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SCREENING OF *ASPERGILLUS NIGER* FOR BIODEGARADATION OF DIFFERENT HYDROCARBONS

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Abstract:

Today hydrocarbons/oil pollution causes serious damage to our environment. Chemical or physical methods are failed to degrade such contaminants hence biodegradation provides simple and cost effective process. In this study the biodegradation potential of Aspergillus niger for different hydrocarbons was analyzed. Two kinds of hydrocarbons i.e. edible (almond oil, mustard oil and cooking oil) and non-edible (engine oil, diesel and petrol) were used.

For the initial screening, culture was inoculated in Bushnell-Haas (BH) plate assay each plate containing respective hydrocarbon. Aspergillus niger displayed highest growth on medium containing cooking oil. While least growth was noted on petrol and diesel. Furthermore to analyze the degradation ability of Aspergillus niger, dextrose media was used. The biodegradable efficiency of Aspergillus niger was noted on the basis of dry weight, total protein, total sugar and reducing sugar in presence of hydrocarbons. The highest growth of Aspergillus niger was noted on media containing 2% cooking oil after 6 days of incubation. Among the non-edible hydrocarbons highest growth was noted on 3% engine oil after 6 days of incubation. Total protein content on edible hydrocarbon was found to be higher than non-edible hydrocarbons. Total sugar content showed great variation among edible and non-edible hydrocarbons. Reducing sugar was found to be very low in both edible and non edible hydrocarbon containing media. 2, 6-dichlorophenol indophenols (DCPIP) dye assay was also used for detection. The highest reduction in absorbance was observed on edible hydrocarbons than non-edible hydrocarbons. Hence Aspergillus niger have ability to degrade hydrocarbons at different rate.

Key words: *Biodegradation, Aspergillus niger, edible hydrocarbons, non edible hydrocarbons.*

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1. INTRODUCTION:

The growing industrial development promotes serious environmental damage due to their toxic waste. Considering the petrochemical industry, pollution by oil and its derivatives imposed serious threat to terrestrial and aquatic ecosystems. Thus, control and treatment strategies to reduce the dangerous effects of oil pollution are needed[1]. Incineration, volatilization or immobilization of pollutants, they all are conventional treatments of pollutants which simply convert the pollutants into another waste and fail to eliminate the problem [2]. These treatments are more expensive, energy demanding and not workable [3]. Bioremediation technology is an attractive alternative method for detoxification and mineralization of pollutants, which in some cases could produce economic benefits[2].

Bioremediation is the use of microbes to detoxify the pollutants and principally based on biodegradation. It may refer to complete degradation of organic contaminants into carbon dioxide, water, inorganic compounds by microbial enzymes due to their diverse metabolic capabilities for the removal and degradation of many environmental pollutants[4]. Biodegradation of hydrocarbon is a different process and its control and optimization depends upon many factors. These factors comprise of the existence of a microbial population capable of degrading the pollutants; the availability of pollutants to the microbial population; the environment factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients) [5]. The rates of uptake and mineralization of many organic compounds by microbial populations in the aquatic environment are proportional to the concentration of the compound [6, 7]. In recent times, many researchers considered the character of different fungi in biodegradation process of petroleum products and the most common fungi which have been verified as biodegraders belongs to following genera: *Alternaria, Aspergillus, Candida, Cephalosporium, Cladosporium, Fusarium, Geotrichum, Gliocladium, Mucor, Paecilomyces, Penicillium, PleurotusPolyporus, Rhizopus, Rhodotolura, Saccharomyces, Talaromyces, Torulopsis* etc [8, 9].

The filamentous fungus *Aspergillus. niger* (black mould) has been used for centuries as industrial versatile microbial cell factory for production of organic acids as well as various extracellular enzymes, native or heterologous proteins and antibiotics. *A. niger* shows an amazing nutritional flexibility and metabolic capacity, and produces high levels of secreted primary and secondary metabolites. Nowadays, it is difficult to think of filamentous fungus where the metabolic capabilities are of greater interest than *A. niger*[10]. Therefore in the present study screening of *A. niger* has been done for the degradation of different hydrocarbons.

