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Selection and Characterization of Bacteria Isolated from Petroleum Refining Effluents with Polycyclic Aromatic Hydrocarbons (PAHs) Removal Capacities and its Conditions

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IN THIS STUDY, we evaluated the effect of different nutritional and environmental conditions on the percentages removal of PAHs present in petroleum refining effluent samples from the corrugated plate interceptor (API) of Cairo Oil Refining Co. (CORC), Egypt. Two hundred twelve and two hundred forty two bacterial strains were isolated from six petroleum refinery oil polluted effluents samples collected from CORC in summer and winter seasons, respectively which were selected due to their growing capacity in the presence of oil as sole carbon source. From these strains four bacterial strains were further selected on the basis of their relatively good growing on hydrocarbon utilizing media, culture characteristics and capacity to biodegrade PAHs. These bacterial isolates were identified on the basis of cell shape, cell arrangement, relation to oxygen and nutritional and biochemical characteristics. The four bacterial strains were found to belong to *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22, *Pseudomonas stutzeri*-S8API12 and *Enterobacter aerogenes*-W5OA31.They were capable to grow on the mineral salts media amended with crude oil as sole carbon source. Our results show that these strains can remove the PAHs by different percentages (%) at different pH values (4-9), NaCl concentrations (1-10%) and different nitrogenous and phosphorous sources. In conclusion, current sequence information provides the basis for a robust tool to estimate the PAHs degradation potential of different petroleum hydrocarbon contaminated sites undergoing *in situ* bioremediation.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous persistent environmental contaminants generated by natural combustion processes and human petroleum activities. They are considered hazardous because of their cytotoxic, mutagenic and carcinogenic effects. The fates of these compounds in the environment and the remediation of PAH-contaminated sites are, therefore, of high public interest (Haeseler *et al.*, 1999 and Yun, *et al.*, 2008). Their environmental importance led the US-Environmental Protection Agency (US-EPA) to identify 16 unsubstituted PAHs as priority pollutants, which must be

monitored in terrestrial and aquatic ecosystems (Keith & Telliard, 1979), 8 of which are possible human carcinogens (Menzi *et al*., 1992). The potential use of microorganisms to clean up contaminated soil, sediments and water can provide efficient, inexpensive and environmentally safe cleanup of the waste material (Bishop, 1998). Bioremediation, a technology that utilizes microorganisms to reduce environmental contaminants, has become a popular and effective remediation technique (Atlas & Bartha, 1992; Bossert & Bartha, 1986; Carmichael & Pfaender, 1997; Pradhan *et al*., 1997 and Wilson & Jones, 1993). The principle processes for their successful removal are currently believed to be microbial transformation and degradation (Abbondanzi *et al*., 2004 and Gibson *et al*., 1975). Biodegradation potentials of microbial strains isolated from hydrocarbon-contaminated environments are as high as, or even higher than those originating from non-contaminated environments since certain bacteria could have acclimatized and become adapted to the contaminated environment (Chaneau *et al*., 1999; Wild & Jones, 1986 and Zhang *et al*., 2006). A large number of consortia or pure bacterial strains with PAH-degrading ability have been isolated from diverse environments, including polluted soils, sediments and water bodies (Chung & King, 2001; Guo *et al*., 2005; Jeon *et al*., 2003; Juhasz & Naidu, 2000 and Kanaly *et al*., 2000). As well, Bioaugmentation, the addition of microorganisms with known metabolic capabilities to contaminated materials, has been used as a remediation technology to enhance the degradation rates in recently contaminated soils and sediments (Juhasz *et al*., 2000 and Venosa *et al*., 1996). The rate and extent of biodegradation is affected by physical, chemical and biological factors of the toxic organic contaminants (Leahy & Colwell, 1990). Nutrients especially of nitrogen and phosphorus are of the most important factors on biodegradation. They appear to be the most common limiting ones (Pritchard *et al*., 1992 and Rosenberg *et al*., 1992). Laboratory and field experiments with inorganic nitrogen and phosphate fertilizers have shown success (Boufadel *et al*., 1999; Harrison *et al*., 2000; Lee *et al*., 1993; Lee *et al*., 1995; Mishra *et al*., 2001; Mulligan *et al*., 2001; Rhykerd *et al*., 1999; Vasudavan & Rajaram, 2001 and Venosa *et al*., 1996), and several optimal ratios were recommended for bioremediation of PAHs in contaminated sites (Bouchez *et al*., 1995 and Wilson & Jones, 1993). As well, Temperature plays a significant role in controlling the nature and extent of microbial hydrocarbon metabolism (Mohn & Stewart, 2000 and Nedwell, 1999). It affects the rate of biodegradation, as well as, the physical and chemical nature of hydrocarbons (Rowland *et al*., 2000). Although microbial activity is generally reduced at low temperature, many of the components in crude oil and diesel can actually be degraded by psychrophilic and psychtrophic microorganisms (Baraniecki *et al*., 2002; Eckford *et al*., 2002 and Gibb *et al*., 2001). The bioavailability of soluble hydrophilic substances, such as aliphatic and polyaromatic hydrocarbons, is temperature dependent. A temperature increase leads to an increase in diffusion rates of organic compounds notably by a decrease of their viscosity (Northcott & Jones, 2000). Thus, higher molecular reaction rates due to smaller boundary layers are expected at elevated temperatures. In counterpart, the increased volatilization and solubility of some hydrocarbons at elevated temperature may enhance their toxicity and therefore may delay the onset of degradation. The

effect of one factor is often influenced by the presence of other factors. For instance, temperature affects the PAH bioavailbility (Northcott & Jones, 2000) and hence affects the activity of microorganisms (Eckford *et al*., 2002). Other factors such as salinity and pH (Kerr & Capone, 1988 and Zhu *et al*., 2001) play important roles on bioremediation. The pH of seawater is generally stable and slightly alkaline. In contrast, the pH of freshwater and soil environments can vary widely. Most heterotrophic bacteria and fungi favor a neutral pH. Studies have shown that degradation of oil increases with increasing pH, and that optimum degradation occurs under slightly alkaline conditions (Zhu *et al*., 2001). Changes in salinity may affect oil biodegradation through alteration of the microbial population. Dramatic variation in salinity may occur in estuarine environments where marine organisms mingle with freshwater forms (Zhu *et al*., 2001). However, reports on interactions among different factors are still limited and the optimal combination of different factors for an efficient bioremediation of PAHs in a specific environment is often not understood.

