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BIOREMEDIATION OF ANTHRACENE IN SEAWATER AND WASTEWATER BY FREE AND IMMOBILIZED MARINE *PSEUDOMONAS FLUORESCENS*

MANAL M. A. EL-NAGGAR AND TAREK O. SAID

National Institute of Oceanography and Fisheries
melnaggar66@yahoo.com

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ABSTRACT

Anthracene is a 3-ring aromatic hydrocarbon with high pollution potential and health hazard. The present study aimed to evaluate the degradation of anthracene by free and immobilized cells of *P. fluorescens.* The bacterial strain was isolated from Abu Qir Bay (Egyptian Mediterranean Sea) and identified using API 20 NE kits. The physiological conditions were optimized (pH 7.5, 30^{o}C and 3ml/100ml inoculum's size with 10^{7}cell/ml). The detection for anthracene degradation was performed using GC/FID technique. The free cells showed degradation rates of 2.08, 2.77 and 2.5 µg/l /h when 100, 200 and 300 µg/l anthracene were applied for 48, 72 and 120h, respectively. A trickle flow column bioreactor packed with a sterilized solid supporter (pumous granules; 25-30g) and aerated with 1.0 L/ min was used to perform the anthracene degradation. This bioreactor showed a high performance towards the treatments of aliphatic and PAHs present naturally in different seawater and wastewater environmental samples. The degradation efficiency% for the aliphatic and PAHs ranged from 28.6% to 93.2% depending on the type of the examined water samples; the treatment of the aliphatic and PAHs is more efficient in wastewaters than that in the seawater samples. On the other hand, the bioreactor showed more than 99% efficiency for PAHs treatment in the examined water samples. Moreover, these immobilized cells of *P. fluorescens* showed efficiency% of 97.4% after 48hrs when 200 µg/l anthracene was applied in absence of other PAHs. While with presence of other PHAs as naphthalene and chrysene in the culture medium the efficiency % of the anthracene degradation was 97.8% after 120h of application.

1. INTRODUCTION

Anthracene, together with other polycyclic aromatic hydrocarbons (PAHs), is a persistent and toxic soil contaminant. Pollution by PAHs is usually found on sites of gas factories and wood preservation plants. Bioremediation is an economically and environmentally attractive solution for cleaning those sites. However, in order to achieve an efficient bioremediation process it is important to perform a complete degradation pathway so that potentially toxic metabolites do not accumulate in the environment (Herwijnen *et al.,* 2003).

Anthracene is a tricyclic aromatic hydrocarbon that is found in high concentrations in PAH-contaminated sediments, surface soils, and waste sites. This hydrophobic contaminant is widely distributed in the environment, occurring as natural constituent of fossil fuels (Cerniglia, 1992). Unlike the higher-molecular-weight PAHs (e.g. benzopyrene, dibenzoanthracene and indenopyrene), anthracene does not pose a direct risk to human health, since it exhibits no genotoxic or carcinogenic effects. However, anthracene showed to be a toxic substance for fish and algae in marine environments (Sutherland *et al*., 1992).

Anthracene is considered as a prototypic PAH and can serve as a signature compound to detect PAH contamination, since its chemical structure is found in carcinogenic PAHs, such as benzo[*a*]pyrene and benz[*a*]anthracene (Melcher *et al*., 2002). It has also been used as a model PAH to determine the factors that affect the bioavailability, biodegradation potential, and the rate of microbial degradation of PAHs in the environment (Jacques *et al*., 2005).

Several studies demonstrated the bioremediation of contaminated environments by seeding and introducing of microorganisms in-situ which is considered as a valuable tool for increasing the rate of biodegradation of pollutants (Atlas, 1991; Grosser *et al.,* 1991; Middledorp *et al.,* 1990). However, the degradation by such introduced bacteria was found to be un-established in other contaminated sites compared to the sites from which such degraders were collected, and the reasons for the failures have not been further investigated (Liu *et al.,* 1990). Moreover, Dott *et al.* (1989) mentioned the advantage of seeding is not generally accepted, since inoculation experiments have shown ambiguous results in comparison with the degradation by indigenous microorganisms.

Thus, the present study aimed to investigate the anthracene degradation using free and immobilized cells of a local marine *Pseudomonas* sp., isolated from a highly contaminated area; Abu Qir bay (Egyptian Mediterranean Sea), according to its ability to utilize the anthracene. Also, a biotreatment will be carried out for the major aliphatic and aromatic hydrocarbons present naturally in some waste and sea water samples using a trickle flow column bioreactor packed with the isolated *P. fluorescens*

2. MATERIALS AND METHODS

2.1 Microorganism

Pseudomonas fluorescens was isolated from Abu Qir contaminated sediment by enrichment on anthracene. The isolate has been described as: a gram-negative, rod-shaped, aerobic, motile bacterium. It showed catalase and oxidase-positive, no organic growth factors are necessary required. The production of diffusible and/or insoluble pigments was investigated as a common characteristic of the fluorescent *Pseudomonas.* The identification process was carried out according to Bergey's manual, section-4 "gram negative aerobic rods and cocci" (Sneath *et al.,* 1984) and Steel (1993), in addition to a biochemical test using the API 20 NE identification kits from bioMérrieux that was used for the identification of non-fastidious gram-negative rods not belonging to the *Enterobacteriacea* (Palmieri, *et al*., 1988; Penna, *et al*., 2002).

