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Surfactant effects on biodegradation of polycyclic aromatic hydrocarbons

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ABSTRACT

The aim of this work was to evaluate the effect of different surfactant type on biodegradation of low molecular weight polycyclic aromatic hydrocarbons (PAHs) (naphthalene and anthracene) in aqueous media by bacterial strain isolated from crude oil-contaminated soil in southern of Algeria. The biodegradative ability of the strain tested was determined by measuring OD 600 and the residual PAH concentration by UV–Vis and GC. The results indicated that 1C strain could degrade both naphthalene and anthracene without addition of surfactants; the degradation rate of PAHs was decreased when surfactants were added. The higher bacterial growth observed in the presence of Tween 80 could be due to the fact that this surfactant can be used as an additional carbon source by 1C strain.

Keywords: Biodegradation; Naphthalene; Anthracene; Surfactant; SDS; CTAB; Tween 80

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that consist of two or more fused aromatic rings in various structural configurations and constitute an important fraction of petroleum hydrocarbons [1]. PAHs are ubiquitous environmental pollutants. Excessive inputs from anthropogenic activities have caused serious contamination and adversely affect the health of aquatic species and humans through bioaccumulation [2–4]. Because of their potential carcinogenicity and mutagenicity, the Environmental Protection Agency has classified PAHs as priority pollutants.

Their elimination could be achieved by physical processes like volatilization, photooxidation, and chemical oxidation, but the better way to clean up PAHs contaminated sites is the microbial biodegradation [5]. Bioremediation, a safe, environmentally friendly, and effective method, uses the ability of organisms, such as bacteria, fungi, algae, or plants, to reduce concentrations of PAHs to an acceptable level by transforming them into less toxic forms or to completely mineralize them into CO_2.

Nevertheless, more PAHs are hydrophobic and have the tendency to adsorb organic materials. In consequence, they present low bioavailability for bacteria and remain particularly persistent in natural environments [6]. Chemical surfactants could increase the

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bioavailability and consequent biodegradation of PAHs. The surfactants assemble into micelles at the critical micelle concentration, and the interior of the micelles provides a hydrophobic environment to solubilize nonpolar compounds such as hydrocarbons.

Several studies revealed that, at laboratory conditions, anionic surfactants (Dowfax, 8390) and non-ionic (Triton X100, Tergitol NP10, Tyloxapol, Brij 35….) can reduce the adhesion of bacteria to hydrophobic surfaces and inhibit bacterial growth. However, cationic surfactants enhance the mobility and apparent solubility of PAHs but they are toxic for micro-organisms [7,8]. The addition of surfactants increases degradation time of naphthalene. Cetyltrimethyl ammonium bromide (CTAB) and SDBS inhibit naphthalene by *Pseudomonas* [9,10].

In the present study, the effect of the cationic cetyltrimethyl ammonium bromide (CTAB), the anionic sodium dodecyl sulfate (SDS), and the nonionic surfactant Tween 80 on the biodegradation of naphthalene and anthracene by the isolated strain were investigated.

2. Materials and methods

2.1. Micro-organism and cultivation conditions

1C bacterial strain used in this study was isolated from a crude oil-contaminated soil (Hassi Messoud, Algeria), it was isolated after enrichment of culture in minimal medium with crude oil as the only carbon and energy source [11,12].

The isolate was grown in Luria–Bertani broth medium composed (g/L): peptone, 10; yeast extract, 5; NaCl, 5; and pH 7 for 24 h at 45˚C. This culture was used as stock culture inoculum (1%, v/v).

Degradation tests were determined in minimal medium containing (g/L): 0.1 yeast extract, 0.4 NH_4Cl, 0.3 K_2HPO_4, 10 NaCl, 0.33 $MgCl_2{\cdot}6H_2O$, 0.05 $CaCl_2{\cdot}2H_2O$, and 1 mL of trace elements solution containing (g/L): 0.25 H_3BO_4, 0.5 $CuSO_4{\cdot}5H_2O$, 0.5 $MnSO_4$ H_2O, and 0.06 $NaMoO_4$ et 0.7 $ZnSO_4{\cdot}H_2O$. The pH of the medium was adjusted to 7.0 ± 0.2.

