Journal of Applied Biological Sciences 5 (2): 9-14, 2011
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Investigation of Metabolite Productions and Degradation of Hazardous Organic Pollutants by *Pseudomonas* spp.

Dilsad ONBASILI[1*] Belma ASLIM[2] Gokcen YUVALI CELIK[1]

[1]Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology 38039, Kayseri, TURKEY

[2]Gazi University, Molecular Biology Research and Application Center, 06830, Ankara, TURKEY

[*]Corresponding Author
e-mail: dilsad@erciyes.edu.tr

Received: January 11, 2011
Accepted: January 27, 2011

Abstract

In this study, 20 strains of *Pseudomonas* spp. were tested for their secondary metabolite and degradation in some organic pollutants [1% (v/v) benzene, BTX and 2% (v/v) gasoline]. All the strains tested exhibited microbial growth and production of rhamnolipid and pyocyanin in presence of hazardous organic pollutants [1% (v/v) benzene, BTX and 2% (v/v) gasoline] as a sole carbon source but 3 strains (*P. aeruginosa* B3, *P. fluorescens* B5 and *P. putida* B15), are higher than others. When these cultures were grown in medium containing organic pollutants, while rhamnolipid production by these strains ranged from52 mg/L to 2 mg/L, the pyocyanin production was 0.02–1.30 µg/mL. While maximal production for rhamnolipid has been obtained in the stationary growth phase, for pyocyanin has been obtained in the exponential growth phase. The results of the study may have important implications in both the industrial and environmental areas as well as it can be a potent source for degradation of hazardous organic pollutants.

Key words: Degradation, Pyocyanin, Rhamnolipid, *Pseudomonas* spp., organic pollutants

INTRODUCTION

The environment is continuously polluted by hazardous organic pollutants with different structures and different toxicity levels that are released from several sources; the main sources of pollution can be identified as industrial activities, munitions waste and agricultural practice. The explosive development of chemical industries has produced a large variety of chemical compounds that include pesticides, fuels, alkanes, polycyclic aromatic compounds, dyes and more. Although these compounds have contributed to modernize lifestyle, several of them may accumulate in soil, water and air. Among hazardous organic compounds resulted from petrochemical and energy-producing industries, aromatic compounds such as benzene, toluene and xylenes are severe contaminants (gasoline). Due to their low water solubility, acute toxicity and genotoxicity, these compounds are classified as priority pollutants by European Environment Agency [15, 17]. Many microorganisms have been reported to use various petroleum hydrocarbons, including BTEX and gasoline as their sole carbon and energy substrate, despite their extreme insolubility in the aqueous phase. Numerous genera of bacteria are known as good hydrocarbon degraders. They tolerate high concentrations of the hydrocarbons and have a high capability for their degradation. Most of them belong to *Pseudomonas*, *Sphingomonas*, *Aeromonas*, *Alcaligenes*, *Acinetobacter*, *Arthobacter*, *Brevibacterium*, *Xantomonas*, *Mycobacterium*, *Rhodococcus* and *Bacillus* species [2, 5, 9]. Bioremediation, technique that utilize the microbial ability to degrade and/or detoxify chemical substance, is relatively low cost, with low-technology level, and a high public acceptance. Much work has been done on the degradation of single pollutants [15, 17]. To obtain an efficient hazardous chemicals-degrading bacterial consortium and monocultures, knowledge of the diversity of the microbial community present in soils contaminated with crude oil, their metabolic features and capacity to degrade crude oil are of paramount importance. One of the factors that limits biodegradation of oil pollutants is their limited availability to microorganisms. However, most of the hazardous organic pollutants degraders produce seconder metabolite such as surface active compounds named biosurfactants (BS) [6, 14], and antimicrobial compounds, pyocyanin [13] *P. aeruginosa* is frequently isolated from petroleum-contaminated sites and is capable of producing metabolites (i.e., alginate, rhamnolipid, pyocyanin) that enhance its competitiveness and survival [13, 18, 29, 32]. Biosurfactants have several potential advantages which are important for bioremediation applications in contaminated environments. These advantages include their biodegradability, low toxicity, better specificity for some applications, the potential for *in situ* production, the ability to be produced from cheap raw materials, effectiveness at extreme conditions of temperature, pH and salinity, and the organisms producing them can be modified genetically to overproduce these compounds [7, 30]. Recently biosurfactants or microorganism-produced biosurfactants have been widely used in environmental protection, including enhanced oil recovery (EOR), oil spill control, biodegradation and detoxification of oil-contaminated industrial effluents and soils [10, 19]. Also,