2.2 Culture conditions

A modified King's B Medium was used as a general medium for routine cultivation of *Pseudomonas*. It composed of (g/100ml): 0.5 peptone; 0.5 tryptone; 0.15 K_2HPO_4; 0.15 $MgSO_4 \cdot 7H_2O$ (Lee *et al*., 2003). The pH value was adjusted at 7.5 and the incubation temperature was 30ºC, 1.5% agar was added to this medium for the maintenance process of the subculture.

Different pH values (5, 6, 7, 8, 9) and different temperatures (4, 25, 30, 35, 40°C) were examined for anthracene degradation using *Pseudomonas fluorescens* according to Sstner *et al.* (1998) and Nievas *et al.* (2005).

2.3 Growth on anthracene

The free cells of *P. fluorescens* were tested for growth on different anthracene concentrations according to Stringfellow and Aitken (1998). A stock solution was prepared

by dissolving 1g of anthracene in 10ml of methylene chloride. Different concentrations (100, 200 and 300 µg/l) of anthracene were added separately and in triplicate to a 250ml solvent-washed sterile bottle. After the methylene chloride had evaporated, 100 ml of the culture medium was added to each bottle, and then the bottles were inoculated using 3ml bacterial suspension with optical density (OD) ≈ 1. Controls were made containing anthracene only as a positive control and methylene chloride only as a negative control. All bottles were sealed and incubated in the dark until complete degradation. The anthracene residues were detected by GC-FID and the degradation rates (µg/l/h) of the added anthracene were calculated. The degraded anthracene concentration was equal the initial concentration – residual concentration/ time, compared to the previous controls (the cell free cultures).

2.4 Estimation of cell growth

The dry weight of the suspended culture was determined in mg/l according to a calibration curve made between the dry weight of the bacterial cell mass and the optical density OD of the broth (turbidity) measured using UV/V-Spectrophotometer at 550nm. The bacterial growth was evaluated and estimated at interval times (0, 24, 48, 72, 96 h) as an increase in the OD relative to the controls. Negative controls exhibited no significant change in the OD.

2.5 Determination of anthracene using Gas Chromatography

2.5.1 Extraction

1000 ml of each collected water samples were extracted three times with 100 ml of dichloromethane in a separating funnel. Sample extracts were combined and concentrated to 5ml using a rotary evaporator under reduced pressure. Finally, samples were concentrated under a gentle stream of pure nitrogen to a volume of 1ml (UNEP/ IOC/ IAEA, 1992).

2.5.2 Clean-up and fractionation process

Clean-up and fractionation was performed prior to gas chromatograph/flame ionization detector (GC/FID). The extracted volume was passed through the silica column prepared by slurry packing with 10g of silica, followed by 10g of alumina and finally 1g of anhydrous sodium sulphate. The aliphatic fraction (F1) was sequentially eluted from the column using 25 ml of hexane. However, the unsaturated aromatic fraction (F2) was eluted with 60ml of hexane and dichloromethane (80:20; V/V). Both of F1 and F2 fractions were concentrated using a gentle stream of pure nitrogen to about 0.2 ml, before being injected into GC/FID (UNEP/ IOC/ IAEA, 1992).

2.5.3 Gas chromatography

All samples were analyzed by a Hewlett Packard 5890 series II GC gas chromatograph equipped with a flame ionization detector (FID). The instrument was operated in split less mode (3µl split less injection) with the injection port maintained at 290°C and the detector maintained at 300°C. Samples were analyzed on a fused silica capillary column HP-1; 100% dimethyl polysiloxane (30 m length, 0.32 mm i.d, 0.17 µm film thickness). The temperature was programmed from 60-290°C, changing at a rate of 3°C /min and maintained at 290°C for 25 min. The carrier gas was nitrogen flowing at 1.2ml/ min (UNEP/ IOC/ IAEA, 1992).

2.5.4 Quantification and mixture preparation

Aliphatic standard mixture (100 µg/ml) brought from MERCK was used for F1 analysis, this standard mixture containing C11,C12, C13, C15, C17, pristane, C18, phytane, C19, C20, C21, C22, C23, C24 and C30. Chlorofluorobenzene (CFB); 20 µg/ml

was used as internal standard for aliphatic fraction. In addition, a stock solution containing the following PAHs was used for quantification of hydrocarbons: naphthalene, acenaphthylene, acenaphthene, fluorene, phenathrene, anthracene, fluoranthene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, pyrene, benzo (a) pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene and indeno(1,2,3-cd) pyrene by dilution to create a series of calibration standards of PAHs at 0.1, 0.25, 0.5, 0.75, 1.0, 2.0, 5.0, and 10 µg/ml. The detection limit was ≈ 0.01 µg/ml for each PAH. For analytical reliability and recovery efficiency of the results, 6 analyses were conducted on PAH reference materials, HS-5 and 2974 (provided by EIMP-IAEA). The laboratory results showed recovery efficiency ranging from 92-111% with coefficient of variation (cv) 8-14% for all studied pollutants (16 PAHs fractions). All solvents were pesticide grade purchased from Merck and appropriate blanks (1000 fold concentrates) were analyzed.