The purposes of this work were to : (I) Collect a number of industrial refinery oil polluted effluent samples from Cairo Oil Refining Co. (CORC), Egypt, (ii) Identify the four most potent bacterial isolates present in the effluent samples to species level, (iii) Investigate the biodegradation activity of each strain by detailed chemical analysis of PAHs residues after cultivation on petroleum-based media, and (iv) Investigate the optimal nutritional and environmental conditions (pH, salinity and temperature) for PAHs biodegradation by the four bacterial strains.

Materials and Methods

Sampling

The CORC under study is located in Cairo, El-Esmaelia Drainage, Egypt, in a petroleum activity site. The study was carried out in two seasons (Summer and Winter) during the period between 2005 and 2006. Representative six crude oil industrial effluent samples were collected every season from different locations viz. outlet area (OA), skim basin inlet (SBI), skim basin outlet (SBO), a corrugated plate interceptor (API), dissolved air floatation inlet (DAFI) and DAF outlet (DAFO), respectively, in sterile dark bottles and transported to laboratory at the same time for microbiological studie (Fig. 1).

Isolation of bacterial strains

Six crude oil industrial effluent samples every season were counted for isolation of crude oil degrading microbial isolates on two types of media: Medium A was prepared according to (Toledo *et al.*, 2006) contained in (g/l): $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.02; KH_2PO4, 1; K_2HPO_4, 1; $NH_4NO_3.6H_2O$, 1 and $FeCl_3$, 0.05. And medium B was prepared according to (Belhaj *et al*., 2002) contained, in (g/l) KH_2PO_4, 0.68; Na_2HPO_4, 1.70; $MgSO_4 \cdot 7H_2O$, 0.35; NH_4NO_3, 1; $CaCl_2 \cdot 2H_2O$, 0.02; $FeSO_4$, 0.04. The media were supplemented with a solution (0.01% final concentration) of trace elements containing, (g/l): $CoCl_2.6H_2O$, 0.1;

$MnCl_2.4H_2O$, 0.425; $ZnCl_2$, 0.05; $CuSO_4$. $5H_2O$, 0.05, $NiCl.6H_2O$, 0.01; Na_2MoO_4. $2H_2O$, 0.01; $Na_2SeO_4.2H_2O$, 0.01 (Shuttleworth & Cerniglia,1996). Solid media contained 15 g/l agar. Each of the previously mentioned media was supplemented with 0.2% (v/v) crude oil used by CORC as only source of carbon and energy.

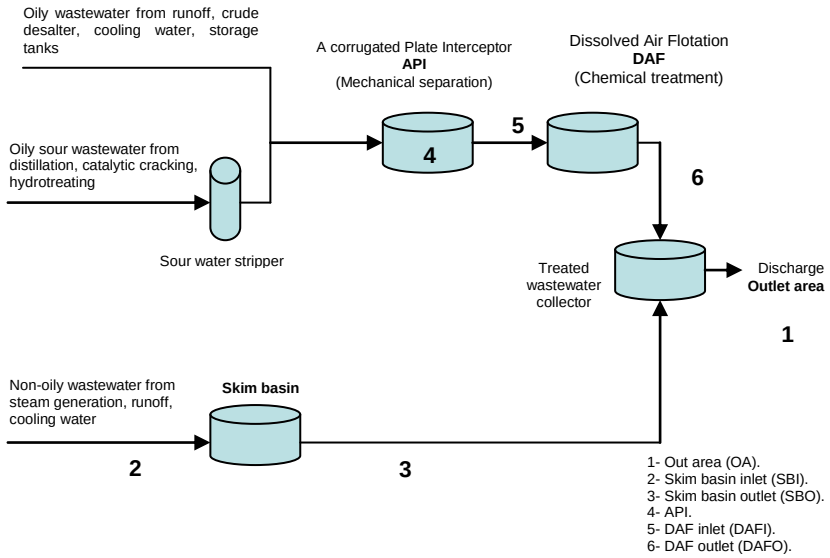

Fig.1. Design of Cairo Oil Refining Co. Industrial effluent plant and sites of industrial effluents sampling localities collection.

One ml from each crude oil industrial effluent sample was spread onto the crude oil utilizing media plates and used for the whole crude oil utilizing microbiological counts. The crude oil utilizing two media plates (three replicates) were incubated for 7 days at 30ºC. The number of viable aerobic heterotrophic microorganisms in each crude oil industrial effluent sample was determined by naked eye using the spread plate technique on the previously two types of media viz. A and B. After inoculation, the plates were kept at 30ºC for 7 days for total population of heterotrophic utilizing crude oil. At the end of incubation, individual colonies were picked out and streaked on solid medium B for conservation, selection and further identification.

A first screening of bacterial strains was done after Gram staining and microscopic examination to eliminate apparently similar strains. This resulted in the collection of nineteen strains. All bacterial strains were submitted to a preliminary test for utilization of hydrocarbons. Each isolate was cultured in 100ml liquid medium B to which 0.2 % crude oil was supplemented as a sole source of carbon. The flasks were incubated for seven days at 30ºC. The strains

that caused visible turbidity were potentially able to use HC as a carbon and energy source. At the end of preliminary test, the number of presumably active strains was eight isolates. A second morphological screening for the elimination of identical isolates reduced this number to the four most potent bacterial isolates which were identified and submitted to quantitative PAHs biodegradation.

Determination of PAHs biodegradation activity

The determination of the biodegradation potential was made according to PAHs removal percentages. Each active strain was grown in duplicate in 250 ml Erlenmeyer flasks containing 100 ml of mineral salts medium (Medium B) supplemented with crude oil and incubated in an orbital shaker at 150 rpm. Sterile controls were used to quantify the abiotic losses due especially to evaporation. The volatilization of the light compounds during steam sterilization and incubation lead to an abiotic loss from the initial oil content. All results of PAHs biodegradation were obtained in reference to the sterile controls. At the end of incubation time of seven days at 30ºC, the residual hydrocarbons were extracted by carbon tetrachloride. After solvent evaporation, the PAHs were determined as ppm by high performance liquid chromatography (HPLC).

Bacterial identification

Polycyclic aromatic hydrocarbons degrading bacterial isolates were characterized based on colony morphology, culture characteristics, pigmentation, Gram staining, biochemical, and physiological testes and identified according to Bergey's Manual of Systematic and Determinative Bacteriology (Holt *et al.*, 1994 and Sneath, 1986).

Growth and maintenance medium

The selected polycyclic aromatic hydrocarbons degrading bacterial isolates were grown in sterile broth medium contained, in (g/l): beef extract 3.0; NaCl 5.0; peptone 5.0 and finally incubated at 30°C for 24 hr.

