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Biodegradation of Crude Oil Using *Aspergillus* species

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Abstract

Biodegradation of crude oil is a process that utilizes the capability of microorganism to degrade toxic pollutant in the environment. In the present study, three fungal species were isolated from the soil contaminated with crude oil in the oil fields in Basrah. The fungal species belongs to the genus *Aspergillus*, which are *A. flavus* ,*A .fumigatus* and *A .versicolar*. Their ability to biodegrade crude oil was tested as single isolates for 15 and 30 days of incubation in the mineral salts medium,the results showed that the fungus *A. flavus* was the best, with biodegradation ability reaching 60% in 15 days and 80% in 30 days .

Keywords:crude oil, fungi, biodegradation.

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1.Introduction

Crude oil is one of the most important energetic resources in the world. It is used as raw material in numerous industries, including the refinery-petrochemical industry ,Oil-derived products are also commonly used in many other chemical processes(Brown *et al* ., 2017 ;Marchand *et al*., 2017).

crude oil are complex mixture of organic compounds basically of paraffinic, oleifinic and polycyclic aromatic hydrocarbons(Santos *etal*., 2014). These hydrocarbons are hazardous to the living organisms and are also carcinogenic, mutagenic and potentimmuno-toxicants which constitutes a serious threat to human and animal health(Alrumman *et al* ., 2015 ; Sajna *et al* ., 2015).

The problems of soil contamination with petroleum hydrocarbons often result in significant decline in its quality and such soils become not useful for use(Kisic *et al* ., 2010 ;Azaizeh *et al.*, 2011).

Their removal becomes essential and several physical and chemical methods are available(Mohammadi-Sichani *et al*., 2017) .

Biodegradation is a process whereby compounds are broken down into smaller constituents or completely broken down into carbon dioxide and minerals by enzymatic ormetabolic processes.(Abdulrazag *et al*., 2016;Dahan*et al*., 2017).Biodegradation of oil contaminated soils, which uses the ability of microorganisms to degrade and/or detoxify organic contamination has been established as oneof the efficient, economic, versatile and environmentally sound treatment(Vidali , 2001 ;Clarkson & Abubakar , 2015).

Microorganisms with specific metabolic capacities have played a significant role in the biodegradation of crude oil and have probably adapted to environments that require treatment(Jussila, 2006 ;Ron& Rosenberg , 2014).

Fungi are one of the best oil-degrading organisms; various studies have identified many fungal species capable of using crude oil as their sole source of carbon and energy(Viswanath *et al .,*2008 ; Shraddha *et al* ., 2011)**.**However, the rate of biodegradation is influenced by several factors such as the type of microorganisms Physical and environmental factors such as nutrients, soil type, pH, temperature, moisture, oxygen water holding capacity and nutrient limitations (Aharoni *et al*., 2017; Avishai *et al*., 2017).This study the efficiency of fungi to degrade crude oil and the effect of time on this process.

2.Materials and methods

2.1 Crude Oil

Crude oil was supplied by Southern Oil Company (Basra, Iraq). It was transferred to laboratory in dark bottle closed tightly and kept in a cold and dark place until to use.

2.2 Sample collection

The Soil samples from the surface layer (5–15 cm) were collected from oil fields in Al-Lahis and Zubair Basra, Iraq, and maintained in plastic bags and storedunder refrigeration at 4ºC until use (Latha & Kalaivani,2012).

2.3 Isolation of fungi

Dilution method Wicklow & Wittingham ,(1974)was used for theisolation of fungi from soil contaminated by crude oil , 10 g of soil was dissolved in 90 ml of distilled water to attain a dilution of 10^{-1} andShake wellby shaker, 1ml transferred by Sterile pipette to the petridishesand then mixed with oil agar medium (OAM)this medium prepared according to Obire&Anyanwu, (2009). Then the petridishes incubated in the incubator under25°Cfor 3 days or more depending on the rate of growth. Then, different fungal colony were isolated and cultured separately in PDA. Fungal species were examined under light microscope and identified using morphological characters and taxonomical keys .