2.6 The application of a trickle flow column bioreactor for hydrocarbons degradation

In this part of work a trickle flow column bioreactor connected with a peristaltic pump was used, it was composed of a glass column (60 cm long, 5 cm internal diameter and a total workable volume of 500 ml) packed with 25-30 g pumous particles; it was connected at the top with an air pump having a flow rate of 1.0 L/min through sterilized air filters. The packed column and its parts were sterilized in an autoclave for 30 min. Then the packed column was inoculated with 15ml of the bacterial suspension, the OD ≈ 1.0 (10^7 cell/ml), and incubated for three days in dark at room temperature till complete adsorption of cells was observed.

2.6.1 Degradation of PAHs naturally present in waste water and seawater samples.

2.6.1.1 Sampling

Four water samples were collected from different sites in Alexandria, Egypt. The first sample (1) was collected from the drainage site of the Extracted Oil Company (EOC). The second water sample (2) was collected from Al-Amya pumping station (where it is situated in Alexandria), where many industrial and agricultural wastes were daily discharged. While the third (3) and the fourth (4) samples which are seawater samples were collected from the Eastern harbor and the Abu Qir harbor of Alexandria, respectively.

2.6.1.2 The degradation process

1000 ml of each examined water sample were sterilized and recycled through the inoculated trickle flow column bioreactor using a peristaltic pump adjusted at 200 ml/h and then incubated in dark at 30°C for 48 h. The aliphatic and the aromatic hydrocarbon residues were extracted separately and estimated at zero and 48 h. The efficiency % of the bioreactor was estimated for the degradation of both the aliphatic and the aromatic hydrocarbon fractions.

2.6.2 Determination of the efficiency% of anthracene degradation in collected samples using the trickle flow column bioreactor

200µg anthracene was added to 1000 ml portion of each collected water sample (1, 2, 3 and 4) under sterile conditions. The inoculated samples were applied separately to the bioreactor and incubated in dark for 48 h at 30°C. The degradation efficiency % was estimated in relation to the original anthracene concentration applied and in comparison with the control (King's B medium).

2.6.3 The degradation of anthracene in absence and in presence of other PAHs by the adsorbed cells of P. fluorescens

The adsorbed cells of *P. fluorescens* in the trickle flow column bioreactor were washed several times using sterile distilled water and inoculated with fresh culture medium (King's B medium). This bioreactor was tested to degrade 200 µg/l anthracene in absence and in presence of a PAHs mixture which was made by adding 100 µg/l of naphthalene and 100 µg/l of chrysene. The degradation rates and the efficiency% of the used bioreactor were estimated as an average for three cycles according to Vadillo-Rodríguez *et al*. (2004).

2.7 Statistical analysis

All experiments were carried out in three replicates for each treatment and arrayed in a completely randomized design. Where all statistical analyses were done by applying ANOVA, F-test, DUNCAN and L.S.D (least significant differences) procedures available with the MSTAT-C software package, Steel and Torrie (1980).

3. RESULTS AND DISCUSSION

The physiological conditions and the medium culture were adjusted to perform more anthracene degradation. It was observed that the degradation was enhanced when no amendment of more carbon sources was performed in the culture medium. This observation was confirmed by Viñas *et al.* (2005) who stated that the moisture content and aeration conditions were the key factors associated with PAH bioremediation and the PAH degraders were more prevalent in samples with low content of nutrient. On the other hand, Quantin *et al.* (2005) found that the presence of the cellulose in the culture medium may lead to stimulate the bacterial respiration process with no effect on PAH dissipation. However, different pH and temperature values were examined to detect their effect on the anthracene degradation process (100 µg/l). It was observed that the optimal pH for *P. fluorescens* is at the neutral range. The maximum degradation rate (2.1 µg/l/h) and cell growth (430 mg/l) were obtained at pH 7.5. These results are in agreement with that observed by Nievas *et al.* (2005). They used three treatments for the anthracene and it was found that the optimum pH ranged from 6.5 to 7.5, and the culture medium should be neutralized to increase the rate of the biodegradation in the treatment unit, Table (1).

On the other hand, the effect of the temperature on the anthracene (100 µg/l) showed a wide workable range (25-35°C), the most suitable temperature was 30°C, where, the degradation rate and the dry weight of *P. fluorescens* were 2.1 µg/l /h and 439 mg/l, respectively, Table (2). This obtained temperature range was also reported by many authors who worked on PAHs degradation using different microbial tools (Kästner *et al.,* 1999; Bugg *et al.,* 2000; Jacques*et al.,* 2005).

However, on studying the anthracene concentration at the isolation site (Abu Qir bay) it was observed that this bay is suffering from high concentrations of different PAHs specially anthracene, its average concentration was about 3.895 µg/l. This result may be the agent which enabled the isolated marine *P. fluorescens* to attack the high concentrations of anthracene investigated in this study. The used microorganism was selected according to its ability to utilize anthracene in the culture medium. The results indicated that the isolated *Pseudomonas fluorescens* was the most potent isolate in degrading anthracene and the abiotic losses can be neglected. The data obtained in Table (3) show that the free cells of *P. fluorescens* were able to utilize 100, 200 and 300 µg/l of anthracene after 48, 72 and 120h of incubation with a maximum degradation rate of 2.08, 2.78 and 2.5 µg/l/h, respectively. Moreover, the estimated bacterial growth showed a dry weight of 402.7, 519.1 and 590 µg/l, respectively. On the other hand, when 400 µg/l anthracene was

applied the free cells of *P. fluorescens* were no longer active towards this concentration, the degradation rate and the bacterial growth dropped to zero level. These results are in a partial agreement with that given by Jacques *et al.* (2005), who tested different *Pseudomonas* species and strains for the anthracene degradation using gas chromatography detector, the results showed that *P. aeruginosa* isolate 312A had the highest degradation rate (3.90 ppm/day), degrading 71% of the anthracene added to the medium (250 mg/l) after 48 days. While *P. citronellolis* 222A and *P. aeruginosa* 332C showed a degradation level of 51% and 24.4%, respectively. Moreover, Herwijnen *et al.,* (2003) and Kästner *et al.* (1994) obtained very low anthracene degradation rates in addition to Kästner *et al.* (1999) showed that 43.8% of [9-^{14}C] anthracene was mineralized by the autochthonous micro-flora in native soil and 45.4% was transformed into bound residues within 176 days.