Parameters controlling the PAHs biodegradation

pH values

This experiment was carried out to determine the optimum pH value for PAHs biodegradation by most potent bacterial isolates. The pH of CORC DAF inlet (DAFI) industrial effluent wastewater was adjusted at different pH values viz. 4, 5, 6, 7, 8 & 9 by using 1N NaOH and HCl. Inoculation and incubation conditions were carried for 5 days in an orbital shaker at 150 rpm and 30°C. At the end of incubation period, the total oils were extracted and analyzed by HPLC.

Sodium chloride concentrations

Different sodium chloride concentrations viz. (g/l %) 1, 2.5, 5, 7.5 &10%, were supplemented with CORC DAF inlet (DAFI) industrial effluent wastewater and inoculated by the most potent PAHs biodegrading bacterial isolates. All other optimal conditions were taken into consideration. At the end of incubation periods, the PAHs in each sample were determined.

Nitrogen and phosphorous sources

This experiment was carried out to investigate the effect of different inorganic nitrogen and phosphorous sources viz. urea, NH_4Cl, K_2HPO_4& $(NH_4)_2HPO_4$ on PAHs biodegrading bacterial isolates. The nitrogen and phosphorous sources were added to the industrial effluent broth and distributed 100 ml per each flask. The conical flasks were inoculated with the bacterial isolates and then incubated at 30°C; the optimum temperature for one week on an orbital shaker operating at 150 rpm. The optimum nitrogen and phosphorous source for each isolate was also determined.

High performance liquid chromatographic analysis

PAHs samples were analyzed using HPLC model Waters 600E equipped with auto-sampler Waters 717 plus and dual wavelength absorbance detector Waters 2487 set 254 nm in Egyptian Petroleum Research Institute (EPRI). The condition of operation is as follows (Lal & Khanna, 1996). Column: supelcosil LC-PAH, 15 cm × 4.6 mm ID,1 µm particles size. Mobile phase: Acetonitrile: Water HPLC grades, gradient from 50:50 to 100% acetonitrile. Flow rate: Gradient program, 0-2 min, 0-2 ml/min.2-45 min 1.0 ml/min.

Results and Discussion

Isolation of hydrocarbon utilizing bacterial isolates from Cairo Oil Refining Company (CORC) industrial effluents in Summer and Winter seasons

Data recorded in Table 1, showed that, different bacterial hydrocarbon (crude oil) utilizing bacterial isolates were obtained from the six water samples collected from outlet area (OA), skim basin inlet (SBI), skim basin outlet (SBO), acorrugated plate interceptor (API), dissolved air floatation inlet (DAFI) and DAF outlet (DAFO) localities, in summer season from Cairo Oil Refining Company (CORC) industrial effluent treatment plant (Fig. 1) on medium A. As well different hydrocarbon (crude oil) utilizing fungal isolates were isolated from OA, SBI, SBO, API and DAFI in the same season (Fig. 1). Also hydrocarbon (crude oil) utilizing bacterial isolates were obtained from the six water samples collected from the previously mentioned localities in summer season on medium B. As well, several hydrocarbon (crude oil) utilizing fungal isolates were isolated. The results revealed that medium B can be selected as the best medium for growth of the hydrocarbon (crude oil) utilizing microbial isolates.

In Winter season medium B which gave the best results was applied only and showed that several hydrocarbon (crude oil) utilizing bacterial isolates were obtained from the six mentioned effluents. However, no hydrocarbon (crude oil) utilizing fungal colonies were isolated.

The first survey was carried out in Summer and Winter seasons and media for growth for the selection of the most potent hydrocarbon utilizing microbial isolates. Summer season gave four bacterial isolates viz. S1AO12, S4DAFI21, S7DAFI22 & S8API12 as well, Winter season gave also four bacterial isolates viz. W5AO31, W7DAFO22, W8API31 & W10DAFO23.

TABLE 1. Microbial growth of the six industrial effluents on two types of medium in Summer and Winter seasons, respectively.

Collection season	Sample no.	Area code no.	Medium A		Medium B	
			Bacterial growth average ($\overline{X}$)	Fungal growth average ($\overline{X}$)	Bacterial growth average ($\overline{X}$)	Fungal growth average ($\overline{X}$)
Summer season	1	OA	73	7	26	2
	2	SBI	50	3	38	7
	3	SBO	29	2	74	4
	4	API	15	2	29	3
	5	DAFI	38	1	23	1
	6	DAFO	6	0	52	1
Winter season	1	OA			86	0
	2	SBI			25	0
	3	SBO			28	0
	4	APII			56	0
	5	DAFI			30	Large colonies
	6	DAFO			32	Large colonies

OA: Out area
SBI: Skim basin inlet
SBO: Skim basin outlet
API: API
DAFI: DAF inlet
DAFO: DAF outlet

These eight selected isolates were applied for the biodegradation of the ∑16 US-EPA listed Poly Aromatic Hydrocarbons (PAHs) using 2 ml of CORC crude oil.

The concentrations of the 16 US-EPA listed PAHs were investigated during 5 days of incubation periods. The 16 PAHs were grouped as two-and three rings PAHs (naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene and anthracene), four rings PAHs (flouranthene, pyrene, benzo (a) anthracene and chrysene), five rings PAHs (benzo (b) flouranthene, benzo (k) flouranthene, benzo (a) pyrene and dibenzo (a,h) anthracene) and six rings PAHs (benzo (g,h,i) perylene and indeno (1, 2, 3-cd) pyrene, and thus also defined as small, medium and large.

Isolation, characterization and physiological behaviour of PAHs degrading bacterial isolates

The four most potent PAHs degrading bacterial isolates were identified as Gram negative and have the following cell morphology (Table 2).

TABLE 2. Morphological and biochemical characteristics of the four PAHs biodegrading bacterial isolates from CORC plant.

Character	Culture Code No.			
	W5OA31	**W7DAFO22**	**S7DAFI22**	**S8API12**
Cell shape	Short rod	Short rod	Short rod	Short rod
Gram reaction	-	-	-	-
KOH (3%) reaction	+	+	+	+
Motility	+	+	+	-
Oxidase	-	-	+	+
Catalase	+	+	+	+
Anaerobiosis	+	+	+	+
Carbohydrate fermentation:				
Cellulose	-	A	-	A
D(-) Mannitol	AG	A	AG	A
D(-) Xylose	AG	A	AG	A
Dextrin	AG	A	AG	-
Galactose	AG	A	AG	A
Glucose	AG	A	AG	A
L(-)Rhamnose	AG	A	AG	-
L(+) Arabinose	AG	A	AG	A
Lactose	AG	A	AG	A
Maltose	AG	A	AG	A
Sorbitol	AG	A	AG	-
Starch	A	-	-	-
Sucrose	AG	A	AG	A
Xylan	-	A	AG	A
Hydrogen sulfide H_2S production	-	+	-	+
Nitrate reduction	+	+	+	+
Nitrite reduction	+	+	-	-
Citrate utilization	+	+	+	+
Enzyme production:				
Amylase	-	-	-	+
Lipase	+	-	-	+
Pictinase	-	-	-	+
Protease	-	-	-	-
Urease	+	+	+	+
Aesculin hydrolysis	+	+	+	+
Indole production	-	+	-	+
Potassium cyanide test (KCN)	+	+	+	+
Growth on :				
Blood agar	+	+	+	+
Blood heamolysis	γ	γ	γ	α
Nutrient agar	+	+	+	+
Mac Conkey agar	+	+	+	+
Oil/Agar medium	+	+	+	+