the water-soluble secondary metabolite pyocyanin (1-hydroxy-5-methylphenazine) has demonstrated antimicrobial activity against a variety of microorganisms [4, 13, 28].

Our studies have shown that among our microbial collection there are microorganisms able to grow hazardous organic compounds. Screening activities performed have allowed the selection of three microbial strains, such as *Pseudomonas aeruginosa* B3, *Pseudomonas fluorescens* B5 and *Pseudomonas putida* B15. In present paper, the above mentioned strains were investigated produce rhamnolipids and pyocyanin and degradation capacity. The outcome of this work can be used for research on remediation of petroleum-contaminated environments.

MATERIAL AND METHODS

Test chemicals

All chemicals used for media preparation and hazardous organic pollutants [Benzene, BTX (Benzene, Toluene, Xylene)] were ensured from Merck. The gasoline used in all experiments was obtained from gas station (Ankara, Turkey).

Bacterial strains, media and growth conditions

The strains of 20 *Pseudomonas* spp. used in this study were obtained from the culture collection of the Biotechnology Laboratuary at Gazi University (Ankara, TURKEY). These strains were previously identified by the Analytical Profile Index (API 20 NE for *Pseudomonas* isolates). All of the strains were stored on Nutrient Agar Medium (Oxoid) slopes at 4 ◦C and stock cultures were maintained at −20 ◦C in 0.5% (v/v) glycerol.

Samples were maintained in 250 mL flasks containing 50 mL of nutrient broth (NB) culture medium [3] (in g/L: peptone, 2.5; NaCl, 2.5; yeast extract 1.0; beef extract 0.5; pH 7.0) for metabolite production as a control and Mineral Salt Medium (MSM) (Atlas, 1997) (in g/L: Na_2HPO_4 4.0, KH_2PO_4 1.5, NH_4Cl 1.0, $MgSO_4$. $7H_2O$ 0.2, $C_6H_8O_7FeNH_3$ 0.05; Modified Hoagland trace element solution g/3,6 L: BH_3 11.0, $MnCl_2$. $4H_2O$ 7.0, $AlCl_3$ 1.0, $CoCl_2$ 1.0, $CuCl_2$ 1.0, KI 1.0, $NiCl_2$ 1.0, $ZnCl_2$ 1.0, $BaCl_2$ 0.5, KBr 0.5, LiCl 0.5, Na_2MoO_4 0.5, $SeCl_4$ 0.5, $SnCl_2.2H_2O$ 0.5, $NaVO_3.H_2O$ 0.1; pH 7.0) for metabolite production and degradation assays.

1 mL of the active cultures was adjusted to Macfarland 5 for whole assays and inoculated inside media. The erlanmayer flasks were incubated at 37 ºC, at regular time intervals (24, 48, 72 and 96 h), by using an incubator shaker (MINITRON) at 100-150 rpm.

NB medium was autoclaved for 15 min at 120 ºC but mineral salt solutions were autoclaved separately to prevent precipitation reactions. The organic pollutants were sterilized through 0.45-µm-pore-size type HA membrane filters (Millipore Corp., Bedford, Mass.).

