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Airlift bioreactor using a bacterial mixed culture improves hydrocarbon degradation in contaminated salty water

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ABSTRACT

Hydrocarbon pollution in salty media is an important and long-lasting problem worldwide, especially it is not attended to. A mixed culture constituted by *Xanthomonas* sp., *Acinetobacter bouvetii*, *Shewanella* sp. and *Defluvibacter lusatiensis* was employed to degrade a hydrocarbon blend, composed of hexadecane (HXD), phenanthrene (PHE) and pyrene (PYR) (25:1:1 v/w/w). The degradation was assayed in two salty conditions (0–35 g L^{-1} NaCl) and the kinetics were described by the reparametrized Gompertz model. The mixed culture was halotolerant and able to degrade the three hydrocarbons, both in serological bottles and in an airlift bioreactor (ALB) in the presence and absence of salt. Hydrocarbon degradation in serological bottles was preferable to HXD and then to PHE and PYR. The presence of salt decreased the Gompertz parameter values for bacterial growth and hydrocarbon degradation. When salty media were used, the maximum degradation extent diminished in both serological bottles and ALB. Hydrodynamic was fundamental to enhance the hydrocarbon degradation efficiency, that is, pneumatic agitation provided in ALB enhanced the interfacial surface reducing the culture time from 14 to 5 d, almost cancelling the lag time required for hydrocarbon degradation. Our results suggest the use of ALB as a good method to remediate hydrocarbon-contaminated water bodies.

Keywords: Bioremediation; Marine and mangrove ecosystems; Bacterial mixed culture; Polycyclic aromatic hydrocarbon; Airlift bioreactor

1. Introduction

Petroleum hydrocarbons are considered as pollutants due to their negative effects and their persistence in the environment. Incidents of leaking tankers, perforated marine equipment and discharge of wastewater from refineries are sources of hydrocarbon contamination. Nowadays, it is widely recognized that petroleum hydrocarbon contamination has impacted and damaged the world's oceans, seas and coastal zones and represents a constant threat to the planet's health [1]. Recent studies have revealed that the Gulf of Mexico is, nowadays, affected by the presence of polycyclic aromatic hydrocarbons (PAH) due to an accident that occurred in 2010 [2]. On the other hand Lizardi-Jiménez et al. [3] found the presence of several PAH in underwater sinkholes associated to tourist activities in Quintana Roo, Mexico; PAH could be transported to mangroves and finally to sea, leaving traces of pollution along three types of water bodies with different salty conditions: underwater sinkholes, mangroves and seawater. The most of reports have mostly focused on the degradation of alkanes, such as hexadecane (HXD), since these are the main components of crude oil. However,

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PAH constitute an important oil fraction, with harmful [4] mutagenic and carcinogenic effects [5]. The PAH constitute a class of hazardous organic chemicals consisting of more than one fused benzene ring. They are products of combustion processes and petroleum refining. Two meaningful molecules of the PAH group are phenanthrene (PHE) and pyrene (PYR) in which volatility is negligible at environmental pressure and temperature.

A wide range of microorganisms have been reported to be involved in the degradation of aliphatic and aromatic hydrocarbons, for example, *Acinetobacter, Bacillus, Mycobacterium, Nocardia, Pseudomonas, Rhodococcus, Sphingomonas, Streptomyces, Vibrio* and *Xanthomonas* [6]. The hydrophobic nature of hydrocarbons requires microorganisms, or consortia of these, with specific mechanisms of substrate consumption. Some authors mention the degradation of HXD by *Acinetobacter* [7], *Rhodococcus equi* [8] and *Pseudomonas aeruginosa* [9]. In the degradation of PHE, some species of the genus *Pseudomonas*, *Mycobacterium* and *Sphingomonas* have been reported [10]. A variety of bacteria such as *Pseudomonas*, *Flavobacterium* and *Cycloclasticus* have been investigated to check if these can metabolize PYR [11].