(+): Positive (-): Negative (α): Alfa heamolysis (γ): Gama heamolysis

(A): Acid production (AG): Acid & gas production

General characteristics

All selected bacterial isolates were Gram negative, aerobic and facultative anaerobic bacteria. Reaction with potassium hydroxide (3%) was positive. All of them were protease negative and urease positive giving orange colour. As well, all of them grow on blood agar, MacConkey agar and oil/agar media. All of them reduced nitrate, utilized citrate and positive in aesculin test. These selected bacterial isolates correspond to section (4): Gram-negative aerobic rods & cocci, tables (4.1, 4.2, 4.7, 4.8, 4.9, 4.15 & 4.16) pages (140, 163, 165, 166, 167, 172 & 173) and section (5): Facultatively anaerobic Gram-negative rods, tables (5.1 & 5.2) pages (408 & 414) in Bergey's Manual of Systematic Bacteriology (Krieg *et al*., 1984). Characterization of these isolates were confirmed by Bergey's Manual of Determinative Bacteriology (Holt, 1994), group (4): Gram-negative aerobic/Microaerophilic rods & cocci, subgroup (4A) page (93), table (4.1) pages (103-116) and group (5): Facultative anaerobic Gram-negative rods, subgroup (1) Family: Enterobacteriaceae page (178), table (5.1) pages (202-222).

Specific characteristics

Isolate W5OA31 : Gram-negative, short rod cell shape, positive reaction with KOH (3%), strongly motile, grow under aerobic and facultative anaerobic conditions. The organism is oxidase negative and catalase positive. It ferments sucrose, sorbitol, mannitol, lactose, maltose, glucose, xylose and arabinose with evolution of gas, while can't ferment xylan and cellulose. The organism does not produce H_2S. Nitrate was reduced by complete reduction as well as nitrite. Citrate was utilized as well as aesculin. The organism produces lipase and urease, and not amylase, pictinase and protease. Indol production was negative and KCN test was positive. Also, it has the ability to grow on blood agar with gamma (γ) heamolysis, Mac Conkey agar and oil/agar media. In view of the previously recorded results and by the aid of Bergey's Manual of Systematic Bacteriology (Krieg, 1984) (Volume 1), section (5): Facultatively anaerobic Gram-negative rods, tables (5.1 & 5.2) pages (408 & 414), this isolate can be considered as being belonging to *Enterobacter aerogenes*, thus it can be given the tentative name *Enterobacter aerogenes-W5OA31*. Characterization of this isolate was confirmed by Bergey's Manual of Determinative Bacteriology (Holt, 1994), group (5): Facultative anaerobic Gram-negative rods, subgroup (1) Family: Enterobacteriaceae page (178), table (5.1) pages (202-222).

Isolate W7DAFO22 : Gram-negative, short rod cell shape, positive reaction with KOH (3%), very weak motile, grow under aerobic and facultative anaerobic conditions. The organism is oxidase negative and catalase negative. It ferments sucrose, sorbitol, mannitol, lactose, maltose, xylan, cellulose, glucose, xylose and arabinose without evolution of gas. The organism strongly produces H_2S; completely reduce nitrate as well as nitrite. It utilizes citrate as well as aesculin. It produces urease and lipase, and not produces amylase, pictinase and protease. Indol production and KCN test were positive. Also, it has the ability to grow on blood agar with gamma (γ) heamolysis, Mac Conkey agar and oil/agar media. In view of the previously recorded results and by the aid of Bergey's Manual of

Systematic Bacteriology (Krieg, 1984) (Volume 1), section (4): Gram-negative aerobic rods & cocci, tables (4.1, 4.2, 4.7, 4.8, 4.9, 4.15 & 4.16) pages (140, 163, 165, 166, 167, 172 & 173), this isolate can be considered as being belonging to *Ps. oleovorans,* thus it could be given the tentative name *Ps. oleovorans – W7DAFO22*. Characterization of this isolate was confirmed by Bergey's Manual of Determinative Bacteriology (Holt, 1994), group (4): Gram-negative aerobic/Microaerophilic rods & cocci, subgroup (4A) page (93), table (4.1) pages (103-116).

Isolate S7DAFI22 : Gram-negative, short rod cell shape, positive reaction with KOH (3%), motile, grow under aerobic and facultative anaerobic conditions. The organism was oxidase positive and catalase positive. It ferments sucrose, sorbitol, mannitol, lactose, maltose, xylan, glucose, xylose and arabinose with evolution of gas, and not ferments cellulose. The organism does not produce H_2S and reduces nitrate to complete reduction, while nitrite does not. Citrate as well as aesculin was utilized. The organism produces urease, and not lipase, amylase, pictinase and protease. Indol production is negative and KCN test is positive. Also, it has the ability to grow on blood agar with gamma (γ) heamolysis, Mac Conkey agar and oil/agar media.

In view of the previously recorded results and by the aid of Bergey's Manual of Systematic Bacteriology (Krieg, 1984) (Volume 1), section (5): Facultatively anaerobic Gram-negative rods, tables (5.1 & 5.2) pages (408 & 414), this isolate can be considered as being belonging to *Enterobacter cloacae,* thus it could be given the tentative name *Enterobacter cloacae–S7DAFI22*. Characterization of this isolate was confirmed by Bergey's Manual of Determinative Bacteriology (Holt, 1994), group (5): Facultative anaerobic Gram-negative rods, subgroup (1) Family: Enterobacteriaceae page (178), table (5.1) pages (202-222).