Biodegradation of organic pollutants

The overnight culture at the log phase of growth were transferred to 250 ml flasks, each containing 50 mL of sterile defined mineral salts medium (MSM) with [1% (v/v) benzene, BTX and 2% (v/v) gasoline] two concentrations of organic pollutants. The flasks were incubated in a shaker at 100-150 rpm at 37 °C. At regular time intervals (24, 48, 72 and 96 h), microbial growth for degradation of 1% (v/v) benzene, BTX and 2% (v/v) gasoline was monitored by measuring the optical density at 600 nm by the spectrophotometer (Hitachi UV-VIS) and the medium without bacteria served as a control. For degradation test, tolerance to various concentration of benzen, BTX and gasoline (1%, 2%, 3%, and 4%, v/v) was carried out by inoculating 96 h bacterial culture to liquid NB medium supplemented with 1% benzen, BTX and gasoline as a sole source of carbon. Concentrations used in this research determined as %1 v/v for Benzen and BTX and %2 v/v for gasoline.

Determination of rhamnolipid

Strains were inoculated on 50 mL of sterile defined NB (as a control) and MSM containing different concentrations of organic pollutants [1% (v/v) benzene, BTX and 2% (v/v) gasoline] for biosurfactant production, followed by incubation on a rotary shaker (130 rpm) at 30ÚC. Bacterial cells were removed from biosurfactant containing medium by centrifugation at 6000 rpm for 10 min. This crude extract was dried with the aid of a rotary evaporator under vacuum. Rhamnolipid concentration was determined according to Dubois et al. (1956) by the colorimetric phenolsulphuric acid method at 480 nm using the spectrophotometer.

Determination of pyocyanin

Pyocyanin was extracted from the broth culture of strains as previously described Essar et al. [12]. The bacteria were removed by centrifugation after incubation. The culture supernatant was mixed with chloroform to remove most nonpyocyanin pigments. The blue pigments in chloroform were extracted by 10 mM HCl followed by neutral water. The pyocyanin was partitioned to the HCl aqueous phase, which was taken for quantification at OD520.

Table 1. Different growth levels of 20 *Pseudomonas* spp. in organic pollutants (1 % (v/v) Benzene, BTX and 2% (v/v) Gasoline)

Genera	Total No	Organic Pollutants	OD[a] (600 nm)						
			% (v/v)	0.0-0.025	0.026-0.05	0.051-0.1	0.11-0.20	0.21-0.40	0.41-0.8
Pseudomonas spp.	20	Benzene	1	4	8	8	-	-	-
		BTX		3	7	9	1	-	-
		Gasoline	2	12	8	-	-	-	-

-a Number of strains reaching this OD, -: none detected, low growth: 0.0-0.050, moderate growth: 0.051-0.1, high growth: 0.11-0.4, excellent growth: 0.41-0.8

RESULTS AND DISCUSSION

The biodegradation of hydrocarbons in polluted environment is mainly through the activities of bacteria and fungi. Typically, individual organisms degrade only a limited range of hydrocarbons. *Pseudomonas* sp. represents one of the most versatile groups of organisms involved in the degradation of hydrocarbons [31]. *Pseudomonas* spp., the organisms used in this study, have been investigated to have specificity for a range of organic pollutants including petroleum products (benzene, BTX and gasoline) commonly used in the Turkey environment. For this purpose, firstly absorbance changes in the mineral salt medium of cultures were monitored by measuring the optical density at 600 nm as spectrophotometric at different time intervals (24, 48, 72 and 96 h). When cultures were grown in 1% (v/v) benzene, BTX and %2 (v/v) gasoline containing MSM medium, absorbance changes generally increased over time. These strains capable of utilizing some organic pollutants as a carbon source showed growth at 24 h and then the growth of these strains became constant. The maximum growth was mostly achieved at 72 h of incubation. Therefore, Table 1 was prepared according to values at 72 h. Growth rates decreased after 4 days of incubation (data not shown). While some strains (total 9 strains) exhibited moderate growth in the range of 0.051-0.1 OD in 1% (v/v) BTX containing MSM medium, the only one strain showed high growth (0.11-0.20 OD) at this concentration when compared with each other. In 1% (v/v) Benzene, while a total 8 strains exhibited moderate growth, the same number of strains showed low growth (0.026-0.05 OD) in 2% (v/v) gasoline containing MSM medium when compared with each other. Later, according to results in Table 1, three strain (B3, B5 and B15) having appreciable some organic pollutants degrading capability were selected. Our research work clearly shows that three strain (B3, B5 and B15) can be a potent source for degradation of some organic pollutants.