2.2. Bacterial growth on surfactants

The degradation of surfactants by 1C strain was carried out by adding 0.02% (w/v) of SDS, 0.02% (w/v) of CTAB, and 0.02% (v/v) of Tween 80 in 100 mL of minimal medium containing 1 mL of inoculum. 1C strain growth was evaluated by measuring the optical density at 600 nm.

2.3. Naphthalene and anthracene biodegradation

One milliliter of 1C strain was inoculated into 100 mL of minimal medium in 250 mL Erlenmeyer flask. The concentration of surfactants was 0.02% (w/v) of SDS, 0.02% (w/v) of CTAB and 0.02% (v/v) of Tween 80. Naphthalene and anthracene were added with an initial concentration of 100 mg/L. Stock solutions for PAHs were prepared by dissolving them in hexane, before use. The solutions were added to the flasks to give the desired final concentrations, which were then left for 3–4 h in a sterile cabinet until all the solvents had evaporated. Erlenmeyer flasks were incubated at 45˚C and 150 tr/min in dark for 3 d.

Control was carried out without surfactant and an abiotic control without bacterial inoculums was performed to evaluate PAHs depletion.

Residual PAHs were extracted from the minimal medium with an equal volume of hexane. The detection of naphthalene and anthracene in hexane solution was performed using UV–Vis spectroscopy by measuring the absorbance.

The same extracts were used to quantify residual naphthalene and anthracene by gas chromatograph equipped with flame ionization detector and column SE-30. Nitrogen served as the carrier gas at a flow rate of 30 mL/min. The column temperature was 175˚C, detector and injector temperature were 200˚C each.

2.4. Statistical analysis

The analysis of variance (ANOVA) was performed and variability was considered only when calculated *F*-value was greater than the tabulated *F*-value at p is less than or equal to 0.05.

3. Results and discussion

3.1. Bacterial growth on surfactants

Growth of 1C strain using cationic, anionic, and non-ionic surfactants as carbon and energy sources is shown in Fig. 1.

Fig. 1 illustrates the bacterial growth of 1C strain in the presence of different surfactants. No growth of micro-organism was observed with CTAB and SDS, indicating that 1C strain could not use these two surfactants as sole carbon and energy sources. However, micro-organisms can utilize Tween 80 as sole carbon and energy source.

Doong and Lei reported that Brij 35 and Tween 80 inhibited the growth of *P. putida*, but this strain can use Triton X-100 and Brij 30 as sole carbon and energy source [13].

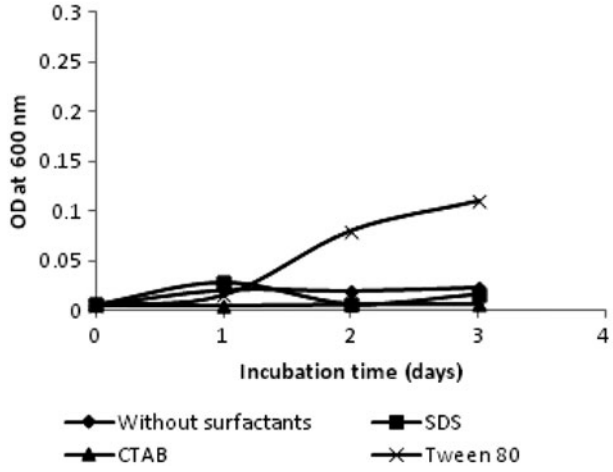

Fig. 1. Bacterial growth of 1C strain on surfactants.

3.2. Naphthalene biodegradation in the presence of surfactants

Many studies reported that surfactants can increase the bioavailability of hydrocarbons and facilitate their uptake. Results displayed in Fig. 2 show CTAB, SDS, and Tween 80 effects on bacterial growth and naphthalene biodegradation by 1C strain.

The results obtained showed that only CTAB surfactant has a positive effect on naphthalene biodegradation by 1C strain. Naphthalene degradation was decreased when Tween 80 was added compared with the results obtained without surfactants addition. 1C strain would preferentially utilize Tween 80 as the carbon source over naphthalene. SDS addition increased bacterial growth, but it does not have a significant effect on naphthalene degradation. However, CTAB addition enhanced bacterial growth and naphthalene degradation.