Isolate S8API12 : Gram-negative, short rod cell shape, positive reaction with KOH (3%), non motile, grow under aerobic and facultative anaerobic conditions. The organism is oxidase positive and catalase positive. It ferments sucrose, cellulose, mannitol, lactose, maltose, xylan, glucose, xylose and arabinose without evolution of gas, and not ferments sorbitol. It produces H_2S. Nitrate was reduced completely, while nitrite was not reduced. Citrate is utilized as well as aesculin. The organism produces urease, amylase, pictinase and lipase, and not protease. Indol production is positive and KCN test is also positive. The organism has the ability to grow on blood agar with Alfa (α) heamolysis, Mac Conkey agar and oil/agar media. In view of the previously recorded results and by the aid of Bergey's Manual of Systematic Bacteriology (Krieg, 1984) (Volume 1), section (4): Gram-negative aerobic rods & cocci, tables (4.1, 4.2, 4.7, 4.8, 4.9, 4.15 & 4.16) pages (140, 163, 165, 166, 167, 172 & 173), this isolate suggestively belongs to *Pseudomonas stutzeri,* thus it could be given the tentative name *Pseudomonas stutzeri –S8APII12*. Characterization of this isolate was confirmed by Bergey's Manual of Determinative Bacteriology (Holt, 1994), group (4): Gram-negative aerobic/Microaerophilic rods & cocci, subgroup (4A) page (93), table (4.1) pages (103-116).

The PAHs-degrading bacteria isolated in this study were identified as *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22, *Pseudomonas stutzeri*-S8API12 and *Enterobacter aerogenes*-W5OA31 on the basis of Gram reaction, relation to oxygen, nutritional requirements, physiological and biochemical tests. There are many reports on bacterial biodegradation of PAHs mainly by bacteria as those our four bacterial isolates selected as the most potent. They were reported to utilize PAHs when supplemented with crude oil, but failed to utilize them as the sole source of carbon and energy (Foght & Westlake, 1988). In contrast, we demonstrated that utilization of PAHs as the sole source of carbon and energy by our *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22, *Pseudomonas stutzeri*-S8API12 and *Enterobacter aerogenes*-W5OA31 strains is possible, perhaps owing to their ability to produce biosurfactants in the culture medium. We have provided further evidence of utilization of PAHs as the sole source of carbon by demonstrating the correlation between increase in bacterial growth and a concomitant decrease (biodegradation) in PAHs content from the culture medium with respect to time.

During the past decade, a variety of microorganisms has been isolated and characterized for the ability to degrade different PAHs and new pathways for PAHs degradation have been elucidated (Cerniglia, 1992 and Zhuang *et al*., 2003). Degradation of PAHs by mesophilic microorganisms under aerobic microorganisms under aerobic conditions has been intensely investigated (Cerniglia, 1992). Little is known about the degradation of PAHs by thermophilic bacteria. Recent studies suggest that specific physiological properties of the microorganisms involved in the degradation of hydrophobic compounds might enhance the availability of these compounds. These mechanisms promoting the transfer of hydrophobic substrate (Garcia-Pena *et al*., 2001), uptake systems with high substrate affinity (Volkering *et al*., 1997) and increased bacterial adherence to the hydrophobic pollutants (Bastiaens *et al*., 2000).

Some physiological properties of the selected crude oil degrading bacteria

Preface

The four most potent crude oil and PAHs biodegrading bacterial isolates were subjected to a special study concerning some of their physiological characteristics. This study include the different factors affecting the rate of growth mainly pH, sodium ion concentrations and incubation temperatures. Data of these parameters are discussed as follow:

Effect of pH value : All the selected crude oil degrading bacterial isolates were capable to grow at different pH values covering a relatively wide range of pH (*i.e*. 4-9). The optimum pH values for their growth were determined as 6, 7 & 9. Data recorded revealed that all of the selected crude oil degrading bacterial isolates grow better within the narrower range of pH from 6-8 (*i.e.* at neutral pH

value and pH 6 as well as pH 8), while only one of the selected crude oil degrading bacterial isolates grew at pH value 5. All the selected crude oil degrading bacterial isolates failed to grow at pH values 4 & 9 at the end of the incubation period of 5 days at 30°C.

Effect of sodium chloride (NaCl) concentration : All the selected crude oil degrading bacterial isolates were allowed to grow in a medium without sodium chloride, but generally speaking, their growth was enhanced when sodium chloride was supplemented in the growth medium. All the selected crude oil degrading bacterial isolates were proved to grow in a medium containing sodium chloride ranging from 0-5% (w/v) sodium chloride.

Effect of incubation temperature : The selected crude oil degrading bacterial isolates were capable of growing at incubation temperatures ranging from 20^{o}C – 50^{o}C. *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22, *Pseudomonas stutzeri*-S8API12 and *Enterobacter aerogenes*-W5OA31 proved to grow very well at this rang of temperatures.

Factors affecting the rate of biodegradation

The four most potent selected bacterial isolates from the second selection subjected to investigate the effect of some factors that affects the rate of biodegradation and to reach to the optimum biodegradation rates. The factors investigated were variation in pH values, different NaCl% (w/v) % concentration and finally, the addition of natural nutrients especially nitrogen and phosphorous. The API industrial effluent was used for the application of these factors.

Effect of pH values

The data recorded in Table 3 show that the PAHs content in the API industrial effluent used was 24ppm. Bacterial isolate *Enterobacter aerogenes*-W5OA31 decreased the sum of PAHs content to 2.34 & 2.66 ppm at pH = 7 & 8, respectively, while at other pH values, it extremely increased. Bacterial isolate *Pseudomonas oleovorans*-W7DAFO22 decreased the sum of PAHs to 8.7, 3.94, 1.9 & 3.11 ppm at pH = 4, 6, 7 & 8, respectively, while at other pH values, it increased. Bacterial isolate *Enterobacter cloacae*-S7DAFI22 was able to decrease the sum of PAHs to 0.52, 0.4, 1.5, 0.38 & 0.61 ppm at pH = 4, 5, 6, 8 & 9, respectively, except at pH = 7 the concentration of PAHs seemed to be the same with out any change. As well, bacterial isolate *Pseudomonas stutzeri*-S8API12 decreased the PAHs to 14.3, 5.57, 4.78, 1.89 & 2.27 ppm at pH = 4, 5, 6, 7 & 8, respectively, at pH = 9, the concentration of PAHs slightly increased to 27.2 ppm. From the data above, pH = 8 is the best value among all pH values for the entire selected PAHs utilizing bacterial isolates.

TABLE 3. Detection of PAHs concentrations (ppm) by HPLC after biodegradation of CORC API industrial effluent by the most potent four selected bacterial isolates from summer and winter seasons at different pH values.