The various advantages of producing seconder metabolites from Pseudomonads include independence from weather conditions, easy and fast growth, colors of different shades, competitiveness and survival, function in evasion of host defense mechanisms, bacterial adhesion and resistance to antibacterial agents and growth on cheap substances [8, 16, 20]. Seconder metabolites-producing *Pseudomonas* spp. are finding vast potential applications in environmental protection, petroleum, food, mining, agriculture, pharmaceutical, textile, leather and other industries [26, 27]. Rhamnolipid and pyocyanin are secondary metabolites. Production of these metabolites by

Table 2. Rhamnolipid and pyocyanin production of 20 *Pseudomonas* spp. in organic pollutants (1 % (v/v) Benzene, BTX and 2% (v/v) Gasoline)

Microorganism	Strain No	Pyocyanin Production (µg/mL)[a]				Rhamnolipid production (mg/L)[a]			
		Control*	Benzene 1% v/v	BTX 1% v/v	Gasoline 2% v/v	Control*	Benzene 1% v/v	BTX 1% v/v	Gasoline 2% v/v
P. aeruginosa	B1	6,50±0,02	0,90±0,00	0,70±0,00	0,6±0,0	388±0,3	-	-	-
	B2	5,40±0,03	0,90±0,00	1,10±0,00	0,7±0,0	385±0,1	-	-	-
	B3	5,86±0,01	**1,30±0,00**	**1,20±0,00**	**0,8±0,0**	452±0,5	-	**0,5±0,0**	0,7±0,0
	B16	2,11±0,05	0,90±0,00	0,90±0,00	0,5±0,0	347±0,0	-	-	20,0±0,1
	B19	10,1±0,01	0,80±0,00	1,10±0,00	0,7±0,0	521±0,3	-	0,3±0,0	-
	B20	9,81±0,03	0,02±0,00	0,03±0,00	0,7±0,0	467±0,2	-	-	-
P. fluorescens	B4	-	-	-	-	334±0,0	-	-	18,0±0,2
	B5	-	-	-	-	339±0,2	**1,5±0,1**	-	3,0±0,0
	B6	-	-	-	-	337±0,4	-	-	5,0±0,0
P. stutzeri	B7	-	-	-	-	339±0,2	-	-	-
	B8	-	-	-	-	298±0,2	-	-	-
	B9	-	-	-	-	257±0,3	-	-	-
	B10	-	-	-	-	219±0,4	-	-	11,0±0,1
	B11	-	-	-	-	263±0,1	-	-	31,0±0,0
P. putida	B12	-	-	-	-	474±0,0	-	-	-
	B15	-	-	-	-	427±0,2	-	-	**52,0±0,2**
	B18	-	-	-	-	320±0,2	-	-	2,0±0,3
P. cepecia	B13	-	-	-	-	327±0,1	-	-	-
	B14	-	-	-	-	336±0,0	-	-	-
P. luteala	B17	-	-	-	-	361±0,0	-	-	5,0±0,0

[a] Values are means±standard deviations of triplicate measurements.
-: none detected, *: It was used nutrient broth medium (v/v) as control (NB medium).

