, temperature, salinity, moisture and pH. Hydrocarbon degradation by microbial organisms is depending on the type and adaptive response of the microorganisms to the presence of hydrocarbons. Fungi are one of the key agents of degradation becoming more important in freshwater and terrestrial environments. In this scenario, apart from the mentioned fungi; *Epicoccum nigrum* can be looked upon as a potential source of mycoremediation in future because it has worked effectively in degradation of hydrocarbons from various samples of oil and petrol. It needs further investigation based upon various parameters.

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REFERENCES

Ahearn DG and Crow SA (1986) Fungi and hydrocarbons in the marine environment, p. 11-18. In S. T. Moss (ed.), The biology of marine fungi. Cambridge University Press, Cambridge.

Atlas RM (1981) Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev*., 45: 180-209.

Atlas RM, Sexstone A, Gustin P, Miller O, Linkins P and Everett K (1980) Biodegradation of crude oil by tundra soil microorganisms, p. 21-28. In T. A. Oxley, G. Becker, and D. Allsop (ed.), Proceedings of the 4th International Biodeterioration Symposium. Pitman Publishing Ltd., London.

Cochrane VW (1958) "Physiology of Fungi." *New York, John Willey and Sons. Pp. 524.*

Colwell RR and Walker JD (1977) Ecological aspects of microbial degradation of petroleum in the marine environment. *Crit. Rev. Microbiol*. 5:423-445.

Cooney JJ, Silver SA and Beck EA (1985) Factors influencing hydrocarbon degradation in three freshwater lakes. *Microb. Ecol*., 11:127-137.

Fedorak PM and Westlake DWS (1981) Microbial degradation of aromatics and saturates in Prudhoe Bay crude oil as determined by glass capillary gas chromatography. *Can. J. Microbiol*., 27:432-443.

Fusey P and Oudot J (1984) Relative influence of physical removal and biodegradation in the depuration of petroleum contaminated seashore sediments. Mar. *Pollut. Bull*. 15:136-141.

Jensen V (1975) Bacterial flora of soil after application of oily waste. *Oikos*, 26:152-158.

Jha DK (1993) A Textbook on Seed Pathology." *Vikas Pulishing House Pvt. Ltd., New Delhi.*

Jobson A, Cook FD and Westlake DWS (1972) Microbial utilization of crude oil. *Appl. Microbiol*. 23:1082-1089.

Jones DM, Douglas AG, Parkes RJ, Taylor J, Giger W and Schaffner C (1983) The recognition of biodegraded petroleum- derived aromatic hydrocarbons in recent marine sediments. Mar. *Pollut. Bull*. 14:103-108.

Kirk PW and Gordon AS (1988) Hydrocarbon degradation by filamentous marine higher fungi. *Mycologia*, 80:776- 782.

Llanos C and Kjoller A (1976) Changes in the flora of soil fungi following oil waste application. Oikos 27:377-382.

Perry JJ (1984) Microbial metabolism of cyclic alkanes, p. 61-98. In R. M. Atlas (ed.), Petroleum microbiology. Macmillan Publishing Co., New York.

Pinholt Y, Struwe S and Kjoller A (1979) Microbial changes during oil decomposition in soil. *Holarct. Ecol*. 2:195-200.

Singer ME and Finnerty WR (1984) Microbial metabolism of straight-chain and branched alkanes, p, 1-60. In R. M. Atlas (ed.), Petroleum microbiology. Macmillan Publishing Co., New York.

Song HG, Pedersen TA and Bartha R (1986) Hydrocarbon mineralization in soil: relative bacterial and fungal contribution. *Soil Biol. Biochem*. 18:109-111.

Thind KS and Randhwa HS (1957) Studies on nutrition of fungi." *Proc. Nat. Acad. Sci. (India) 27 : 39 – 46.*

Tomita Y, Uomori A and Minato H (1970) Steroidal sapogenesis and sterols intissue culture of *Dioscorea tokoro." Phytochemistry, 9 : 111 – 114.*

Walker JD, Colwell RR and Petrakis L (1976) Biodegradation rates of components of petroleum. *Can. J. Microbiol*. 22:1209-1213.