However, a trickle flow column bioreactor was applied according to Dror and Freeman, (1995) in order to degrade the anthracene as a biological treatment for the contaminated areas. It is based on cell immobilization and simulation of stationary-phase conditions. The combination of an appropriate immobilization technique, operational conditions and medium composition provided a stabilized cell environment resulting in stable performance for several days or weeks. Figure (1) shows the adhesion process of *P. fluorescens* cells on the used inert physical supporter (pumous particles). These particles were examined by a scanning electron microscopy according to a method given by Bisdom (1981). It was observed that these bacterial cells tend to occupy the grooves of the supporter rather than adhering on the surface of these particles and the adhesion of these cells took place in about three days.

Table (1): Effect of different pH Values on anthracene (100 µg/l) degradation by the free cells of *Pseudomonas fluorescens*

pH Value	Degradation time (h)	Dry weight (mg/l)	Degradation rate (µg/l /h)	Residual anthracene conc. (µg/l)
5	72	50.6^{c*}	0.09^{c}	93.52
6	72	125^{c}	0.23^{d}	83.5
7	48	355^{b}	1.87^{ab}	10.2
7.5	45	430^{a}	2.1^{a}	5.4
8	48	339^{b}	1.19^{b}	42.8
9	72	103.6^{c}	0.14^{c}	89.9

*The values with same letter are insignificantly different at P<0.01 while the values with different letters have a significant difference at ($L.S.D_{0.01}$) for the dry weight and the degradation rate are 79.92 and 0.82, respectively.

Table (2): Effect of different temperatures on anthracene (100 µg/l) degradation by the free cells of *Pseudomonas fluorescens.*

Temperature (°C)	Degradation time (h)	Dry weight (mg/l)	Degradation rate (µg/l/h)	Residual anthracene (µg/l)
4	72	50.6^{c}	0.07^{c}	95.3
20	72	253.6^{b}	0.78^{bc}	43.76
25	48	376^{a}	1.19^{b}	43.04
30	45	439^{a}	2.11^{a}	4.7
35	48	380^{a}	1.7^{ab}	16.8
45	72	125^{c}	0.09^{c}	93.52

*The values with same letter are insignificantly different at P<0.01 while the values with different letters have a significant difference at p<0.01, the ($L.S.D_{0.01}$) for the dry weight and the degradation rate are 79.62 and 0.86, respectively.

Table (3): Degradation of different anthracene concentrations (av.± SD) by free cells of a marine *Pseudomonas fluorescens.*

Degradation time (h)	Initial anthracene concentration (mg/l)							
	100		200		300		400	
	Degradation rate (µg/l/h)	cell dry weight (mg/l)	Degradation rate (µg/l/h)	cell dry weight (mg/l)	Degradation rate (µg/l/h)	cell dry weight (mg/l)	Degradation rate (µg/l/h)	cell dry weight (mg/l)
0	0	50.6±6.4*	0	50.1±7.1	0	50.7±6.4	0	50.7±6.4
24	1.5±0.05	270.3±13.6	1.51±0.06	272.2±34.4	0.89±0.02	250.2±14.5	0.09±0.02	50.2±4.5
48	2.08±1.1	402.7±21.5	1.82±0.32	447.3±23.4	1.07±0.01	295.3±25.9	0.07±0.01	45.3±5.9
72			2.78±1.5	519±40.2	1.65±0.03	344.6±37.5	0.05±0.03	44.6±7.5
96					2.05±0.03	440.7±28.3	0.05±0.03	40.7±8.3
120					2.5±0.01	590±22.9	0.02±0.01	34.9±2.9

*The experiments were made in three replicates.