2. MATERIALS AND METHODS:

2.1. PREPARATION OF INNOCULUM:

All chemicals used were of analytical grade and purchased from Sigma/ E. Merck, and Fluka and were prepared in distilled water throughout the study. The *Aspergillus niger* was taken from the Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro and grown in Enzyme and Fermentation Biotechnology Research Laboratory. The culture was maintained on glucose agar medium at 37±2°C.

2.2 INITIAL SCREENING DONE ON BH PLATE ASSAY:

The degradation ability of *A. niger* was first screened using the Bushnell-Haas (BH) plate assay, it contains following ingredients; Magnesium Sulfate (0.02%), Calcium Chloride (0.002%), Monopotassium Phosphate (0.1%), Dipotassium Phosphate (0.1%),Ammonium Nitrate (0.1%),Ferric Chloride (0.005%) Agar (2%) for screening purpose and pH of medium was maintained at 6.5 ± 2. BH plate assay detects ability of degradation based on the growth of microbe[11]. Each carbon source was added separately into the BH medium agar plates. In order to screen for hydrocarbon utilizing ability of *A. niger*, sterile filter papers soaked in hydrocarbons(cooking oil, almond oil, mustard oil, diesel, petrol and engine oil) and were aseptically placed into the lids of each inoculated Bushnell-Haas agar plates; this technique is called the vapour phase transfer[12].In control plates hydrocarbons were not added. All plates incubated for 3 and 7 days at 37°C in incubator.

2.3. SCREENING WITH ADDITIONAL CARBON SOURCE:

The following ingredients were used for the preparation of SDB culture medium; Dextrose (4%), Peptone (1%). Different hydrocarbons also introduced in 1%, 2% and 3% respectively. The following table 2.1 shows the list of hydrocarbons. No hydrocarbons were introduced in control flasks.

Table 2.1: Hydrocarbons	
Edible Hydrocarbons	**Non-Edible Hydrocarbons**
Almond Oil	Engine Oil
Mustord Oil	Diesel
Cooking Oil	Petrol

Incubated for 5, 6 and 7 days at 37°C. Biodegradation analysis was done in terms of total weight, total protein, total sugar and reducing sugar. Mycelial biomass was collected on Whattman filter paper, dried and weighed on electrical balance. Total sugar content of all filtrates was determined by phenol sulfuric acid method[13]. Total protein content of all filtrates was determined by Lowry method [14]. The reducing sugar content of all filtrates was determined by Dintrosalicyclic acid (DNS) method [15].

2.4. COLORIMETRIC ASSAY FOR OIL DEGRADATION ANALYSIS:

The biodegradability of *Aspergillus niger* was also estimated using the technique based on the redox indicator 2, 6-dichlorophenol indophenols (DCPIP) [16, 17]. 0.05mM DCPIP dye was added in Bushnell Haas broth from the stock of 3mM DCPIP dye. Microbial cultures were inoculated in presence of hydrocarbons.

Each flask was incubated for 7 days and reading was taken at 600 nm in spectrophotometer at 24 hours interval. Each flask contain following content as shown in table 2.2:

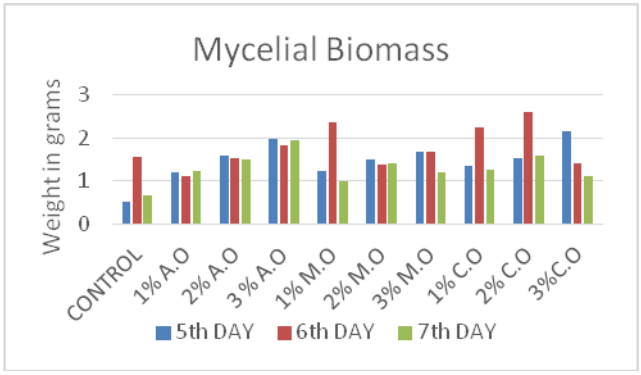
Table 2.2: Contents		
DCPIP	**BH**	**Hydrocarbons**
1. 0.66ml	39.4 ml	No oil
2. 0.66ml	38.84 ml	0.5 ml Almond oil
3. 0.66ml	38.84 ml	0.5 ml Cooking oil
4. 0.66ml	38.84 ml	0.5 ml Mustard oil
5. 0.66ml	38.84 ml	0.5 ml Engine oil
6. 0.66ml	38.84 ml	0.5 ml Diesel
7. 0.66ml	38.84 ml	0.5 ml Petrol

