Detection and evaluation of hydrocarbon mixtures degrading bacteria isolated from desiel polluted soils

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Abstract :

Four hydrocarbon degrading bacterial species were isolated from diesel contaminated soil in Hilla city .The isolates were identified according to cultural and biochemecal tests .It showed that they belong to gram negative species and identified as *Pseudomonas aeruginosa ,Serratia marcescens , Pseudomonas flouresence and Alcaligens eutrophus.* The ability of isolates to degrade hydrocarbons were tested towards three HC compounds , engine oil,gasoline and kerosene at 37˚C for 30 days . These isolates exhibited significant differences in their efficiency of degradation depending on total viable count (CFU/ml) , electrical conductivity (EC µS/cm) and emulsification index (E_{24}). The more efficient isolate was *Pseudomonas aeruginisa* as indicated from the increase in the viable count to reach 2.8×10^9 ,3.7×10^9 and 5.3×10^9 (CFU/ml) using a given hydrocarbon component , as a sole source of carbon respectively. Bacterial consortium of the isolated species was prepared. The capability of hydrocarbon consumption was studied. It showed an increase in their activity for biodegradation by increasing of electrical conductivity which reached 82 compared to 52 , 43 , 38 and 34 for the previous mentioned species.

الخلاصة:

عزلت أربعة أنواع بكتيرية محللة للهيدروكاربونات من تربة محلية ملوثة بالمشتقات النفطية (الديزل) في مدينة الحلة . شخصت العزلات بالاعتماد على الصفات المزرعية والفحوصات البايوكيميائية . وقد أظهرت النتائج إن العزلات تعود إلى الأنواع البكتيرية السالبة لصبغة غرام وهي *Pseudomonas flouresence ,Serratia marcescens , Pseudomonas aeruginisa* و *Alcaligens eutrophus* . وقد درست قابلية الأنواع على تفكيك ثلاث أنواع من الهيدروكاربونات وهي زيت المحركات والكازولين والكيروسين بدرجة حرارة ٣٧ °م ولمدة ٣٠ يوم . بينت النتائج وجود فروقات بكفاءة العزلات اعتمادا على العدد البكتيري الحي(CFU/ml) والتوصيلية الكهربائية(EC µS/cm) ودليل الاستحلاب (E_{24}) . وقد كان النوع *Pseudomonas aeruginisa* هو الأكثر كفاءة حيث ازداد العدد الحي الى 2.8×10^9 و 3.7×10^9 و5.3×10^9 مستعمرة /مل بوجود المركبات الهيدروكاربونية المستعملة بالبحث كمصدر وحيد للكربون ، على التوالي . , وقد حضر خليط بكتيري من هذه الانواع ودرست فعاليته في التكسير الحيوي للهيدروكاربونات . وقد اظهرت النتائج زيادة معنوية في هذه القابلية بدلالة التوصيلية الكهربائية حيث وصلت الى 82 مقارنة مع ٥٢ ،٤٣، ٣٨ ، ٣٤ للانواع المذكورة على التوالي .

Introduction:

Crude oil is a complex mixture of hydrocarbons, composed of aliphatic, aromatic and asphaltene fractions along with nitrogen, sulfur and oxygen-containing compounds. The constituents of these hydrocarbon compounds are present in varied proportions resulting in high variability in crude oil from different sources (Salam *et al* ., 2010).

Bioremediation of contaminated aquatic and soil environments has arisen as an effective technology, with a range of advantages compared to more traditional methods. Bioremediation of waste materials, which contain hydrocarbons and their derivatives, is based on the ability of microorganisms to increase their biomass growing on these substrates and degrading them to non-toxic products, such as H2O and CO2 (Jain *et al*., 2005).

Bioremediation has been evaluated in several studies as an option to treat the oil pollution resulting from the spillage or leakage of crude oil and fuels in the environment (Sutiknowati, 2007) .

Contamination of soils and aquifers by oil is a persistent and widespread pollution problem ravaging almost all compartments of the environment and imposing serious health implication and ecological disturbances (Bundy *et al*., 2002;). Petroleum pollutants are not only toxic to biological component of the environment, but some hydrocarbon components are indeed carcinogenic and mutagenic, with immunomodulatory effects on humans, animals and plant life (Obayori *et al*., 2009). Mechanical and chemical methods for remediation of hydrocarbon-polluted environment are often expensive, technologically complex and lack public acceptance (Vidali, 2001).