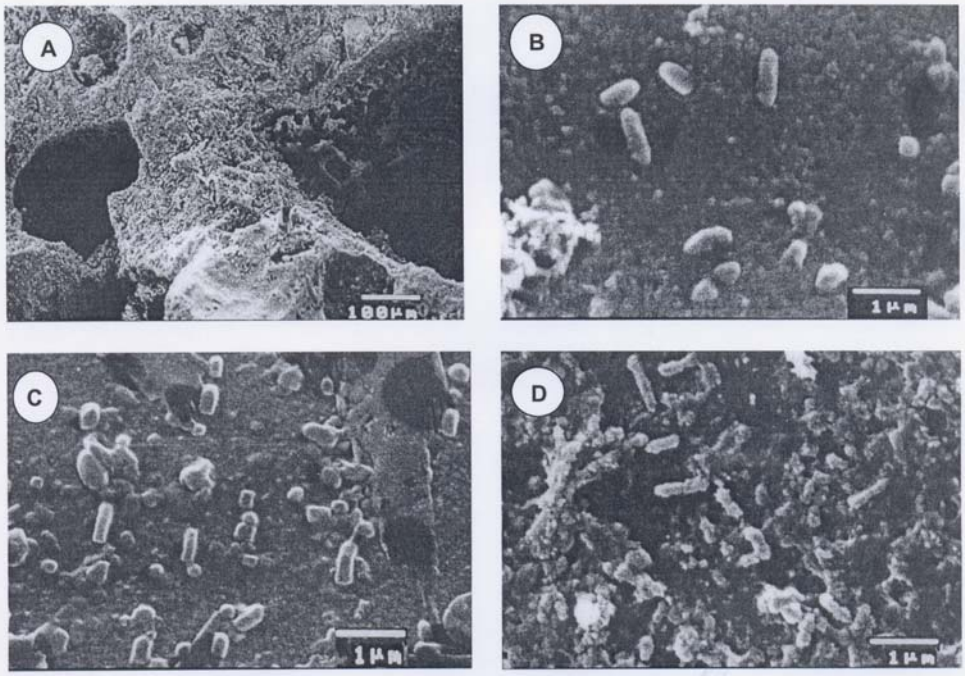

Figure 1. Scanning electron micrographs showing the solid supporter (pumous particles inside the used trickle flow column bioreactor (A). The adhesion of the *Pseudomonas fluorescens* after; 1-day (B), 3-days (C) and 5-days (D).

However, it was noticed that, each collected environmental sample has its own hydrocarbon profile, sample-1 (EOC) characterized by having 14059.99 ng/l as a total aromatic hydrocarbon content and the most dominant fractions were anthracene, and naphthalene with 11683.2 and 2280 ng/l, respectively. While the total aliphatic hydrocarbon content was 16846.73 ng/l and the most dominant fraction was C24 with 14426.78 ng/l. However, the other three samples 2, 3 and 4 were characterized by having a total aromatic hydrocarbon of 1051.1, 2156.1 and 3825 ng/l, respectively, and total aliphatic hydrocarbons of 3815.6, 2564.7 and 3705.6 ng/l, respectively. However, the dominant aromatic fraction in these environmental samples was naphthalene, while the dominant aliphatic fraction varied according to the collection site; they were C22, C23 and C30, respectively. On testing the efficiency% of this bioreactor to degrade such hydrocarbon fractions (which are naturally present in these water samples collected from different contaminated sites in Alexandria) it was observed that there is a great tendency for the cells of *P. fluorescens* towards the degradation of the aromatic hydrocarbons compared to the aliphatic fractions regardless to the collection site, where the efficiency% was always > 99%. On the other hand, the degradation of the aliphatic hydrocarbons in these environmental samples by the adsorbed cells of *P. fluorescens* showed a great variation depending on the collection site, where the efficiency % ranged from 28.9% to 93.22% (Tables 4 and 5).

However, the results in Figure (2) indicated that the immobilization process for these bacterial cells led to a great effect in enhancing the anthracene degradation process. The efficiency% of these cells was 97.6% when 200 µg/l of anthracene was applied for 48h using the King's B medium (the control), while, when comparing the results of the free cells they showed only 44% efficiency in degrading 200 µg/l anthracene after the same time of incubation (Table –3). These results are in a great agreement to a great extent with that of Vadillo-Rodriguez *et al.* (2004) who observed the physical adsorption of the bacterial cells on modified substrata may promote rearrangements in the bacterial cell surface structures and the mechanical trapping of these cells. In addition, they mentioned the use of physical supporters appears to be the most reliable and economic method for immobilization. In addition, in Figure (2) it was noticed that *P. fluorescens* was more efficient for the anthracene degradation when applied in the waste water samples (1 and 2) compared to its application in the seawater samples (3 and 4). The degradation efficiency % of these adsorbed cells showed an average of 70% and 35%. Moreover, the cell growth estimated by detecting the cell leakage from the bioreactor during the degradation process, showed a parallel behavior to the anthracene degradation and it decreased with increasing the residual concentration of the anthracene.

On the trial to use the adsorbed cells of this marine *P. fluorescens* as a tool for degrading the anthracene even with the addition of other PAHs (100 µg/l naphthalene and 100 µg/l chrysene) in the culture medium, it was clearly observed that, this bacterium has a specific and selective action towards the anthracene. The data presented in Figure (3 i and ii) and Table (6) showed the great tendency of the isolated *P. fluorescens* towards the anthracene degradation compared to the other tested PAHs especially naphthalene which is considered as more structurally simple. The efficiency% of these adsorbed cells to degrade 200 µg/l anthracene in the culture medium was 94.7% after 48h of incubation. While on addition of the other PAHs they showed 95.6% after 120h of incubation. This unexpected behavior was also observed by Foght and Westlake (1996) and Bugg *et al.* (2000). They mentioned that there are two conflicting mechanisms for transport PAHs out of the *P. fluorescens* LP6a cells: uptake by passive diffusion and

an energy-driven efflux system. However, this efflux system in *P. fluorescens* LP6a was examined by Hearn *et al*. (2003) and showed to be chromosomally encoded and not only transport nontoxic growth substrates, but it also displayed unusual hydrocarbon substrate selectivity. In addition, they observed that phenanthrene, anthracene, and fluoranthene were exported from the cell while naphthalene was not. Similarly, Moody *et al.* (2003) and Herwijnen *et al*. (2003) found such unexpected behavior in *Mycobacterium vanbaalenii* PYR-1, it was found to be a highly regio- and stereo-selective in degradation of dimethylbenz[*a*]anthracene and anthracene through a *noval* pathway. On the other hand, in this study it was observed that these adsorbed cells of *P. fluorescens* are able to utilize other aromatic hydrocarbons with efficiency matching up to 50% (Figure 3 i and ii).