A positive effect on bacterial growth was observed with the three surfactants tested, possible reasons for this could be the use of surfactants as an additional carbon source.

Surfactant effect on hydrocarbons biodegradation was described by many authors. Chen et al. reported that SDS addition has not changed naphthalene degradation, in the contrast, rate of degradation was decreased in the presence of T-maz 80 and CA-620 and naphthalene degradation was inhibit by SDBS addition [8].

Similar results were reported by Pathak et al. naphthalene degradation by *Pseudomonas* sp. was decreased in the presence of the three types of surfactants (SDS, CTAB, and Tween 80) [14]. However, Mukesh Kumar et al. reported that both bacterial

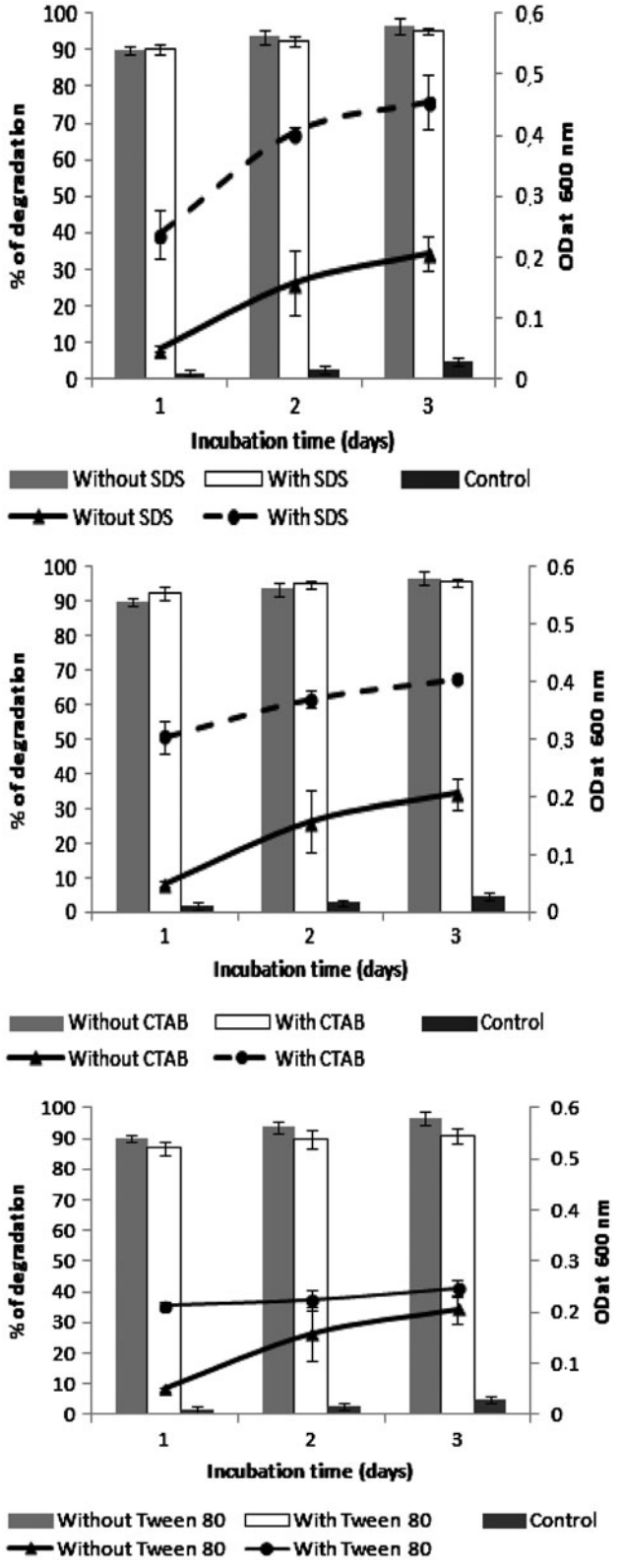

Fig. 2. Surfactant effect on naphthalene degradation and bacterial growth.

growth and naphthalene degradation by *Pseudomonas* sp. PSS6 were increased by Brij 30 addition [15].