Thus, bioremediation remains the method of choice for effective removal of hydrocarbon pollutant in the environment (Okoh & Trejo-Hernandez, 2006). Hydrocarbon degrading bacteria are mainly responsible for the mineralization of oil pollutants and are distributed in diverse ecosystems (Adebusoye *et al*.,2007).

It is uncommon to find organisms that could effectively degrade both aliphatics and aromatics possibly due to differences in metabolic routes and pathways for the degradation of the two classes of hydrocarbons. However, some reports have suggested the possibility of bacterial species with propensities for degradation of both aliphatic and aromatic hydrocarbons simultaneously (Obayori *et al*., 2009). This rare ability may be as a result of long exposure of the organisms to different hydrocarbon pollutants resulting in acquisition of appropriate degradative genes (Okerentugba & Ezoroye,2003).

Individual microorganisms can metabolize only a limited range of hydrocarbon substrates, so assemblages of mixed populations with overall broad enzymatic capacities are required to bring the rate and extent of petroleum biodegradation further. Microbial populations that consist of strains that belong to various genera have been detected in petroleum-contaminated soil or water (Habe *et al*.,2001).

Material & Methods

Soil sample collection :

Five top layer (5-10 cm) soil samples were collected aseptically from diesel polluted sites in different regions of Hilla city .

Isolation of degrading bacteria :

Environmental samples were cultured for searching of hydrocarbon degrading bacteria . A mineral salts medium ($(NH_4)_2SO_4$. 1g , K_2HPO_4 . 1g , KH_2PO_4 .1g , $MgSO_4$. $7H_2O$ 1g , $CaCl_2$. 0.01g , $FeCl_3$. 0.0001 , Distilled water 1L) has been chosen and supplement with different hydrocarbon mixture (medium A : Mineral medium + 10% gasoline , medium B : Mineral medium + 10% kerosene, medium C : Mineral medium + used engine oil one g of each soil sample were incubated in 5 parallels into the mineral media and incubated for 20 days at 37˚ C .

Biodegradation capacity of strains :

Threes kinds of hydrocarbonic compounds (gasoline , kerosin and used oil engine) were selected as representation of hydrocarbons and to check whether the isolated strains are able to grow on hydrocarbon compounds . Mineral salts medium (MSM) was used and supplemented with filter sterilized petroleum derivatives to give a final concentration of 10% , 2% and 10% respectively (Woodhull & Jerger ,2006).

Identification of bacterial isolates :

Several basic morphological and biochemical tests were carried out according to MacFaddin (2000) in order to identify the isolated strains.

Assay of imulsification activity :

The emulisification activity of the biosurfactant was measured as described by Cooper and Cooper ,1987 . Three milliliters of different samples was added to 2.0ml of the biosurfactant solution in graduated test tube and vortexes at high speed for 2 min . Control sample was distilled water instead for biosurfactant of emulsification index (E24) was made 24 hr as the height of emulsion layer, divided by the total height of the mixture and multiplied by 100 .

Bacterial consortium preparation :

The bacterial consortium was prepared by inoculating nutrient broth by aloopfull of *Pseudomonas aeruginisa ,Serratia marcescens , Pseudomonas flouresence and Alcaligens eutrophus*. These strains were obtained earlier from diesel contaminated soils and have high utilizing potential of hydrocarbons.

Biosurfactant production

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The capacity of the microbial consortium to degrade hydrocarbons was evaluated in 50 ml of MSM media plus 10%of gasoline ,kerosene ,and used engine ,separately . a control samples were run in parallel in each case and incubated in triplicate at 37 C for 30 days . Thereafter , each flask containing the growing culture was acidified to pH 2.0 and extracted with 20 ml dichloromethane (EM Science ;>99.8%) in a separating funnel . Excess water was removed by adding sodium sulfate (Shuttleworth and Ceringlia ,1996) .

Electrical conductivity :

Electrical conductivity of the culture broth was evaluated using WTW cond 720 /Germany according to (Atekwana *et al* ., 2004) .

Results and Discussion :

Soil samples were used as a source of hydrocarbons degrading bacterial strains by plating them on mineral salts medium supplemented with gasoline 10%, kerosin 10% and engine oil 10% .