Table (4): Biodegradation of different aromatic hydrocarbons present naturally in wastewater and seawater samples by adsorbed cells of *P. fluorescens* using a trickle flow column bioreactor.

Hydrocarbon Fraction	Concentration in ng/l							
	1*		2*		3**		4**	
	zero –h[a]	48h[b]	zero –h	48h	zero -h	48h	zero -h	48h
Naphthalene	2280	<DL	3796	<DL	2498.3	<DL	3678.3	<DL
Acenaphthylene	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Acenaphthene	<DL	<DL	<DL	1.656	<DL	<DL	0.304	<DL
Fluorene	<DL	<DL	<DL	<DL	<DL	<DL	4.14	<DL
Phenanthrene	17.35	<DL	<DL	5.712	16.164	<DL	2.184	1.112
Anthracene	11683.2	<DL	0.491	0.828	<DL	<DL	0.714	<DL
Fluoranthene	24.987	0.308	4.26	<DL	<DL	<DL	0.366	<DL
Pyrene	0.455	<DL	1.589	2.88	4.104	<DL	1.932	<DL
BaA	12.67	<DL	0.887	<DL	<DL	<DL	<DL	<DL
Chrysene	3.93	<DL	<DL	<DL	<DL	<DL	10.81	<DL
BbF	19.87	<DL	<DL	<DL	<DL	<DL	0.356	<DL
BkF	2.725	<DL	<DL	<DL	10.961	<DL	<DL	<DL
BaP	2.166	<DL	0.232	<DL	32.5	<DL	5.054	2.208
Dibenzo(a,h)anthracene	0.747	0.159	4.104	<DL	<DL	<DL	0.639	0.47
Benzo(ghi)Perylene	9.71	<DL	<DL	<DL	0.198	<DL	0.281	<DL
Indeno(1,2,3-cd)Pyrene	2.218	<DL	8.028	<DL	2.436	<DL	0.526	<DL
Total PAHs	14059.987	0.467	3815.59	11.076	2564.66	<DL	3705.61	3.79
Efficiency%	99.9		99.71		100		99.9	

<DL: below the detection limit, *1 and 2: waste water samples collected from EOC and El-Amya pumping stations, respectively ** 3 and 4: seawater samples collected from the Eastern Harbor and Abu Qir Harbor, respectively. [a] mean before treatment and [b] mean after 48h of treatment.

Table (5): Biodegradation of different aliphatic hydrocarbons present naturally in wastewater and seawater samples by adsorbed cells of *Pseudomonas florescence* using a trickle flow column bioreactor.

Hydrocarbon fraction	Concentration in ng/l							
	1*		2*		3**		4**	
	Zero-h[a]	48h[b]	zero-h	48h	zero-h	48h	Zero-h	48h
C11	<DL	<DL	<DL	<DL	86.1	43.17	<DL	<DL
C12	<DL	<DL	<DL	<DL	26.71	<DL	<DL	<DL
C13	10.24	<DL	<DL	<DL	<DL	16.944	13.224	12.58
C15	154.1	16.48	9.456	<DL	<DL	<DL	<DL	<DL
C17	<DL	<DL	<DL	<DL	<DL	<DL	10.99	9.694
Pristine	70.11	5.42	<DL	<DL	205.57	137.44	<DL	<DL
C18	<DL	<DL	<DL	<DL	<DL	<DL	17.66	<DL
Phytane	217.18	<DL	43.416	37.57	<DL	<DL	<DL	<DL
C19	10.55	<DL	<DL	<DL	<DL	<DL	<DL	<DL
C20	452.58	<DL	43.43	<DL	15.3	7.089	<DL	<DL
C21	46.29	<DL	<DL	<DL	<DL	<DL	620.213	118,63
C22	1458.9	53.23	982.176	339.32	264.82	207.45	133.692	118.26
C23	<DL	<DL	<DL	<DL	1614.98	1020.03	70.09	<DL
C24	14426.78	11903	571.11	23.58	<DL	<DL	<DL	<DL
C30	<DL	<DL	50.21	<DL	<DL	<DL	3029.2	<DL
Total	16846.73	11978.13	1051.096	400.47	2156.11	1498.9	3824.98	259.164
Efficiency %	28.9		76.44		30.9		93.22	

<DL: below the detection limit, *1 and 2: waste water samples collected from EOC and El-Amya pumping stations, respectively ** 3 and 4: seawater samples collected from the Eastern Harbor and Abu Qir Harbor, respectively. [a] mean before treatment and [b] mean after 48h of treatment.

Table (6): Degradation of anthracene* in absence and in presence of other PAHs by the adsorbed cells of *P. flourescens* using a trickle flow column bioreactor.