The use of bioreactors for bioremediation is a good option, in which excellent degradation efficiency can be obtained, depending on the type of pollutant, the type of reactor and the applied loading rate. Recently, Lizardi-Jiménez et al. [12] proposed the use of airlift bioreactors (ALB) as an effective alternative for ex situ oil-contaminated water bioremediation. ALB have several advantages such as a larger mass transfer capacity without imposing mechanical stress on bacterial cells; moreover, the installation and operation costs are relatively low. Tzintzun-Camacho et al. [13] reported on the degradation of HXD by a defined mixed culture constituted by *Acinetobacter bouvetii*, *Xanthomonas* sp., *Shewanella* sp. and *Defluvibacter lusatiensis*, in which the role of each-one strain within the mixed culture was clearly defined using a bubble column bioreactor; however, the capability of this mixed culture to degrade PAH in a hydrocarbon blend (HCB; aliphatic-PAH) was unknown. Moreover, their hydrocarbon degrading capabilities in variable salty conditions were not demonstrated. Based on the importance of hydrocarbon degradation in marine and mangrove ecosystems, this work aimed to demonstrate the HCB (aliphatic-PAH) biodegradation capability of the aforementioned mixed culture in two different salty media using serological bottles and ALB.

2. Materials and methods

2.1. Mixed culture

Four Gram negative bacterial strains, previously identified as *Xanthomonas* sp., *A. bouvetii*, *Shewanella* sp. and *Defluvibacter lusatiensis* [13] were used. The strains were originally isolated from the rhizosphere of *Cyperus laxus* Lam, a native plant capable of growing in a highly contaminated swamp adjacent to an operating refinery in Veracruz (Mexico) [14]. Strains were separately preserved in trypticasein soy agar (TSA; Bioxon, Mexico) and activated in liquid nutrient broth (Bioxon, Mexico; 30°C, 200 rpm, 48 h) before using.

2.2. Culture media composition

Mineral medium (MM), as described by Lizardi-Jiménez et al. [15], contained (in g L^{-1}): $NaNO_3$, 0.675; K_2HPO_4, 0.215; KCl, 0.113 and $MgSO_4{\cdot}7H_2O$, 0.110. According to Wen-Ta et al. [16], to the MM medium was supplemented with the following trace elements (in g L^{-1}): $CaCl_2{\cdot}2H_2O$, 0.368; $CuSO_4{\cdot}5H_2O$, 0.642; $Na_2MoO_4{\cdot}2H_2O$, 0.604; $CoCl_2{\cdot}2H_2O$, 0.594; $ZnSO_4{\cdot}7H_2O$, 0.718; $FeSO_4{\cdot}7H_2O$, 0.09 and $MnSO_4{\cdot}H_2O$, 0.422. Finally, in order to mimic seawater composition, salt medium (SM), NaCl (35 g L^{-1}) was added to MM [17]. The initial pH was adjusted to 6.5 with 1 N HCl solution.

2.3. Hydrocarbon blend

The HCB consisted of HXD (reagent grade 99%), PHE (96%) and PYR (98%), Sigma-Aldrich, USA, in the ratio of 25:1:1 v/w/w. The HCB was dissolved in dichloromethane (DCM) and added to the medium (MM or SM).

2.4. Hydrocarbon biodegradation

The biodegradation studies were carried out in two experimental devices: (i) serological bottles with 50 mL of medium (MM or SM) and HCB (1.3 g L^{-1}) in which the cultures were maintained (30°C, 48 h, 200 rpm) in a rotatory shaker (Series I 26; New Brunswick Scientific Co., NY, USA) and (ii) a cylindrical glass ALB (7 cm diameter, 50 cm length), provided with a concentric tube (4.2 cm diameter, 20 cm length) located 2.8 cm above the bottom with 1 L of medium (MM or SM) supplemented with 1.3 g L^{-1} of HCB. Pneumatic agitation was supplied by air sparging into the concentric tube at 0.6 cm s^{-1}. For both devices, the four strains were inoculated at a 1:1:1:1 ratio. The total initial bacterial concentration was 1×10^6 CFU mL^{-1}. The cultures were performed at 30°C and 48 h. Analyses were completed in triplicate.