Code no. / PAHs	Crude	Control	*Enterobacter aerogenes*-W5OA31						*Pseudomonas oleovorans*-W7DAFO22					
			4	5	6	7	8	9	4	5	6	7	8	9
Naphthalene	104469.3	17.8	0.0	12.2	21.3	1.6	0.0	36.3	2.1	0.17	3.5	1.7	2.87	0.0
Sum di-PAHs	104469.3	17.8	0.0	12.2	21.3	1.6	0.0	36.3	0.0	0.17	3.5	1.7	2.87	0.0
Acenaphthylene	0.0	0.0	57.9	22.3	0.0	0.0	0.0	0.0	6.6	4.3	0.0	0.0	0.0	20.3
Acenaphthene	46213.7	0.0	0.17	0.0	1.4	0.4	1.3	2.9	0.0	0.0	0.44	0.27	0.24	0.0
Fluorine	27158.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.92	0.0	0.0	0.0	0.0
Phenanthrene	5178.4	1.51	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Anthracene	50096.6	0.0	0.0	0.0	0.0	0.0	0.96	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum tri-PAHs	128647.2	1.51	0.0	22.3	1.4	0.4	2.26	2.9	0.0	5.22	0.44	0.27	0.24	20.3
Flouranthene	22628.6	0.0	0.0	0.84	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pyrene	7289.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo(a)anthracene	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.59	0.0	0.0	0.0	0.0	0.0	0.0
Chrysene	39673.6	0.0	1.11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum tetra-PAHs	69591.5	1.5	0.0	0.84	0.0	0.0	0.0	0.59	0.0	0.0	0.0	0.0	0.0	0.0
Benzo(b)flouranthene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo(k)flouranthene	2863.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo(a)pyrene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 3. Continued.

Code no. / PAHs	Crude	Control	*Enterobacter aerogenes*-W5OA31						*Pseudomonas oleovorans*-W7DAFO22					
			4	5	6	7	8	9	4	5	6	7	8	9
Dibenzo(a,h) anthracene	58475.2	2.9	0.0	0.25	0.11	0.34	0.4	0.09	0.0	0.0	0.0	0.0	0.0	0.07
Sum pent-PAHs	61338.3	2.9	0.0	0.25	0.11	0.34	0.4	0.09	0.0	0.0	0.0	0.0	0.0	0.07
Benzo(g,h,i)perylene	11460370	0.0	659	0.0		0.0	0.0	0.0	0.0	164	0.0	0.0	0.0	48.3
Indeno(1,2,3-cd)pyrene	6491849	0.0	9045	0.0	40.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum hex-PAHs	17952219	0.0	0.0	0.0	40.1	0.0	0.0	0.0	0.0	164	0.0	0.0	0.0	48.3
Sum PAHs	18316266	23.7	9763	35.7	63	2.34	2.66	39.9	8.7	169	3.94	1.97	3.11	68.7
PAHs Residue%			412	151	266	9.9	11.2	168	36.7	715	16.6	8.3	13.1	290
PAHs Removal%						90.1	88.8		63.3		83.4	91.7	86.9	

TABLE 3. Continued.

Code no. / PAHs	Crude	Control	*Enterobacter cloacae*-S7DAF122						*Pseudomonas stutzeri*-S8AP112					
			4	5	6	7	8	9	4	5	6	7	8	9
Naphthalene	104469.3	17.8	0.52	0.0	0.55	19.8	0.0	0.6	11	0.97	0.0	0.56	1.34	27
Sum di-PAHs	104469.3	17.8	0.52	0.0	0.55	19.8	0.0	0.6	0.0	0.97	0.0	0.56	1.34	27
Acenaphthylene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.65	0.0	0.0	0.0
Acenaphthene	46213.7	0.0	0.0	0.0	0.95	1.6	0.38	0.01	2.6	2.7	0.0	0.66	0.93	0.0
Fluorine	27158.5	0.0	0.0	0.04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phenanthrene	5178.4	1.51	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Anthracene	50096.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum tri-PAHs	128647.2	1.51	0.0	0.04	0.95	1.6	0.38	0.01	0.0	2.7	1.65	0.66	0.93	0.0
Flouranthene	22628.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pyrene	7289.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.64	0.0	0.0
Benzo(a)anthracene	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chrysene	39673.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.13	0.0	0.0	0.0
Sum tetra-PAHs	69591.5	1.5	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	3.13	0.64	0.0	0.0
Benzo(b)flouranthene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo(k)flouranthene	2863.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo(a)pyrene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dibenzo(a,h) anthracene	58475.2	2.9	0.0	0.0	0.0	1.14	0.0	0.0	0.65	1.9	0.0	0.03	0.0	0.17
Sum pent-PAHs	61338.3	2.9	0.0	0.0	0.0	1.14	0.0	0.0	0.0	1.9	0.0	0.03	0.0	0.17
Benzo(g,h,i)perylene	11460370	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Indeno(1,2,3-cd)pyrene	6491849	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum hex-PAHs	17952219	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum PAHs	18316266	23.7	0.52	0.4	1.5	24.1	0.38	0.61	14.3	5.57	4.78	1.89	2.27	27.2
PAHs Residue%			2.2	0.2	6.3	102	1.6	2.5	60.1	23.5	20.2	7.9	9.6	114
PAHs Removal%			97.8	99.8	93.7		98.4.	97.5	39.9	76.5	79.8	92.1	90.4	

Effect of different NaCl concentrations

The data recorded revealed that the PAHs concentration in the API industrial effluent used was 24ppm. At NaCl concentration = 1%, the four most potent selected bacterial isolates *Enterobacter aerogenes*-W5OA31, *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22 & *Pseudomonas stutzeri*-S8API12 were capable to almost biodegrade the PAHs, as well as at NaCl concentrations = 2.5%, 5% & 10%. At NaCl concentration = 7.5% only bacterial isolates *Enterobacter aerogenes*-W5OA31 and *Pseudomonas oleovorans*-W7DAFO22 were capable to almost biodegraded the PAHs, while at NaCl concentration = 7.5% bacterial isolates *Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8API12 decreased the PAHs concentration to 60 & 68%, respectively.

Effect of different nutrient sources nitrogen and phosphorous (Urea, NH_4Cl, K_2HPO_4& $(NH_4)_2HPO_4$)

The data recorded showed that the PAHs concentration in the API industrial effluent used was 228ppm. Data for the addition of urea show that bacterial isolate *Enterobacter aerogenes*-W5OA31 extremely increased the PAHs concentration while the other bacterial isolates *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8API12 decreased it to 1-2%. Data for the addition of NH_4Cl show that bacterial isolates *Enterobacter aerogenes*-W5OA31 and *Pseudomonas oleovorans*-W7DAFO22 extremely increased the PAHs concentration while the other bacterial isolates *Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8API12 decreased the PAHs concentration to 0.1-1%.