3. RESULTS AND DISCUSSION:

Result showed that *Aspergillus niger* has efficacy to degrade different hydrocarbons.

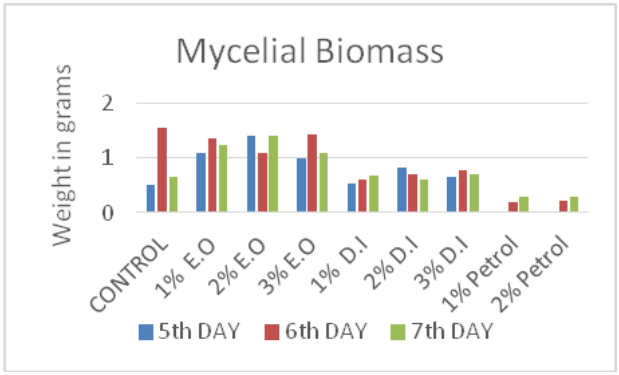
3.1. INITIAL SCREENING OF *APERGILLUS NIGER*:

During initial screening, *A. niger* showed different growth rate at different hydrocarbons. It was observed that the rate of fungal growth in the B.H media containing hydrocarbon as a carbon source is high as compared with media without hydrocarbons. This might be due to the fact that the fungi use hydrocarbon as a substrate for their existence, growth and using extra cellular enzymes to break down the intractable hydrocarbon molecules, by demolishing the long chains of hydrogen and carbon, thereby, converting hydrocarbons into simpler forms or products that can be absorbed for the growth, development and nutrition of the fungi[18].However it easily used edible hydrocarbon as a carbon source than non-edible hydrocarbons. In vapour phase transfer method medium which contain cooking oil, Aspergillus niger showed the highest growth and least growth was observed on petrol, in addition it take more time for biodegradation of non-edible hydrocarbons. Also, displayed the variation in growth under different percentages of particular hydrocarbon. Incubation time period also varies the degradation rate of *A. niger*. It was noted that the highest growth of *A. niger* on different hydrocarbons obtained after 6-days of incubation. In petrol medium *A. niger* took longest time for degradation while on 3% petrol medium it did not show growth even after 7 days of incubation.



A.O= ALMOND OIL, M.O=MUSTARD OIL, C.O= COOKING

Figure 3.1: Total biomass of Aspergillus niger in presence of edible hydrocarbons

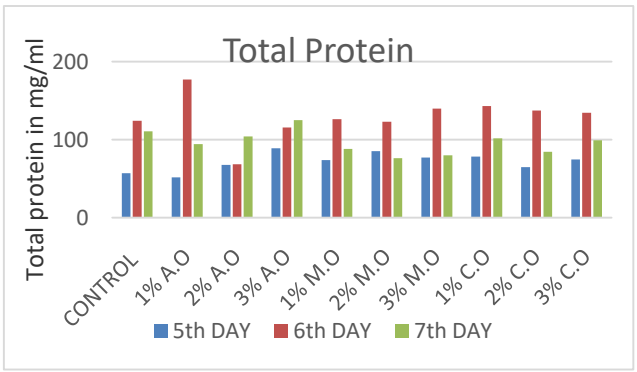


E.O= ENGINE OIL, D.I= DIESEL

Figure3.2: Total biomass of Aspergillus niger in presence of non-edible hydrocarbons.