2.5. Analytical methods

2.5.1. Bacterial growth

Bacterial growth was determined by counting viable cells in Petri dishes with TSA. The dishes were incubated at 30°C and counted after 48 h of culture. Bacterial growth was expressed as colony forming units (CFU) mL^{-1}.

2.5.2. Residual hydrocarbons

The samples from serological bottles were washed three times with 50 mL of DCM in separated funnels and allowed to stand for 30 min. On the other hand, the samples from the ALB (10 mL) were mixed with 10 mL of DCM–acetone (1:1 v/v); the mixture was stirred and allowed to stand for 30 min. The concentration of the residual HCB was determined by gas chromatography using a FID detector (Varian; Star 3900 GC; California, USA) and an AT-1HT column (15 m × 0.25 mm × 0.10 μm, Alltech Heliflex, Illinois, USA) with helium as the carrier gas (30 mL min^{-1}; 40 psi). The furnace was heated to 100°C (maintained for 2 min); then, the temperature was increased at 20°C min^{-1} up to 200°C and held at the end for 1 min. The injector and detector temperature was 300°C. The injection volume was 2 μL.

2.5.3. Kinetics of bacterial growth and hydrocarbon degradation

The bacterial growth kinetics were described using the reparametrized Gompertz model proposed by Zwietering et al. [18]. The model is shown in Eq. (1).

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

where y is the dependent variable, defined as the logarithmic ratio between population at time t (N) and the initial population (N_0) (Eq. (2)):

$$y = \ln\left(\frac{N}{N_0}\right) \quad (2)$$

where A is an asymptotic value that represents the maximum population reached during the bacterial growth, μ_m represents the maximum specific growth rate and λ represents the lag time; defined as the abscissa axis intercept of the tangent evaluated in the inflection point, into the exponential growth phase.

The Gompertz model was also reparameterized and used to describe the hydrocarbon biodegradation kinetics according to Yanzhen et al. [19] as shown in Eq. (3):

$$s = 1 - \exp\left\{-\exp\left[\frac{R_m e}{S_0}(\lambda - t) + 1\right]\right\} \quad (3)$$

where s is the dependent variable, defined as the ratio between hydrocarbon concentration at time t (S) and the initial concentration (S_0):

$$s = \frac{S}{S_0} \quad (4)$$

where R_m represents the maximum specific degradation rate and λ represents an equivalent lag time for hydrocarbon degradation.

2.6. Statistical analysis

All determinations were performed in triplicate and are presented as the average value and standard deviation. The obtained data for bacterial growth and hydrocarbon biodegradation were analyzed by non-linear regression using the Marquardt–Levenberg (1944) algorithm; the statistical package IBM SPSS 18 was used. The estimated parameters are presented with the standard error. The estimated parameters were compared by analysis of variance (ANOVA) and multiple comparisons were assessed by the Holm–Sidak method ($\alpha = 0.05$), using the statistical package SigmaPlot 12.5.

3. Results

3.1. Hydrocarbon biodegradation and surface tension in serological bottles

Hydrocarbon biodegradation was corroborated by the determination of microbial growth. The number of CFU mL^{-1} during growth is shown in Fig. 1 for serological bottles in both culture media (MM and MS). Growth was clearly observed, demonstrating that at least one of the hydrocarbons (HXD, PHE and PYR) was used as a bioavailable carbon source. The observed colonies in Petri dishes showed that *A. bouvetii* and *Xanthomonas* sp. were predominant (results not shown); however, a mixed culture, including all four strains, acts as synergistic hydrocarbon degrader [13]. Fig. 1 shows that, for both culture media, bacterial growth was higher during the first 5 d without a significant lag phase. The growth kinetics (Table 1) were significantly lower using SM than MM, that is, the estimated values for μ_m and A were decreased by 33% and 15%, respectively, in the presence of salt.