Bacterial growth curves showed that 1C strain can use the three surfactants as carbon and energy source with naphthalene.

3.3. Anthracene biodegradation in the presence of surfactants

Anthracene is considered as a hazardous pollutant with low water solubility of 0.04 mg/L at $T = 25$°C. Surfactant-mediated biodegradation is a promising alternative to remove hydrocarbons from contaminated soil. The biodegradation of anthracene in the presence of surfactants is shown in Fig. 3.

In the case of anthracene biodegradation experiments, the best bacterial growth was observed in the presence of Tween 80 when compared to CTAB and SDS. The results showed also that anthracene degradation by 1C strain has not increased by SDS addition, and increased just in the first day of incubation in the presence of CTAB and Tween 80.

Similar results were reported by Deschênes et al. anthracene degradation in soil has not increased by SDS addition [16]. A decrease in anthracene degradation by *P. putida* was observed in the presence of SDS and CTAB [17]. Bautista et al. study revealed that Tween 80 increases naphthalene and anthracene degradation by *Pseudomonas* sp., *Enterobacter* sp., *Stenotrophomonas* sp. strains [18].

The analysis of variance of PAH degradation and bacterial growth are shown in Table 1.

The results indicated that the addition of surfactants had no significant effect on PAHs degradation. A significant effect was observed on bacterial growth on naphthalene-surfactants system.