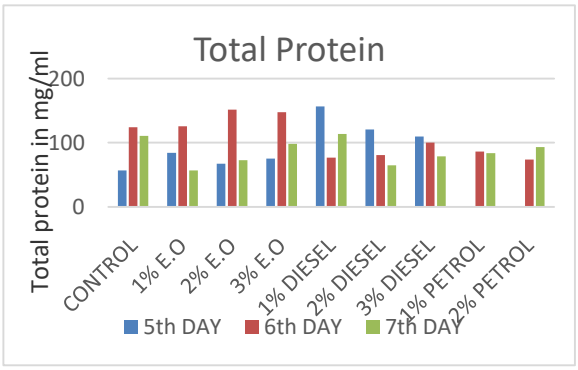
3.2. SCREENING WITH ADDITIONAL CARBON SOURCE:

A. niger is a non-ligninolytic fungus so it showed better growth on medium which contain readily degradable carbon source dextrose as mentioned by Chung[19]. *A. niger* showed highest protein, total sugar and reducing sugar content in edible hydrocarbons while showed least on non-edible hydrocarbons. Highest total protein content obtained after 6 days of incubation with exception of petrol and diesel. Total sugar content varies according to the hydrocarbon nature and its quantity in media and also depend upon incubation time period. Reducing sugar content was found to be very low in edible as well as non-edible hydrocarbons.



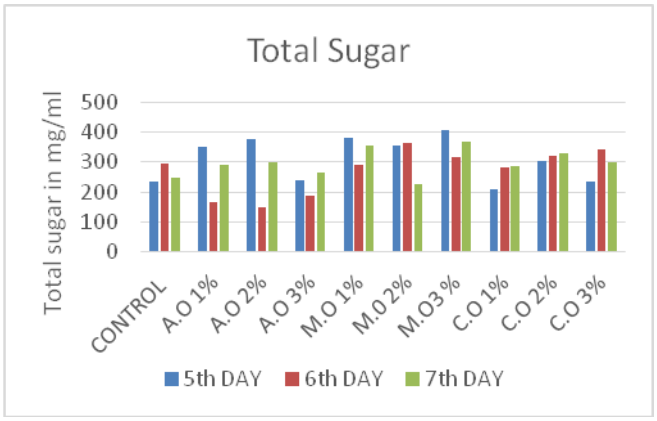
A.O= ALMOND OIL, M.O=MUSTARD OIL, C.O= COOKING OIL

Figure 3.3: Total protein content of Aspergillus niger in presence of edible hydrocarbons



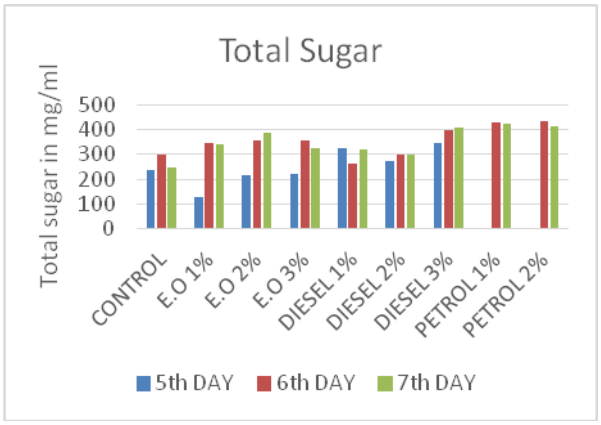
E.O= ENGINE OIL

Figure 3.4: Total protein content of Aspergillus niger in presence of non-edible hydrocarbons



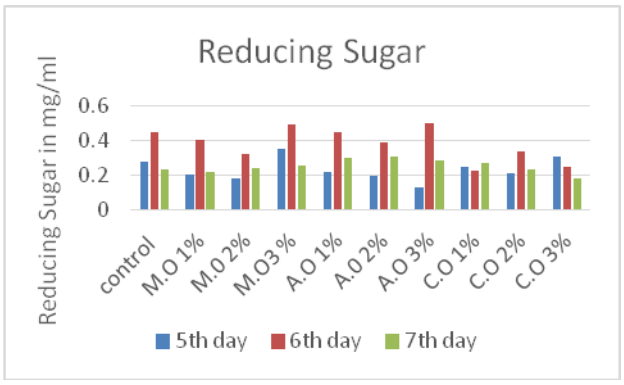
A.O= ALMOND OIL, M.O=MUSTARD OIL, C.O= COOKING OIL