Four different bacterial isolates were obtained as apart of direct plating effort to estimate three effectiveness by plate count , emulsification index (E_{24}) , electrical conductivity (EC) and Optical density (OD_{600}).

All these isolates were tested for the ability to degrade individual hydrocarbons which are common in petroleum. Biodegrading rates of bacterial consortium showed increased ability according to (OD_{600}) values compared with control sample .

It is widely believed that that individual organisms could only metabolized limited range of hydrocarbon substrates (Adebusoy et al ., 2007).This led to the assertion that mixed culture exhibited superior degrative competence than pure culture strains (Debajit,2011).

The results of colony morphological characterization and biochemical tests of selected strains exhibited that the isolated strains were belonged to four bacterial species *Pseudomonas aeruginisa ,Serratia marcescens , Pseudomonas flouresence and Alcaligens eutrophus*

Emulsification index of the produced biosurfactant was studied. The results revealed that the high values of this index reach (98%) with gasoline , while the least degree exhibited with engine oil (58%) (Table -2-).

Most of the literacy (Borah, 2011 ;Sutiknowati , 2007) shows *Pseudomonas sp.* and *Alcaligenes sp.* having the best potential to degrade the used engine oil.

The hydrocarbon degrading microorganisms are identified by growing the isolates on a medium containing engine oil . The bacterial growth is monitored by measuring the absorbance at 600 nm . One bacterial species found to give the maximum absorbance and identified as *P. aeruginosa* .

The degradation capacity of the four isolates were tested by allowing them to grow on the mineral salts medium containing different type of hydrocarbon mixtures (Mohd *et al* .,2011).

The bacterial strains isolated in this study were capable to produce biosurfactant when grown on various hydrocarbon mixtures as a sole carbon source . The production was seen within 30 days and peak of biosurfactant activity [E24(68± 4) %] was obtained after incubation period with gasoline by *P. aeruginosa*

The results showed increasing in conductivity during incubation period among the different bacterial species ,but the highest value appeared with bacterial consortium to reach 89 µS/cm (Fig.3).These observations consistence with the findings from geochemical study carried out by Legall,(2002) that revealed existence of higher levels of dissolved ions have been documented at sites contaminated with hydrocarbons .

Table -2- emulisification index (%) of isolated hydrocarbon dgrading bacteria with different hydrocarbon compounds (E_{24}(%)).

Bacterial species	HC	emulisification index
P. aeruginosa	Engine oil	82.3
	Gasoline	96.4
	kerosen	94.1
S.marcescens	Engine oil	77.2
	Gasoline	81.5
	kerosen	80.5
A. eutrophus	Engine oil	81.5
	Gasoline	95.3
	kerosen	91.0
P .flouresence	Engine oil	74.3
	Gasoline	86.3
	kerosen	82.7
consortium	Engine oil	84.5
	Gasoline	98.1
	kerosen	95.3