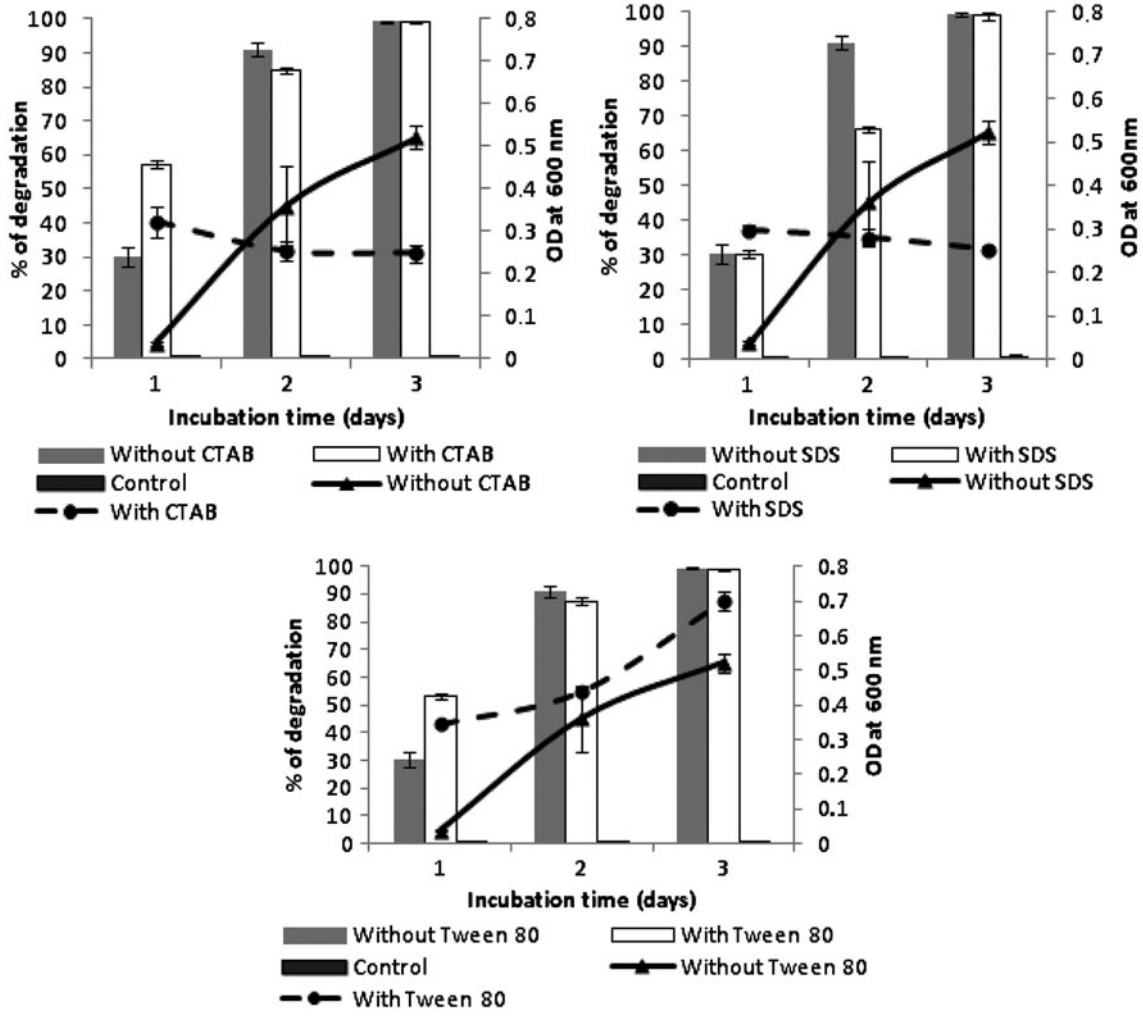

Fig. 3. Surfactant effect on anthracene degradation and bacterial growth.

Table 1
Statistical analysis (ANOVA)

Factor	Source of variation	df	MS	Calculated *F*-value	Table *F*-value	Level of significance
Naphthalene degradation	Surfactant addition	3	15.5385111	2.48376081	4.07	Not significant
	Incubation time	8	6.25604167			
Anthracene degradation	Surfactant addition	3	156.201502	0.1728342	4.07	Not significant
	Incubation time	8	903.765015			
Bacterial growth on naphthalene	Surfactant addition	3	0.03405197	5.2874965	4.07	Significant
	Incubation time	8	0.00644009			
Bacterial growth on anthracene	Surfactant addition	3	0.0417346	1.98084404	4.07	Not significant
	Incubation time	8	0.0210691			

The relationship between PAHs biodegradation and surfactant in pure culture is still not clear. Both positive and negative effects have been reported on surfactants on microbial utilization of PAHs [19]. The positive effects are generally attributable to the increased solubility/dissolution of these compounds by surfactants which enhances their bioavailability.

The negative effects are contributed by a variety of factors, such as toxicity of surfactants to micro-organism, preferential degradation of surfactants, and limited bioavailability of substrate solubilized in surfactant micelles [20].

Triton X100, SDS, and Tween 80 have a positive or negative effect on phenanthrene biodegradation when different micro-organisms are involved [10,21,22] so, the effect of surfactant was also dependent on the specific bacteria used.

Effect of surfactants on the biodegradation of PAHs depends not only on the nature of the surfactant but on the PAH itself. Chen et al. reported that pyrene biodegradation by *B. cepacia* was increased in the presence of Tween 80 [23], similar result was observed by Zhang and Zhu, Tween 80 addition enhanced pyrene biodegradation by *K. oxytoca* [24]. However, a negative effect of Tween 80 on naphthalene biodegradation was reported by Pathak et al. [14].

Rodrigues et al. reported that the same surfactant had different effects on biodegradation of anthracene and fluoranthene. So, it is very important to choose the correct combination between the micro-organisms, the PAHs substrate and surfactant molecule [17].

4. Conclusion

The present study describes the effect of non-ionic, anionic, and cationic surfactants on the biodegradation of naphthalene and anthracene. The results obtained in these experiments show that the effect of surfactants on the biodegradation of PAHs depends on the nature of the surfactant and on the PAH itself. In our case, the addition of surfactants has a positive effect on bacterial growth but a negative effect was revealed in the PAHs biodegradation. Possible reasons for this could be the competitive substrate utilization.

The bacterial strain 1C isolated from crude oil-contaminated soil is able to degrade naphthalene and anthracene without addition of surfactant. The biodegradation potential of this strain gives it an advantage for possible application on bioremediation of water and hydrocarbon-contaminated sites.

Acknowledgments

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