Hydrocarbon degradation (Fig. 2) was preferable for HXD for which a significant lag phase was not observed (Table 1). Fig. 2(a) shows that the HXD degradation rate was highest during the first 3 d; then, the rate decreased. The lag phase for PHE and PYR degradation was close to 2.5 d (Table 1), that is, PAH were degraded after aliphatic degradation ceased. This could be due to minimal specific hydrocarbon surface and PAH were dissolved into the superficial organic phase in the rotatory shaker. When HXD concentration decreased, PHE and PYR were transferred to the aqueous phase and degraded. The estimated maximum degradation rate (R_m) for HXD was decreased by 60% with respect to MM in the presence of salt (SM), while the R_m for PHE and PYR were not significantly affected. However, the maximum degradation (A) decreased by 11.6%, 24.9% and 27.1% for HXD, PHE and PYR, respectively, with respect to MM, in the presence of salt.

Since the strain *A. bouvetii* has been previously reported to be a bioemulsifier producer [13], and an apparent emulsified pseudo-phase was also visualized, surface tension was measured during the hydrocarbon degradation assays. Fig. 3 shows surface tension throughout the culture time. Surface tension was significantly increased in the presence of salt, suggesting the importance of improving the culture hydrodynamics.

3.2. Hydrocarbon biodegradation in the ALB

As described in the previous section, hydrocarbon assimilation (degradation) was demonstrated by bacterial growth;

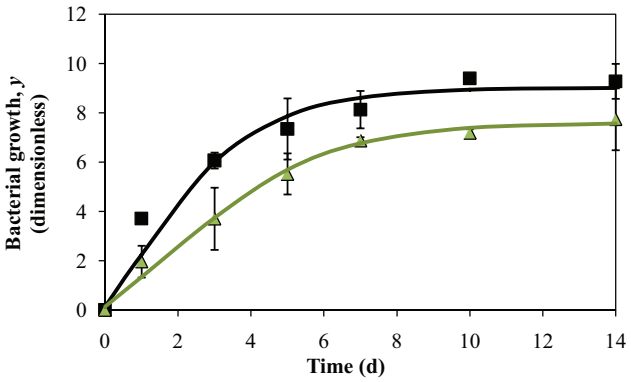

Fig. 1. Bacterial growth kinetics in serological bottles using both: MM (-■-) and SM (-▲-) culture media. Each value is the mean of three replicates ± standard deviations (error bars). Continuous curves represent the reparametrized Gompertz estimated growth kinetics.

Table 1
Kinetic Gompertz parameters estimated for bacterial growth and hexadecane, phenanthrene and pyrene degradation in serological bottles (SB) and airlift bioreactor (ALB)

Bacterial growth					
		μ_m (d^{-1})	λ (d)	A (CFU mL^{-1})	R^2
SB	MM	1.79 ± 0.27^{b}	0.00 ± 0.36^{b}	9.02 ± 0.35^{a}	0.93
	SM	1.20 ± 0.17^{c}	0.00 ± 0.43^{b}	7.64 ± 0.36^{b}	0.93
ALB	MM	2.93 ± 0.52^{a}	0.41 ± 0.27^{a}	8.83 ± 0.49^{a}	0.92
	SM	5.47 ± 2.47^{a}	0.49 ± 0.29^{a}	7.32 ± 0.43^{b}	0.88
HXD biodegradation					
		R_m (d^{-1})	λ (d)	A (%)	R^2
SB	MM	0.62 ± 0.17^{b}	0.10 ± 0.23^{d}	89.2 ± 2.5^{a}	0.87
	SM	0.24 ± 0.04^{c}	0.00 ± 0.22^{d}	78.8 ± 2.3^{b}	0.95
ALB	MM	1.31 ± 0.32^{a}	0.08 ± 0.10^{d}	93.0 ± 2.4^{a}	0.93
	SM	0.95 ± 0.02^{a}	0.14 ± 0.09^{cd}	92.0 ± 2.2^{a}	0.96
PHE biodegradation					
		R_m (d^{-1})	λ (d)	A (%)	R^2
SB	MM	0.25 ± 0.30^{c}	2.27 ± 0.20^{b}	80.0 ± 2.2^{b}	0.98
	SM	0.22 ± 0.05^{c}	2.54 ± 0.25^{b}	60.1 ± 2.0^{d}	0.97
ALB	MM	1.24 ± 0.12^{a}	0.28 ± 0.02^{c}	92.0 ± 0.1^{a}	0.99
	SM	0.28 ± 0.08^{c}	0.00 ± 0.33^{d}	62.6 ± 4.4^{c}	0.85
PYR biodegradation					
		R_m (d^{-1})	λ (d)	A (%)	R^2
SB	MM	0.32 ± 0.03^{c}	2.82 ± 0.14^{a}	74.4 ± 1.4^{bc}	0.99
	SM	0.29 ± 0.10^{c}	2.51 ± 0.24^{ab}	54.2 ± 2.1^{e}	0.95
ALB	MM	1.20 ± 0.28^{a}	0.38 ± 0.16^{c}	90.2 ± 1.1^{a}	0.99
	SM	0.25 ± 0.12^{c}	0.09 ± 0.51^{cd}	51.8 ± 5.4^{e}	0.72