Data for the addition of K_2HPO_4 show that the four most potent selected bacterial isolates *Enterobacter aerogenes*-W5OA31, *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8API12 decreased the PAHs concentration to 0.1-0.5% as well as the addition of $(NH_4)_2HPO_4$. From the above three factors applied on API industrial effluent used data show that the optimum conditions recorded were at pH = 8, NaCl concentration = 5% and the addition of K_2HPO_4 & $(NH_4)_2HPO_4$ (*i.e*. using equal volumes of both).

Survival of microorganisms in petroleum hydrocarbons medium after their inoculation is a key deciding factor in the rate of biodegradation of hydrocarbons either in soil or in liquid phase (Ramos *et al*., 1991). Since all the bacteria in the present study were isolated from a petroleum contaminated soil/liquid environment very easily as also reported by other authors (Rahman *et al*., 2003 and Sugiura *et al*., 1997). This was evident from the significant increase in the bacterial population of *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22, *Pseudomonas stutzeri*-S8APII12 and *Enterobacter aerogenes*-W5OA31 in liquid medium supplemented with Refining effluents a corrugated plate interceptor inlet (APII) samples polluted with petroleum crude

oil as sole source of carbon and energy sources as compared with control. However, higher growth rate of *Pseudomonas* strains compared with *Enterobacter* strains might be related to higher breakdown and utilization of petroleum hydrocarbons and especially PAHs by former bacterial strains. Different *Pseudomonas* strains were isolated from petroleum contaminated soil sample of North-East India grew on a large number of hydrocarbon compounds as a source of carbon and energy demonstrating these strains might be efficient hydrocarbon degraders (Das & Mukherjee, 2005 and Mukherjee & Das, 2005). Besides, the necessity for seeding with hydrocarbon or PAHs degrading bacteria might have a risen from the fact that indigenous microbes of Refining industrial effluents samples in CORC plant were efficient degraders of a wide range of complex compounds of crude oil and PAHs and therefore, introduction of efficient hydrocarbon degraders would be essential in order to effectively degrade most or all of the hydrocarbons in a complex petroleum mixture (Atlas, 1977).

Quantitative determination of the different combinations of the four most potent bacterial isolates on the crude oil degradation power

The effect of the combinations between the four selected bacterial isolates viz. *Enterobacter aerogenes*-W5OA31, *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8API12 on the API industrial effluents were studied and then incubated in an orbital shaker for 5 days at 30°C and 150 rpm, at the end of which HPLC analysis of the extracted samples was performed in the Central labs of Egyptian Petroleum Research Institute, Cairo.

Seven combinations viz. (*Enterobacter aerogenes*-W5OA31 and *Pseudomonas oleovorans* -W7DAFO22), (*Enterobacter aerogenes*-W5OA31 and *Enterobacter cloacae*-S7DAFI22), (*Enterobacter aerogenes*-W5OA31 and *Pseudomonas stutzeri*-S8API12), (*Pseudomonas oleovorans*-W7DAFO22 and *Enterobacter cloacae*-S7DAFI22), (*Pseudomonas oleovorans*-W7DAFO22 and *Pseudomonas stutzeri*-S8API12), (*Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8API12) and (*Enterobacter aerogenes*-W5OA31, *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8API12) as well as control were studied.

Data recorded in Table 4 revealed that combination between bacterial isolates viz. *Pseudomonas oleovorans*-W7DAFO22 and *Enterobacter cloacae*-S7DAFI22 as consortium completely biodegraded the residual PAHs present. Combination between bacterial isolates viz. *Pseudomonas oleovorans*-W7DAFO22 and *Pseudomonas stutzeri*-S8API12 as consortium decreased the PAHs present to 0.83 ppm. Consortium between *Enterobacter aerogenes*-W5OA31 and *Pseudomonas stutzeri*-S8API12 decreased the PAHs present to 0.96 ppm. Consortium between the four bacterial isolates decreased the PAHs present 1.95 ppm.

Table4. Biodegradation of PAHs presented in API industrial effluents by seven combinations as consortium for the four selected bacterial isolates

PAHs \ Consorti	**Control**	Consortium1	Consortium2	**Consorium3**	**Consortium4**	**Consortium5**	Consortium6	Consortium7
Naphthalene	**0.018**	11.1	2.2	**0.0**	**0.0**	**0.0**	0.0	0.0
Acenaphthylene	**0.11**	0.0	0.0	**0.0**	**0.0**	**0.0**	0.0	0.0
Acenaphthene	**0.007**	0.0	0.0	**0.79**	**0.0**	**0.0**	0.0	0.015
Fluorine	**0.0**	0.16	0.003	**0.0**	**0.0**	**0.0**	0.14	0.0
Phenanthrene	**0.16**	0.008	0.009	**0.0**	**0.0**	**0.23**	0.03	0.08
Anthracene	**3.63**	0.73	0.012	**0.03**	**0.0**	**0.6**	4.8	1.9
Flouranthene	**0.0**	1.28	0.11	**0.12**	**0.0**	**0.0**	0.0	0.0
Pyrene	**0.24**	0.0	0.042	**0.016**	**0.0**	**0.0**	0.0	0.0
Benzo(a)anthracene	**0.055**	0.0	0.0	**0.0**	**0.0**	**0.0**	0.0	0.0
Chrysene	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.0**	0.0	0.0
Benzo(b)flouranthene	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.0**	0.0	0.0
Benzo(k)flouranthene	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.0**	0.0	0.0
Benzo(a)pyrene	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.0**	0.0	0.0
Dibenzo(a,h) anthracene	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.0**	0.0	0.0
Benzo(g,h,i)perylene	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.0**	0.0	0.0
Indeno(1,2,3-cd)pyrene	**0.0**	0.0	0.0	**0.0**	**0.0**	**0.0**	0.0	0.0
Sum PAHs	**4.22**	13.278	2.376	**0.96**	**0.0**	**0.83**	5.02	1.95
PAHs Residue%		315	56.3	**22.6**	**0.0**	**14**	119	46.2
PAHs Removal%			43.7	**77.4**	**100**	**86**		53.8

Consortium1: *Enterobacter aerogenes*-W5OA31 and *Pseudomonas oleovorans*-W7DAFO22

Consortium2: *Enterobacter aerogenes*-W5OA31 and *Enterobacter cloacae*-S7DAFI22

Consortium3: *Enterobacter aerogenes*-W5OA31 and *Pseudomonas stutzeri*-S8APII12

Consortium4: *Pseudomonas oleovorans* -W7DAFO22 and *Enterobacter cloacae*-S7DAFI22

Consortium5: *Pseudomonas oleovorans* -W7DAFO22 and *Pseudomonas stutzeri*-S8APII12

Consortium6: *Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8APII12

Consortium7: *Enterobacter aerogenes*-W5OA31, *Pseudomonas oleovorans* -W7DAFO22, *Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8APII12

As well, consortium between *Enterobacter aerogenes*-W5OA31 and *Enterobacter cloacae*-S7DAFI22 decreased the PAHs present to 2.376 ppm. The consortium between *Enterobacter aerogenes*-W5OA31 and *Pseudomonas oleovorans*-W7DAFO22 increased the PAHs present to 13.278 ppm, as well, combination between bacterial isolates viz. *Enterobacter cloacae*-S7DAFI22 and *Pseudomonas stutzeri*-S8API12 as consortium increased the PAHs present to 5.02 ppm. Consequently, viz. bacterial isolates as consortium for (*Pseudomonas oleovorans*-W7DAFO22 and *Enterobacter cloacae*-S7DAFI22), (*Pseudomonas oleovorans*-W7DAFO22 and *Pseudomonas stutzeri*-S8APII12) and (*Enterobacter aerogenes*-W5OA31 and *Pseudomonas stutzeri*-S8APII12) were selected as the best bacterial consortium for PAHs biodegradation.