In absence of other PAHs			In presence of other PAHs		
Degradation time (h)	Concentration residue (µg/l)	Efficiency %	Degradation time (h)	Concentration residue (µg/l)	Efficiency %
0	200±12..8	0	0	200±10.5	0
4	183.2±21.7	8.5±1.91	36	187.6±26.4	6.2±5.3
8	163.2±18.3	18.4±2.7	48	163.2±18.7	18.4±8.4
12	134±23.9	33±3.4	72	110.8±19.3	44.6±9.1
18	85.6±27.1	57.2±9.6	96	38±10.7	81±3.3
24	58.9±15.9	70.6±7.3	120	8.8±2.8	95.6±0.9
36	40.4±11.6	79.8±4.9			
48	10.6±4.2	94.7±2.4			

*The applied concentration was 200 µg/l and the experiments were made for three cycles.

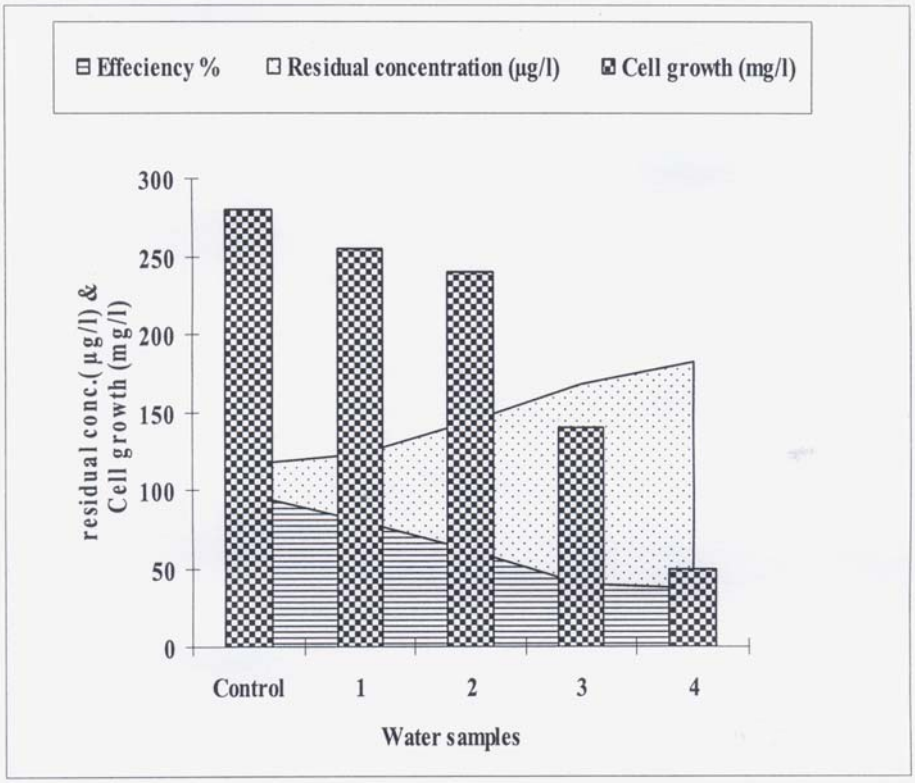

Fig. (2): The efficiency% for 200 µg/l anthracene degradation by the adsorbed cells of *P. fluorescens* using a trickle flow column bioreactor for 48hrs of incubation in waste water (1 and 2) and in seawater (3 and 4) samples compared to the control (King's B medium).