Pseudomonas spp. are affected from various carbon sources in medium [23, 25]. Onbasili and Aslim [22] found that diversities of organic compounds (2,4-D, benzene, BTX and gasoline) as carbon source affected the monomer composition of EPS that is a seconder metabolite produced by some *Pseudomonas* spp. cultures. Similary, in our study rhamnolipid and pyocyanin productions of the 20 strains were studied, with organic pollutants [1% (v/v) benzene, BTX and %2 (v/v) gasoline] being used as the sole source of carbon at different time intervals (24, 48, 72 and 96 h). The results showed that these strains produced some metabolites (pyocyanin and rhamnolipids) when grown with some organic pollutants as the carbon source (Table 2). Several authors reported that pyocyanin and rhamnolipid substances had antimicrobial effect against some bacteria [1, 20, 21]. Hence, these data suggest that rhamnolipid and pyocyanin may contribute to the protective effects against organic pollutants toxicity. Osman et al. [24] reported that *Pseudomonas* BOP 100 has the capabilities for production of rhamnolipid and pyocyanine when grown on ethanol as sole carbon source. Maximum coproduction capacity was observed at a concentration of 3% ethanol; yield of rhamnolipids was 3 g/L, and of pyocyanine 0.2 g/L. Osman et al. [24] and Tuleva et al. [29] reported that while maximal production for rhamnolipid has been obtained in the stationary growth phase, for pyocyanin has been obtained in the exponential growth phase. Norman et al. [20] were identified the antibacterial compound pyocyanin, in an oil-degrading culture containing *P.* a*eruginosa* and it was further demonstrated that pyocyanin reached a concentration of 9.5 µM in the culture supernatants. Zhang et al. [33] found that *Pseudomonas aeruginosa* produced 15.4 g/L rhamnolipids when cultured in a basal mineral medium using glycerol as a sole carbon source. Similary, Rashedi et al., [25] reported that maximal surfactant production occurred after 96 h of incubation, when cells reached the stationary phase of growth. This study is supported by studies reported in the literature. While the maximum rhamnolipid production by 20 strains was mostly achieved at 72 h of incubation, maximum pyocyanin production was realized at 48 h of incubation. Table 2 was prepared according to values at 48. and 72. h and results were lower than that of control. When these cultures were grown in medium containing organic pollutants, while rhamnolipid production by these strains ranged from52 mg/L to 2 mg/L, the pyocyanin production was 0.02–1.30 µg/mL. The amounts of rhamnolipid [*P. fluorescens* B5 (1,5 mg/L in Benzene), *P. aeruginosa* B3 (0,5 mg/L in BTX) and *P. putida* B15 (52 mg/L in gasoline)] and pyocyanin [B3 (1,3 µg/mL in Benzene) (1,2 µg/mL in BTX) (0,8 µg/mL in gasoline)] produced by three strains were highest than the production amount of other strains in organic pollutants [1% (v/v) benzene, BTX and %2 (v/v) gasoline]. The results showed that these three strains produced both maximal rhamnolipid (B3, B5 and B15) and pyocyanin (B3), as well as degraded some organic pollutants efficiently when compared to the individual bacterial cultures tested. Also, the cell growth and production curves obtained in MSM medium with organic pollutants [1% (v/v) benzene, BTX and %2 (v/v) gasoline] being used as the sole source of carbon at different time intervals of strains (B3, B5, B15) are shown in Fig 1-6. The highest metabolite productions of selected strains occurred at 48. h for pyocyanin (exponential phase) and 72. h for rhamnolipid (stationary phase) of incubation, when some organic pollutants were used (Fig 1-6). However, the optical density increased with the increase of the metabolites. It was apparent from the fact that pyocyanin and rhamnolipid were produced in stationary phases of growth, that growth rate was an important parameter in theirs synthesis.

This paper describes preliminary studies to examine the data on degradation capability and seconder metabolite productions by *Pseudomonas* spp. of toxic organic pollutants commonly used in Turkey. However, further research is needed to determine advanced method of degradation and structure and properties of the rhamnolipid and pyocyanin produced by these strains during a bioremediation process. The results of the study may have important implications in both the industrial and environmental areas.