Note: All parameters are presented as estimated value ± standard error.

Fig. 4 shows the bacterial growth kinetics using ALB. First, it can be observed that the culture time was reduced from 14 to 5 d due to the hydrodynamics (pneumatic agitation) within the bioreactor. This is related to the estimated maximum growth rate, which was 1.68-fold higher than observed using serological bottles with MM and 4.56-fold higher with SM. Apparently, bacterial growth rate was higher using SM than MM during the first 3 d; however, since the standard error associated with μ_m, using SM, was 45% based on estimated value, it was not significantly different to μ_m, using MM (Table 1). Fig. 5 shows that hydrocarbon degradation using ALB was non-specific, that is, all three hydrocarbons were degraded from the beginning of culture, according to the Gompertz estimation (λ values were not significantly different, Table 1). However, R_m and A were diminished by the presence of salt: R_m decreased by 27.5%, 77.4% and 79.2% for HXD, PHE and PYR, respectively; A decreased by 1%, 32.6% and 43.3% for HXD, PHE and PYR, respectively, all with respect to the estimated values for MM. Same as experiments in serological bottles, in the ALB, surface tension was not significantly changed along the culture time.

4. Discussion

Due to the complexity of oil product composition, several enzymatic capabilities are necessary to achieve significant degradation. The presence of various microorganisms provides enhanced versatility of metabolic pathways, which can promote the synergistic degradation of hydrocarbons and their intermediary compounds, resulting in enhanced biotransformation and mineralization [20]. Previous studies in our laboratory, using the same mixed culture, showed that HXD was the limiting substrate due to its low bioavailability [21]. Lizardi-Jiménez et al. [15] simultaneously evaluated transfer rates to oxygen and HXD over time in ALB, concluding that the transfer of HXD to the aqueous phase limited biodegradation; similar results were observed by Tzintzun-Camacho et al. [13].

In this work, the mixed culture was capable of growing in SM, demonstrating it is halotolerant and able to grow in