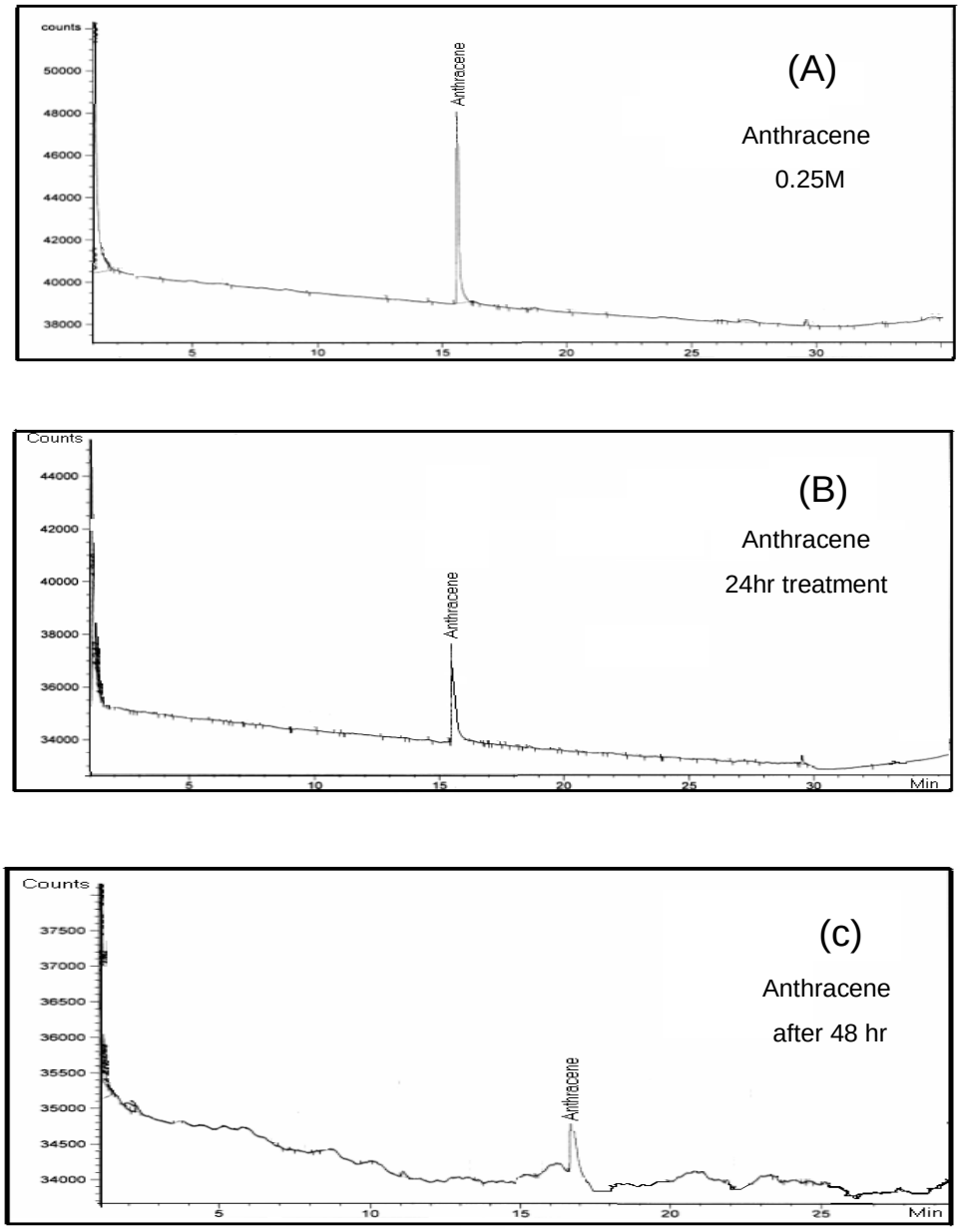

Figure (3 i). GC-chromatograms showing the anthracene degradation by the adsorbed cells of *P. fluorescens* on the puomus particles using a trickle flow column bioreactor in absence of other PAHs (A,B and C).

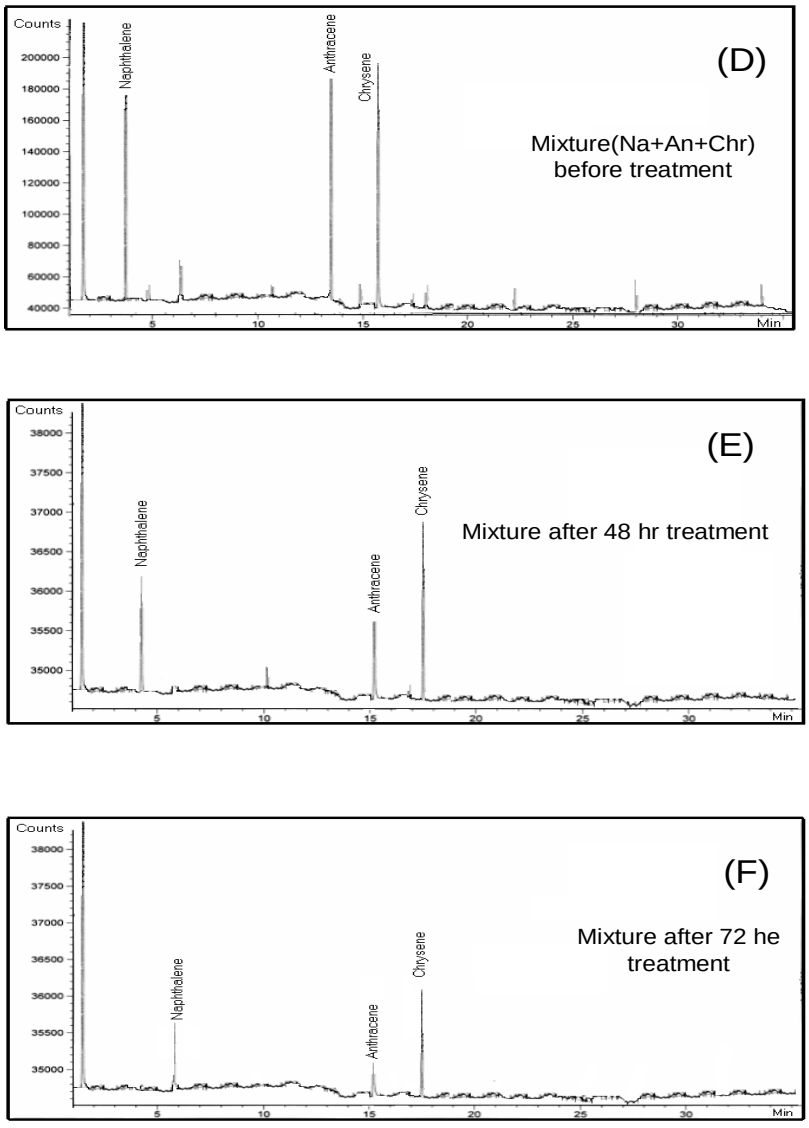

Figure (3 ii). GC-chromatograms showing the anthracene degradation by the adsorbed cells of *P. fluorescens* on the puomus particles using a trickle flow column bioreactor in presence of PAHs mixture (D,E and F).

4. CONCLUSION

It is suggested that this trickle flow column bioreactor may be useful in the treatment of anthracene residues in the highly contaminated environments especially in the wastewater treatment. In addition, it is clear to state that, there are complex interactions between bacterial species and medium conditions that influence the biodegradation capacity of *P. fluorescens*.

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