2.4 Testing the ability of fungal species to biodegradecrude oil

mineral salt medium (MSM) were used which contain :10g Na Cl, 0.42g MgSO4,0.12gKCl,0.83gKH2PO4,0.42g NaNO3,1.25g Na2HPO4 dissolve in 1 L of distilled water,the pH was adjusted to 4.5,then autoclaved at 120°C for 20 min. The (MSM) supplemented with 1% (v/v)crude oilwas used as carbonsource and energy for the biodegradation.Two agar plugs $1cm^2$ from the pure cultures of each fungal isolates were inoculated into MSM medium (100 ml/250 ml Erlenmeyer flask)containing sterile crude oil 1% (v/v) as a sole source of carbon and energy.All flasks were Incubated with constant shaking about 120 rpm for tow periods15 and 30 days at 25°C.Control flasks had no organism were incubated at same condition.

2.5 Crude oil extraction

After the end of the incubation period, fungal activities were stopped by adding 1% 1N HCl for the extraction of crude oil. Extraction of crude oil from the liquid medium was done by following the method of Mittal & Singh, (2009),with some adjustments. The leachate was transferred to separating funnel then 80 ml of Petroleum ether andAcetone 1:1 was added to it,the funnel was shacked well several times for (5-10) minutes with the opening of valve to exit the gases and left to settle downuntil two layer were formed .The upper layer represent theoil hydrocarbonsand the lower represent the (water +Acetone), the upper layer was taken and passed through colum containing glass wool and sodium sulfate anhydrous to remove the residual water. The solvent was vaporized overnight. This procedure carried out under same condition on the control flask .The percentage degradation of the crude oil was then calculated gravimetrically according to Oudot (1984).

$$\text{Degradation\%} = \frac{mg\ of\ \ crud\ oil\ control - mg\ of\ crud\ oil\ test}{mg\ of\ \ crud\ oil\ control} * 100$$

The descending from the separating column stored in closed sterile container until analysis by Spectrofluorometer.

2.6 Statisticalanalysis

Analysis Of Variance (One- way ANOVA) was applied by Minitab ver.16

software and Relative Least Significant Differences (RLSD) values were calculatedto identify the fungal degradation significant differences the design used was complete random design.

3.Results

3.1 Identification of fungal species

Three fungal species were isolated from contaminated soil in this study, all isolates were showed potentials for hydrocarbon biodegradation and identified as *Aspergillus flavus , A .fumigatus* and *A .versicolar* .

3.2 Crude oil biodegradation

All of the fungal species showed a good growth in the (MSM) medium. The biomass of the fungal species increased over the time and varied between the three species,also the fungal species transformed the form of crude oil from a liquid and luminous layer into semi-solid and non-shiny parts. The results showed that the species*A. flavus* gave the best degradation abilityin 15 days and in the 30 days table1.

ANOVA test- showed that there were no significant differences ($p>0.05$) between the three fungi in the mean concentrations of total petroleumhydrocarbons (TPH) remaining in salt medium after 15 days of incubation, while there were significant differences ($p<0.01$) between the three fungi in the mean concentrations of total petroleum hydrocarbons (TPH) remaining in the (MSM) medium after 30 days of incubation, also there were significant differences ($p<0.01$) in the mean concentrations of total petroleumhydrocarbons (TPH) in the (MSM)

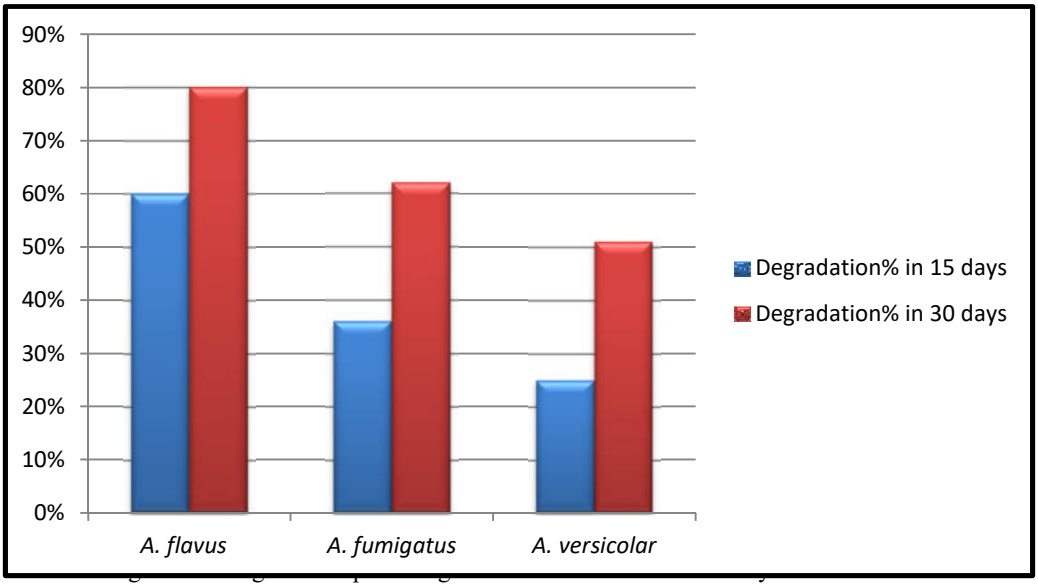
mediumbetween 15 and 30 days of incubation.