As all the bacteria in the present study were isolated from crude oil polluted Refinery effluents samples in CORC plant, they survived and were adapted to the crude oil liquid environment very easily. This was evident from the significant increase in the population of bacterial strains when compared with control.

It has been observed that the PAHs compounds in different crude oil polluted Refining effluents of a corrugated plate interceptor (API) samples were degraded to different extents by the four bacterial isolates, implying that the bioavailability of particular compound in a crude oil polluted Refining effluents of a corrugated plate interceptor (API) samples. HPLC analysis demonstrated that PAHs were preferentially degraded in crude oil by the four most potent bacterial strains used in this study. Crude petroleum oil pollutents of Refining effluents a corrugated plate interceptor (API) samples in CORC is a complex mixture of hydrophobic components like n-alkanes, aromatic, resins and asphalthene and microorganisms are known to attack and degrade a specific components as compared with other components of oil.

Diammonium hydrogen phosphate was selected as best nitrogen source to increase the rate of biodegradation of PAHs by four most potent selected bacteria. However, present results were in contradiction to report of Chhatre *et al*. (1996) describing addition of nutrients in the soil was unlikely to have dramatic effect on the microbial degradation of crude oil. PAHs biodegradation potential of *Enterobacter* and *Pseudomonas* strains from CORC Refining effluents a corrugated plate interceptor (API) samples were far higher than other bacterial isolates, even for mixed cultures (Chhatre *et al*., 1996 and Sugiura *et al*., 1997).

The growth of *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22, *Pseudomonas stutzeri*-S8APII12 and *Enterobacter aerogenes*-W5OA31 strains at the expense of PAHs inoculation indicated a greater assimilation of PAHs by these bacterial strains isolated from polluted Refining industrial effluents samples in CORC plant. Therefore, we agree with the finding of Vila *et al*. (2001) that all PAHs lost from the growth medium. Furthermore, it might be reasonable to assume that several key factors such as optimum growth condition of bacteria.

The degree of degradation of PAHs was inversely proportional to the number of rings in the PAHs molecules degradation. This observation is consistent with previous studies on PAHs degradation (Leblond *et al*., 2001). The present study shows that addition of nutrient increase the degradation rate of each group of components of the PAHs even up to 100% as in using consortium between *Pseudomonas oleovorans* -W7DAFO22 and *Enterobacter cloacae*-S7DAFI22.

Conclusion

Refinery effluents samples collected from the corrugated plate interceptor (API) of CORC were used in this study. The indigenous microbial population present displayed a good capability to degrade the different US- Environmental Protection Agency (US-EPA) 16 unsubstituted PAHs. The finding in this study showed that *Pseudomonas oleovorans*-W7DAFO22, *Enterobacter cloacae*-S7DAFI22, *Pseudomonas stutzeri*-S8APII12 and *Enterobacter aerogenes*-W5OA31 strains could be useful in the biodegradation of PAHs present in API industrial effluents of sites in CORC highly contaminated with crude petroleum oil hydrocarbons under all optimal conditions. The nature of these bacteria could add further advantages for their use in bioremediation of petroleum contaminated industrial effluents in CORC plant.

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اختيار وتوصيف بكتيريا معزولة من مياه صرف تكرير البترول لها القدرة على نزع المركبات الأروماتية متعددة الحلقة وظروفها

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تم فى هذه الدراسة تقييم تأثير العوامل الغذائية والبيئية المختلفة على نسب نزع المركبات الأروماتية متعددة الحلقة المتواجدة فى عينات من مياه صرف تكرير البترول الناتجة من مياه الخرج الصناعى لشركة القاهرة لتكرير البترول – مصر. تم عزل عدد مائتان ومائتان وثمانية وأربعون سلالة بكتيرية معزولة من عدد ست عينات مياه صرف تقطير بترول ملوثة بزيت البترول تم تجميعها من شركة القاهرة لتكرير البترول خلال فصلى الصيف والشتاء على التوالي حيث تم اختيارها لقدرتها على النمو فى وجود زيت البترول كمصدر وحيد للكربون . تم اختيار أقوى أربع سلالات مرة أخرى من هذه العزلات لقدرتها على النمو بصورة جيده على الهيدروكربونات والصفات المزرعية وقدرتها على التكسير الحيوي للمركبات الأروماتية متعددة الحلقة. تم تعريف هذه العزلات على أسس شكل وترتيب الخلايا وعلاقتها بالأكسجين والخواص الغذائية والبيوكيميائية حيث وجد أن هذه السلالات الأربعة قريبة الشبة من *سيوموناس اوليوفورنس* *Pseudomonas oleovorans*-W7DAFO22 و*انتيروباكتر كلواكا* *Enterobacter cloacae*-S7DAFI22 و*سيوموناس ستتزرى* *Pseudomonas stutzeri*-S8API12 و*انتيروباكتر ايروجينز* *Enterobacter aerogenes*-W5OA31 . وتستطيع هذه السلالات النمو على الأوساط الغذائية والمكونة من الأملاح المعدنية مزوده بزيت البترول الخام كمصدر وحيد للكربون. وتشير النتائج إلى إمكانية هذه السلالات على نزع المركبات الأروماتية متعددة الحلقة بنسب مختلفة عند قيم مختلفة من الأس الهيدروجيني (٤-٩) وتركيزات مختلفة من كلوريد الصوديوم (١-١٠٪) ومصادر مختلفة من النيتروجين والفسفور. وتخلص هذه الدراسة إلى إمكانية تكوين قاعدة بيانات كأدوات لإمكانية تكسير المركبات الأروماتية متعددة الحلقة فى المواقع الملوثة بالهيدروكربونات البترولية المختلفة عند تطبق تكنولوجيا المعالجة الحيوية.