Figure3.5: Total sugar content of Aspergillus niger in presence of edible hydrocarbons



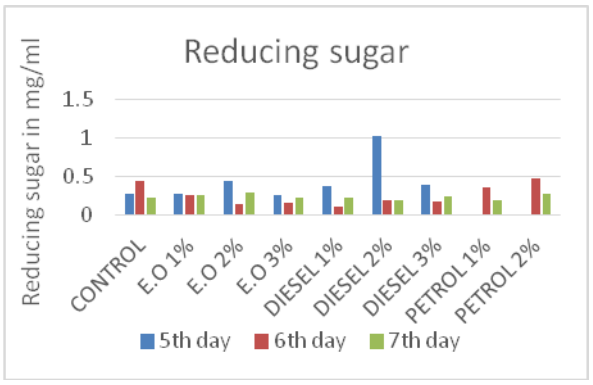
E.O= ENGINE OIL,

Figure 3.6: Total sugar content of Aspergillus niger in presence of non-edible hydrocarbons



A.O= ALMOND OIL, M.O=MUSTARD OIL, C.O= COOKING OIL

Figure3.5: Total sugar content of Aspergillus niger in presence of edible hydrocarbons



E.O= ENGINE OIL,

Figure 3.6: Total sugar content of Aspergillus niger in presence of non-edible hydrocarbons

3.3. COLORIMETRIC ASSAY FOR HYDROCARBON DEGRADATION ANALYSIS:

To analyze hydrocarbon degradation capacity of *A. niger*, DCPIP colorimetric method was used which indicates the utilization of hydrocarbons by the cell. DCPIP is an electron acceptor, when it is introduced into the culture media DCPIP changes from blue (oxidized form) to colorless (reduced form), which shows the exploitation of the substrate by the fungal specie[17]. During this study, *A. niger* displayed the fastest onset color disappearance on edible hydrocarbons than non-edible hydrocarbon The decrease in absorbance verifies the degradation ability of *A. niger* for different hydrocarbons. Meanwhile petrol medium showed least decrease in absorbance followed by diesel. Petrol medium showed reduction in absorbance after 4 days of incubation. The total color change strengthens the verity that, *A. niger* is a potential hydrocarbon oxidizer. Also, Okerentugba and Ezeronye demonstrated that *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. were capable of degrading hydrocarbons especially when single cultures were used [20]