Table1.Mean concentrations of total petroleum hydrocarbons (TPH) andthe percentage of biodegradation of fungal species inLiquid Medium for 15 and 30 days of incubation

Species	TPHµg/L after 15 days	Degradation%	TPHµg/L after 30 days	Degradation%
A. flavus	1.32[a]	60%	0.94[a]	80%
A. fumigatus	1.93[a]	36%	1.36[a]	62%
A. versicolar	2.11[a]	25%	1.73[b]	51%
Control	2.4	-	2.2	-
RLSD	1.02	-	0.5	-

The similar letter means no difference and the different letter means there is differences between them.

AndANOVA test- one way showed thatthere was also a significant difference in the percentage of biodegradation of crude oil between 15 and 30 days of incubation figure 1.



4.DISCUSSION

Allthe fungal species which isolated during this study were belonging to the genus *Aspergillus*, this species is widespread and isolated from all environments and it is commonly found in warm regions, and has the ability to produce large numbers of reproductive units which spread easily in air and soil(Sabah *et al.* , 2016).Also the widespread prevalence of these species may be due to their various enzymatic capacities (Sharaddah *et al*., 2011).In addition to the found that some of these species have the ability to produce enzymes such as Cytochrome P-450 monooxygenase and Lignin &Manganese Peroxides (Durairaj *et al* ., 2016).These fungi have been reported as hydrocarbon bio-degraders isolated from soil(April *et al.,*2000).Isolated of fungal species from contaminated soil refer to the adaptation of these fungal strains to petroleum compounds and the ability to degrade a wide range to these compounds(Al- Jawhari , 2014 ;Burghal *et al* ., 2016).

Their ability to biodegrade crude oil, *A. flavus* gave the highest ability to biodegrade crude oil during 15 and 30 days andthe percentage of biodegradation was 60% and 80% respectively during the incubation periods .This may be due to the fact that it has an active and efficient enzymatic system and its high ability to consume petroleum compounds. Several studies have demonstrated the possibility of this species to secret of more than one type of enzymes to biodegrade the different fraction of crude oil, as well as its ability to grow in various difficult environmental conditions and its ability to grow in different environments and the exploitation of different resource

for growth(Fredrick *et al*., 2012 ;Mohsenzadeh *et al*., 2012).The species *A.fumigatus*and *A .versicolar* showed less ability for crude oil biodegradation, this may be due to the weakness of their enzymatic ability to analyze various components of crude oilwhich led to a decline in their ability to show a result similar to the first fungus.Or they may need more time to degrade crude oil better(Clemente *et al*., 2001; Diana *et al*., 2011) .The incubation period had a significant role in the processof biodegradation, the results after the incubation period 30 dayswere better in the three species, this is because the fungal mycelium were in attachment for a longer period with crude oil and this enables it to degrade hydrocarbons into simpler compounds and use it as a source for carbon and energy(Nyer *et al*., 2002;Chigu*et al*., 2010).

5.Conclusion

The results of this study appeared that the isolated fungi showeda good biodegradation efficiency and the species *Aspergillus flavus* was the best one, also the results showed that the time play an important time on the biodegradation process, in which the percentage of biodegradation increased with time.

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