Acknowledgements

This work was supported by FEF Gazi University BAB project no: 05/2006-18.

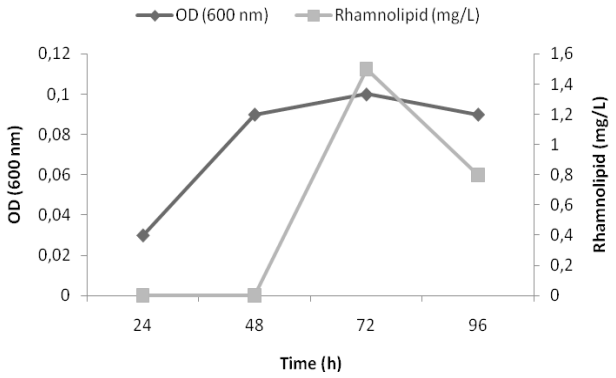

Figure 1. Growth and rhamnolipid production of B5 in Benzene at different times.

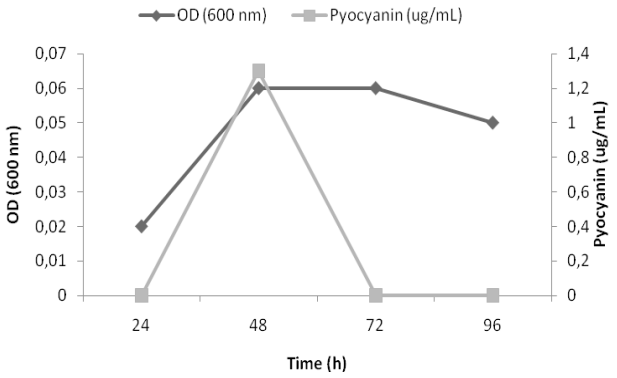

Figure 2. Growth and pyocyanin production of B3 in Benzene at different times.

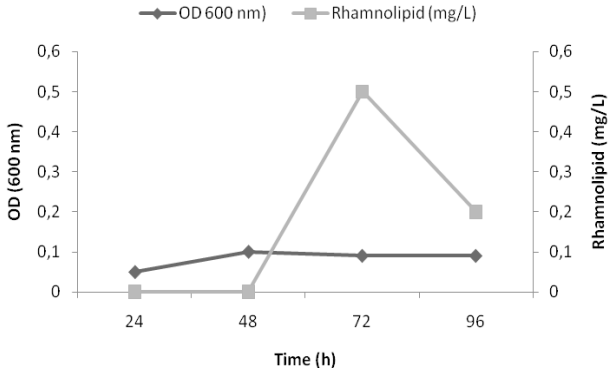

Figure 3. Growth and rhamnolipid production of B3 in BTX at different times.

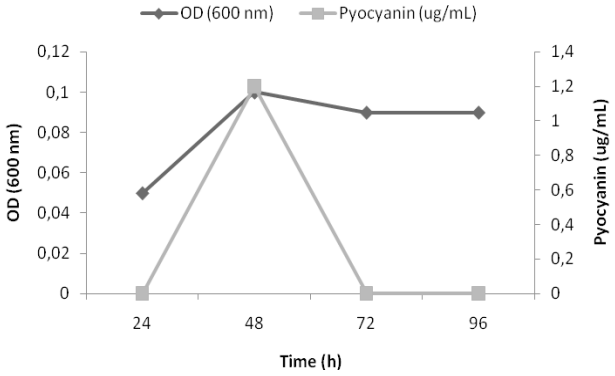

Figure 4. Growth and pyocyanin production of B3 in BTX at different times.