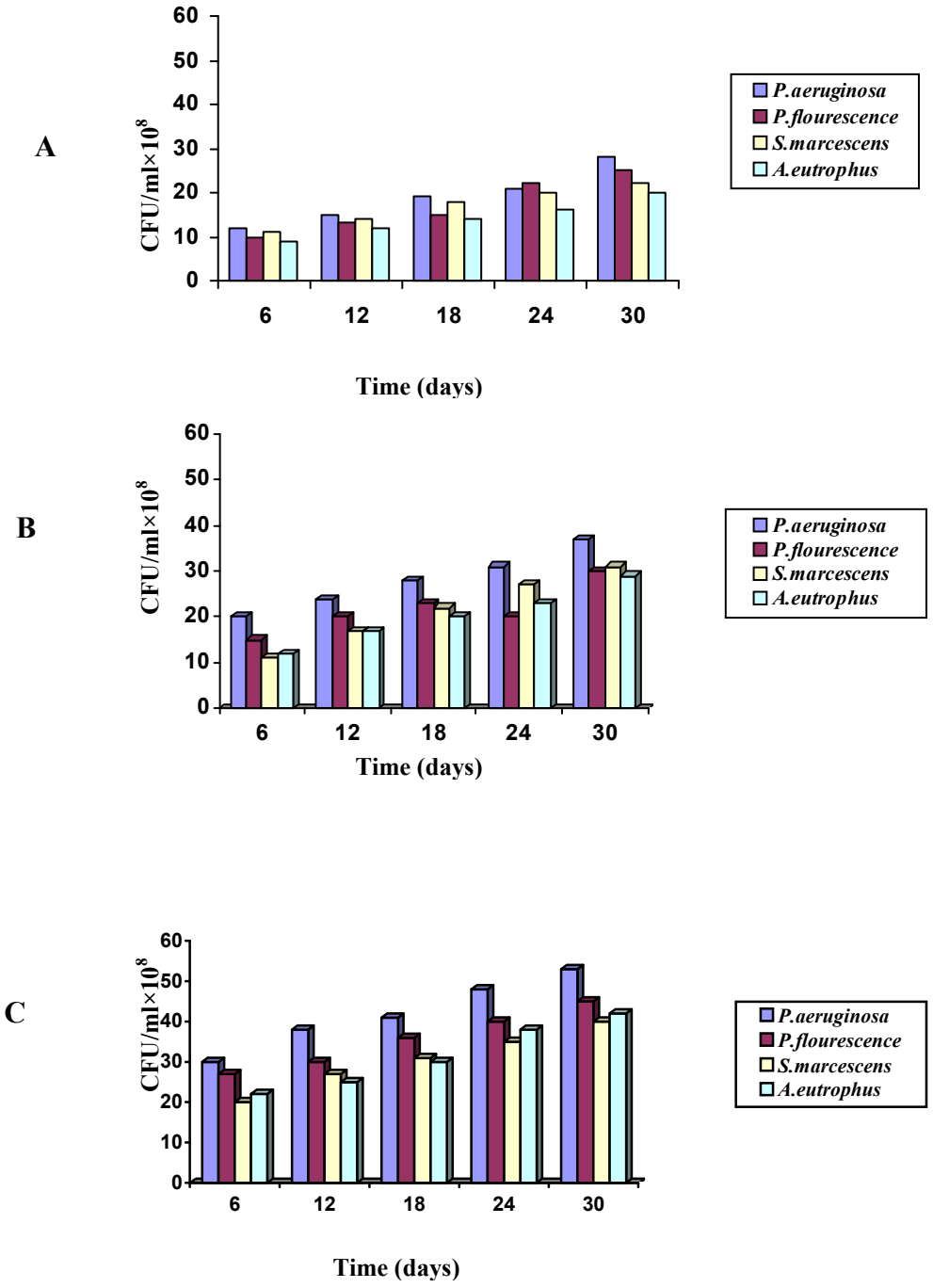

Figure.1.Colony Forming Unit /ml for bacterial isolate grown on MSM with 10% A-engine oil B-Gasoline C-Kerosine as a carbon source