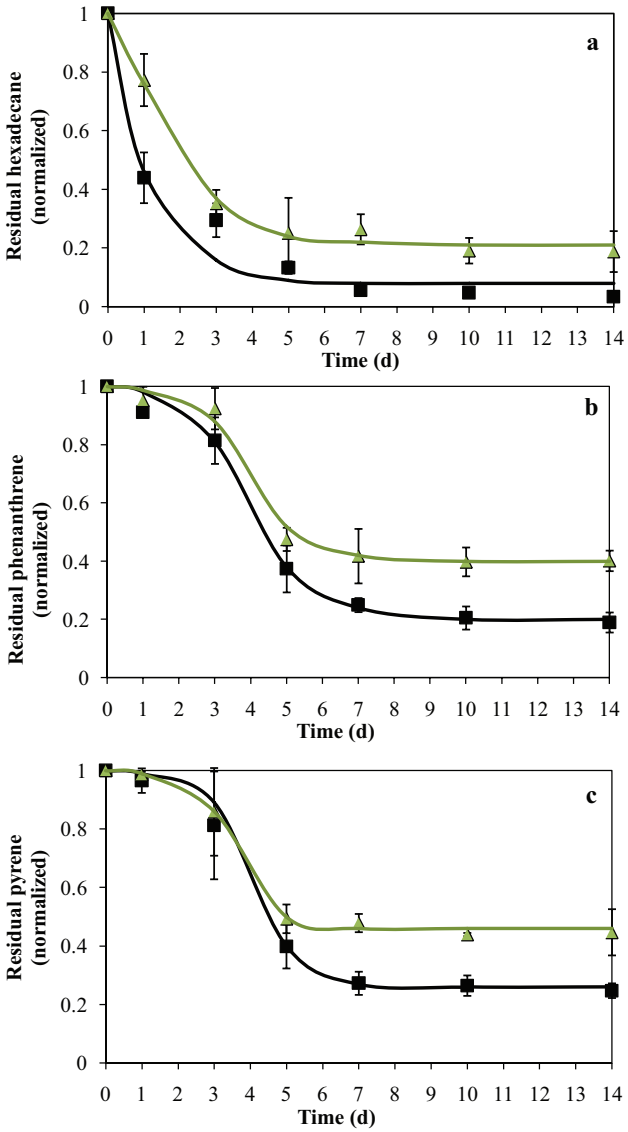

Fig. 2. Hexadecane (a), phenanthrene (b) and pyrene (c) biodegradation kinetics in serological bottles using both: MM (-■-) and SM (-▲-) culture media. Each value is the mean of three replicates ± standard deviations (error bars). Continuous curves represent the reparametrized Gompertz estimated hydrocarbon biodegradation kinetics.

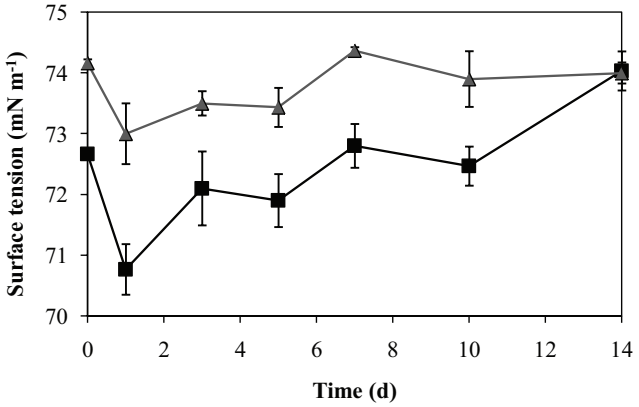

Fig. 3. Surface tension kinetics in serological bottles using both: MM (-■-) and SM (-▲-) culture media. Each value is the mean of three replicates ± standard deviations (error bars).

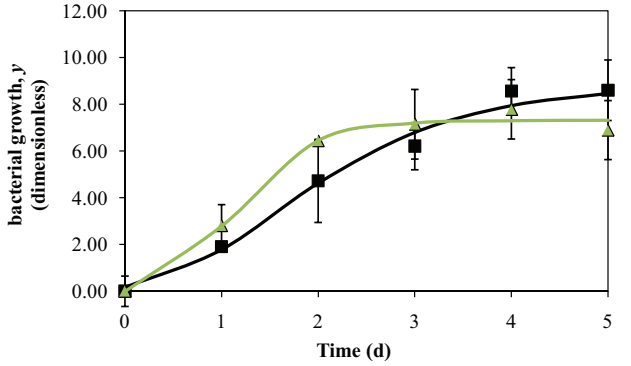

Fig. 4. Bacterial growth kinetics in airlift bioreactor in both: MM (-■-) and SM (-▲-) culture media. Each value is the mean of three replicates ± standard deviations (error bars). Continuous curves represent the reparametrized Gompertz estimated growth kinetics.