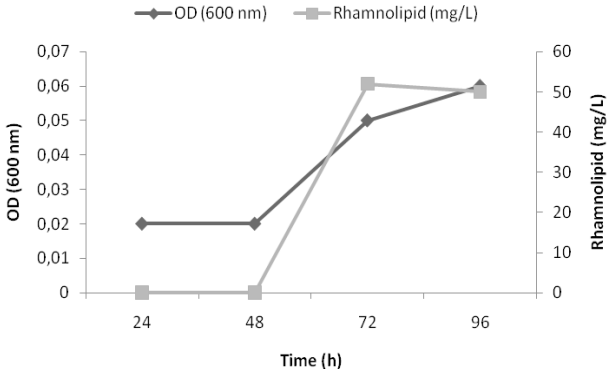

Figure 5. Growth and rhamnolipid production of B15 in gasoline at different times.

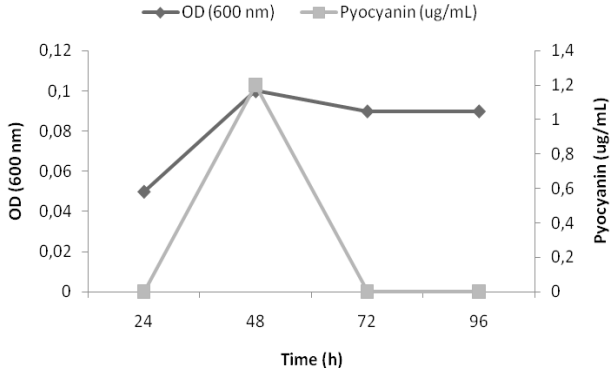

Figure 6. Growth and pyocyanin production of B3 in gasoline at different times.

REFERENCES

[1] Asthana S, Rusin P, Gerba CP. 1997. Infuluence of hydrocarbons on the virulence and antibiotic sensitivity associated with *Pseudomonas aeruginosa*. International Journal of Environmental Health Research, 7: 277-287.

[2] Atlas R.M. 1984. Petroleum microbiology. (New York: " McGraw-Hill)

[3] Atlas R.M. 1997. Handbook of Microbiological Media. (New York:CRC).

[4] Baron SS, Rowe JJ. 1981. Antibiotic action of pyocyanin. Antimicrob. Agents Chemother., 20: 814-820.

[5] Bartha R. 1986. Biotechnology of petroleum pollutant biodegradation. Microbial Ecology, 12:155-172.

[6] Bielicka K, Kaczorek E, Olszanowski A, Voelkel A. 2002. Examination of biodegradation of hydrocarbons in emulsified systems. Polish Journal of Environmental Studies, 11:11-16.

[7] Bodour AA, Maier RM. 2002. Biosurfactants: types, screening methods and application. In: Encyclo. Environ. Microbiol. 2nd edn, Vol.2, ed. , pp.750-770, NewYork: Wiley.

[8] Cerantola S, Bounery JD, Segonds C, Marty N, Montrozier H. 2000. Exopolysaccharide production by mucoid and non-mucoid strains of *Burkholderia cepecia*. FEMS Microbiology Letters, 185:243-246.

[9] Chaillan F, Chaineau CH, Point V, Saliot A, Oudot J. 2006. Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cuttings. Environmental Pollution, 144:255-265.

[10] Christofi N, Ivshina IB. 2002. Microbial surfactants and their use in field studies of soil remediation. Journal of Applied Microbiology, 93:915-929.

[11] Dubois M, Gilles KA, Hamilton JK, Peters PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. Analitical Chemistry, 28:350-356.

[12] Essar DW, Eberly L, Hadero A, Crawford IP. 1990. Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: Interchangeability of the two anthranilate synthases and evolutionary implications. Journal of Bacteriology, 172:884-900.

[13] Hassan HM, Fridovich I. 1980. Mechanism of the antibiotic action of pyocyanine. Journal of Bacteriology, 14:156–163.

[14] Hommel RK. 1990. Formation and physiological role of biosurfactants produced by hydrocarbon-utilizing microorganisms. Biodegradation, 1:107-119.