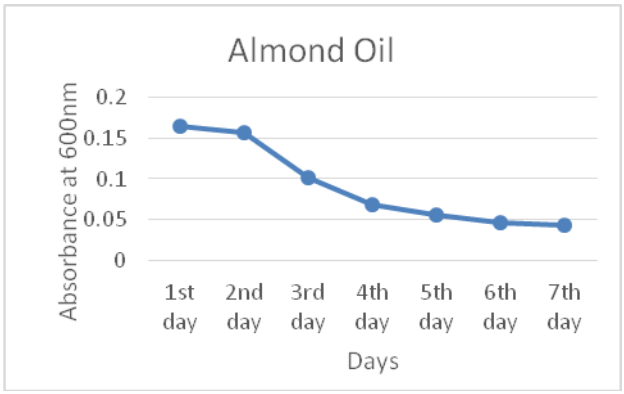


Figure3.7: Reduction in absorbance of DCPIP in presence of Almond oil

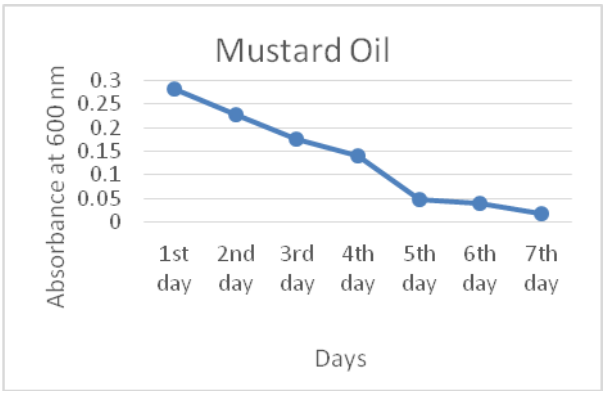


Figure3.8: Reduction in absorbance of DCPIP in presence of Mustard oil

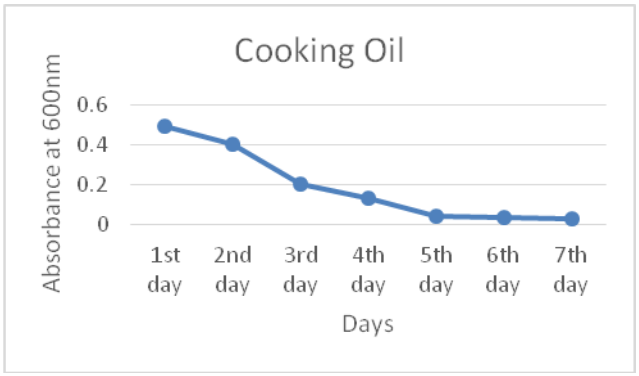


Figure3.9: Reduction in absorbance of DCPIP in presence of Cooking oil

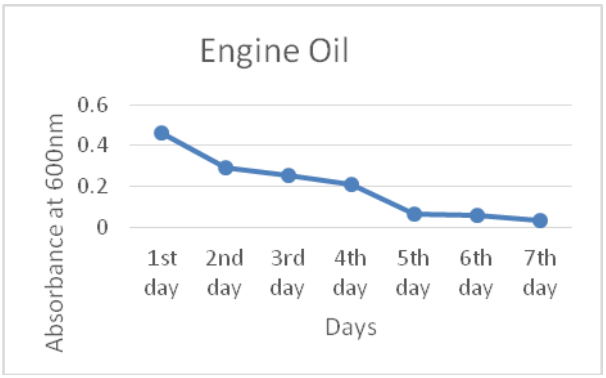


Figure3.10: Reduction in absorbance of DCPIP in presence of Engine oil

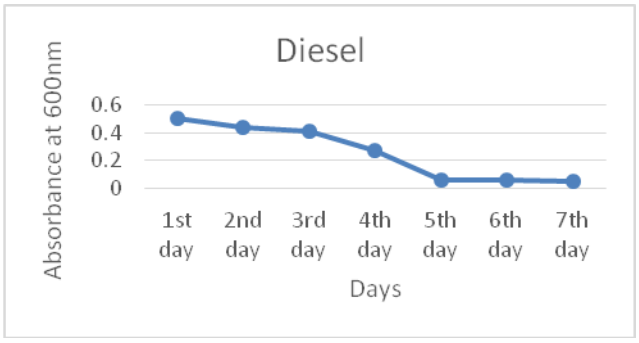


Figure3.11: Reduction in absorbance of DCPIP in presence of Diesel

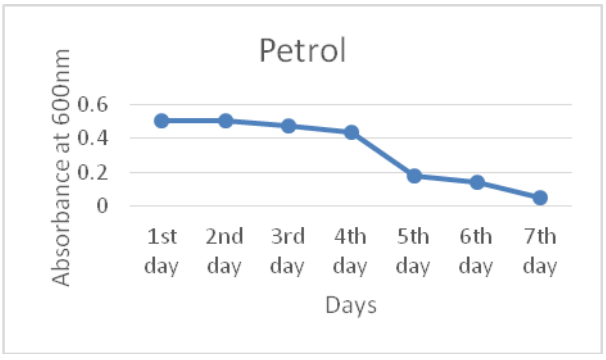


Figure3.12: Reduction in absorbance of DCPIP in presence of Petrol

4. CONCLUSION :

It has been observed in present study that *A. niger* has ability to degrade the hydrocarbons at different rates. The highest biodegradation efficiency encountered by *A. niger* is on edible hydrocarbon than on non-edible hydrocarbons.

5. ACKNOWLEDGEMENTS:

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