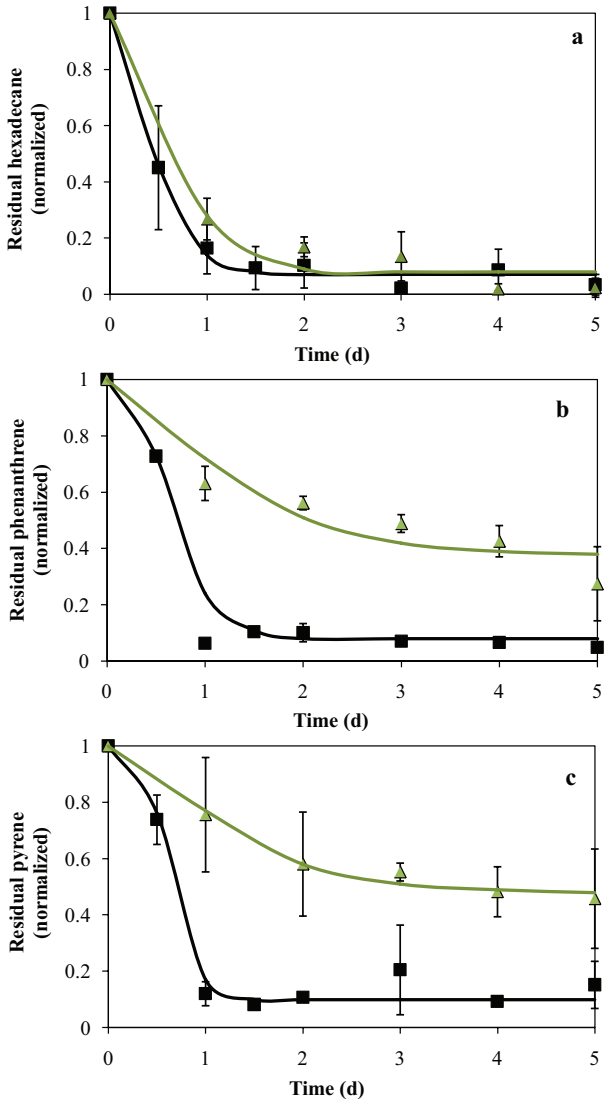

Fig. 5. Hexadecane (a), phenanthrene (b) and pyrene (c) biodegradation kinetics in airlift bioreactor using both: MM (-■-) and SM (-▲-) culture media. Each value is the mean of three replicates ± standard deviations (error bars). Continuous curves represent the reparametrized Gompertz estimated hydrocarbon biodegradation kinetics.

the presence and absence of salt [22]. The genus *Shewanella* has been found as part of the bacterial community in Arctic sea ice [23]. De la Rosa-García et al. [24] reported *Shewanella* sp. as capable of growing in 75 g L^{-1} salt, thus classifying the strain as moderately halotolerant. Vimala et al. [25] isolated *Acinetobacter* sp. from a type of marine sponge, demonstrating its halotolerant nature. The genus *Xanthomonas* grows in salt concentrations lower than 6% [26]. Several species of *Acinetobacter* [27] and *Xanthomonas* [28] have been reported to be alkane degraders. Also, it is known [13] that a mixed culture is capable of degrading HXD and, in our work, it was demonstrated that the same mixed culture also degrades PAH such as PHE and PYR in the presence of salt. Preferential HXD removal by the mixed culture was observed, that is, PAH biodegradation began when more than 50% of HXD had been degraded. Low molecular weight alkanes are biodegraded quickly as compared with multiple branched and long chain alkanes, and the enzymatic systems involved in degradation are varied, for example, monooxygenase and cytochrome P450. The fundamental enzymes that catalyzes alkanes of long chains and PAH could be dioxygenase. *Acinetobacter* belongs to a bacterial genus that produces alkane monooxygenase and dioxygenase enzymatic systems [29]. Doyle et al. [30] reported that the genera *Acinetobacter* spp. and *Xanthomonas* spp. are PAH degraders. When a specificity is observed for the consortium to degrade HXD, PAH are also degraded; this characteristic does not change in the presence of salt, as shown by our results.

In our work, 27 mg of PHE was degraded within 6 d, similar to a report by Tam et al. [31] in which a bacterium from mangrove sediments in Hong Kong was able to remove 20 mg of PHE (initial concentration of 50 mg L^{-1}) within 6 d with 35 g L^{-1} salinity. Other authors have reported on halotolerant bacteria (*Ochrobactrum* sp.) degrading 2.76 mg of PHE (initial concentration of 3 mg L^{-1}) in 4 d with NaCl, in a concentration similar to this work (30 g L^{-1} of NaCl) [32].