[15] Jn-Gyung J, Chang-Ho P. 2004. Characteristics of *Rhodoccocus pyridinovorans* PYJ-1 for the biodegradation of benzene, toluene, m-xylene (BTEX), and their mixtures. Journal of Bioscience and Bioengineering, 97:429.

[16] Joshi VK, Attri D, Bala A, Bhushan S. 2003. Microbial pigments. Indian Journal of Biotechnology, 2:370-377.

[17] Machnicka A, Suschka J. 2001. Activity of selected microorganisms and mixtures in BTX biodegradation. Polish Journal of Environmental Studies, 10: 341

[18] Maneerat S. 2005. Biosurfactants from Marine Microorganisms, Journal of Science Technology, 27:1263-1272.

[19] Mulligan CN. 2005. Environmental applications for biosurfactants. Environmental Pollution, 133:183-198.

[20] Norman RS, Moeller P, McDonald TJ, Morris, PJ. 2004. Effect of pyocyanin on a crude-oil-degrading microbial community. Applied Environmental Microbiology, 70: 4004-4011.

[21] Omar S, Rayes A, Eqaab A, Voß I, Steinbüchel A. 2001. Optimization of Cell Growth and Poly (3-hydroxybutyrate) Accumulation on Date Syrup by a *Bacillus megaterium* Strain. Biotechnology Letters, 23:1119-1123.

[22] Onbasili D, Aslim B. 2009. Effects of some organic pollutants on the exopolysaccharides (EPSs) produced by some *Pseudomonas* spp. strains. Journal of Hazardous Materials, 168:64–67.

[23] Osman SF, Fett WF, Fishman ML. 1986. Exopolysaccharides of the phytopathogen *Pseudomonas syringae* pv. glycinea. Journal of Bacteriology, 166:66-71.

[24] Osman M, Ishigami Y, Someya J, Jensen HB. 1996. The bioconversion of ethanol to biosurfactants and dye by a novel coproduction technique. Oil Chemist Society, 73:851-856.

[25] Rashedi H, Assadi MM., Jamshidi E, Bonakdarpour B. 2006. Production of rhamnolipids by *Pseudomonas aeruginosa* growing on carbon sources. International Journal of Environmental Science Technology, 3:297-303.

[26] Raza ZA, Khan MS, Khalid ZM, Rehman A. 2005. Production of Biosurfactant Using Different Hydrocarbons by *Pseudomonas aeruginosa* EBN-8 Mutant. Zeitschrift für Naturforschung C, 61:87-94.

[27] Royan S, Parulekar C, Mavinkurve S. 1999. Exopolysaccharides of *Pseudomonas mendocina* P2d. Letters Applied Microbiology, 29: 342-346.

[28] Schoental R. 1941. The nature of the antibacterial agents present in *Pseudomonas pyocyanea* cultures. British Journal of Experimental Pathology, 22:137–147.

[29] Tuleva BK, Ivanov GR, Christova NE. 2002. Biosurfactant production by a new *Pseudomonas putida* strain. Zeitschrift für Naturforschung C, 57:356-360.

[30] Vardar-Sukan F, Kosarıc N. 2000. Biosurfactants. In: Encyclopedia of Microbiology, 2ed ed. vol.1, pp. 618-635, A-C. Academic Press,

[31] Wackett LP, Hershberger LCD. 2001. Biocatalysis and biodegradation: microbial transformation of organic compounds. pp. 228 Washington ASM Press.

[32] Zhang Y, Miller R M. 1992. Enhanced octadecane dispersion and biodegradation by a *Pseudomonas* rhamnolipid surfactant (biosurfactant). Applied Environmental Microbiology, 58: 3276–3282.

[33] Zhang G, Wu Y, Qıan X, Meng Q. 2005. Biodegradation of crude oil by *Pseudomonas aeruginosa* in the presence of rhamnolipids. Journal of Zhejiang University, 8:725-730.