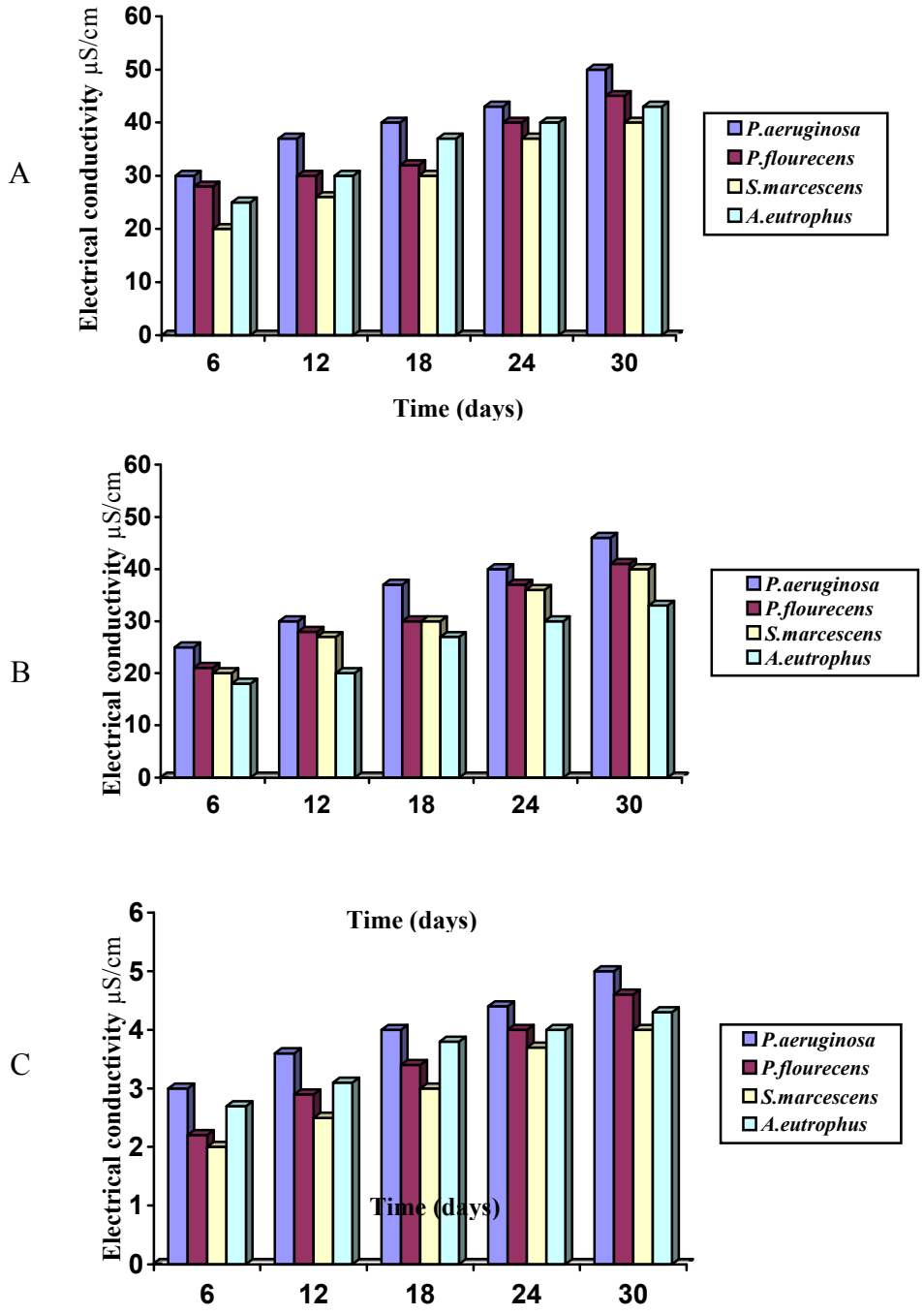

Figure.2.Electrical conductivity for bacterial isolate grown on MSM with 10% A-engine oil B-Gasoline C-Kerosine as a carbon source

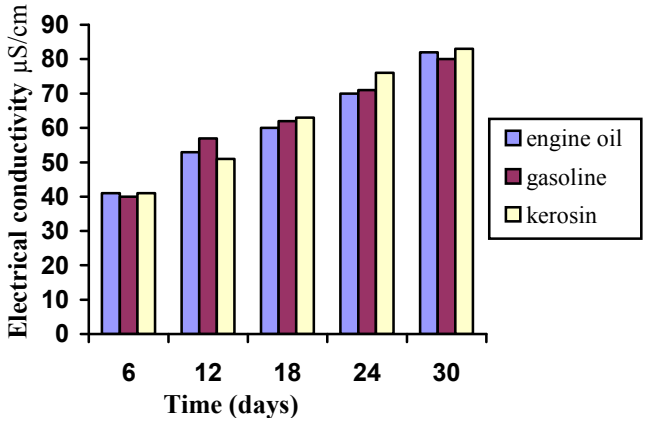

Fig.4.Electric conductivity of consortium after 30 days o growth

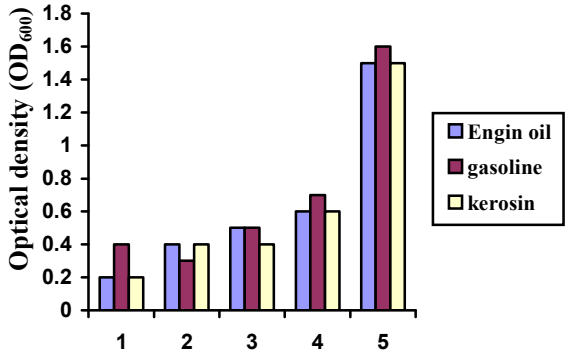

Fig.4.Optical density values of bacterial isolated compound with consortium after 30 days o growth

1- *S.marcescens* , 2- *P.flourecence* , 3-*A.eutrophus* ,4- *P.aeruginosa* ,5-Consortium

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