Ceyhan [33] used *Proteus vulgaris* to degrade 375 mg L^{-1} of PYR (initial concentration of 500 mg L^{-1}) within 7 d, which is equivalent to 75% degradation. In this study, PYR degradation was similar (350 mg of PYR using MM). Darmawan et al. [5] reported PYR degradation (100 mg L^{-1}) by *Burkholderia fungorum*, *Mycobacterium vanbaleeni* and *Mycobacterium gilvum*, showing high degradation percentages such as 96.2%, 82.2% and 100%, respectively, in 16 d.

The production of a bioemulsifier is usually viewed as an obvious criterion for the existence of emulsion-mediated hydrocarbon uptake. Our results indicate that emulsion-mediated hydrocarbon removal by the consortium occurred after the third day, thereby improving the bioremediation efficiency due to an enhancement of the interfacial surface, thus allowing for a better association between the hydrophobic substrate and the bacterial cell [34].

Previous studies have shown that *A. bouvetii* (used as a pure culture) is capable of producing a bioemulsifier, detected after 2 d of culture, using HXD (13 g L^{-1}) as the only source of carbon and energy [13]. The observed ability of *A. bouvetii* to reduce the surface tension in this work was minimal, probably because its bioemulsifier does not necessarily reduce the surface tension [35]. Although the surfactant facilitates the interaction between hydrophobic and hydrophilic phases, the use of biosurfactant producer bacteria is better than chemical surfactants since a chemical surfactant as Tween 80 can inhibit the microbial growth and biodegradations kinetics [36].

With respect to the use of bioreactors, it is evident that hydrodynamics are fundamental to observe bacterial growth rates and non-specific hydrocarbon high efficient degradation. The reason for non-preferential hydrocarbon degradation when ALB was used can be related with the obtained interfacial surface. In serological bottles, hydrocarbon phase is formed as a scum on the aqueous phase; in contrast, in the ALB the hydrocarbon phase is dispersed in droplets pseudo-homogeneously distributed throughout the aqueous phase. In this work, the degradation time was reduced by up to 35% when ALB was used, and all three Gompertz parameters were better than those computed for serological bottles. Moreover, hydrocarbon degradation is apparently mediated by bioemulsifier production. Wen-Ta et al. [16] characterized the operational conditions and demonstrated that hydrocarbon mass transfer is the limiting step in biodegradation. In order to enhance mass transfer, they proposed a difference in Reynolds number between the aqueous and organic phases of about 3,000; under these conditions, Lizardi-Jiménez et al. [12] proposed the use of ALB for the remediation of hydrocarbon water pollution. The hydrodynamics under operational conditions into the ALB can enhance the interfacial hydrocarbon surface area by forming micrometric droplets; besides, the droplets are covered by the bioemulsifier, stabilizing the extended interfacial surface and then, increasing the biodegradation rates as observed by Sánchez-Vázquez et al. [37] with a protein bioemulsifier of *Aspergillus brasiliensis* degrading an HCB using an ALB.

5. Conclusions

A defined mixed culture, which was previously characterized in HXD biodegradation, was used to biodegrade a hydrocarbon blend composed of HXD, PHE and PYR in an MM supplemented with 35 g of NaCl L^{-1}. The mixed culture was able to degrade all three hydrocarbons in the absence and presence of salt, demonstrated to be halotolerant. Hydrocarbon degradation in serological bottles occurred preferably for HXD and then for PHE and PYR. The presence of salt negatively affected the Gompertz kinetics for bacterial growth and hydrocarbon degradation. The maxima degradation diminished by 11.6%, 24.9% and 27.1% for HXD, PHE and PYR, respectively, using serological bottles and diminished by 1.0%, 32.6% and 43.3% for HXD, PHE and PYR, respectively, using ALB. The bioreactor hydrodynamic played an important role in enhancing the hydrocarbon degradation efficiency. Pneumatic agitation provided in ALB reduced the culture time from 14 to 5 d, almost eliminating the lag time period for PAH degradation. Our results suggest the use of ALB as a good method to remediate hydrocarbon-contaminated ecosystems such as mangroves or seawater.

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