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Bioremediation of naphthalene in water by *Sphingomonas paucimobilis* using new biodegradable surfactants based on poly (ε-caprolactone)

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A B S T R A C T

New amphiphilic block surfactants ABA based on a central segment of polycaprolactone with different molecular composition were evaluated in the bioremediation of naphthalene in water by *Sphingomonas paucimobilis* and compared with sodium dodecyl sulphate as reference surfactant (SDS). Also the biodegradation of the new surfactants by bacteria, *S. paucimobilis* and a mixture of bacteria (*Pseudomonas aureginosa*, *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Bacillus megaterium*) was studied by indirect impedance technique and carbon dioxide determination. All the bacteria biodegraded in solution and micellar phase the central segment of PCL with mineralization rates in the range of 0.024–0.036 mg of CO_2 per day.

S. paucimobilis biodegraded naphthalene in the presence of the new surfactants and GC analysis demonstrated that conversion to products started immediately after inoculum. In all the experiments, except for SDS, at 140 h of incubation time, the remaining naphthalene concentration was about 10% of the initial concentration. In contrast, the production of CO_2 was delayed 4–7 days and values around 75%of naphthalene mineralization degree were achieved in three weeks. The addition of PCL-surfactants, in solution and in micellar phase, not interfered in the naphthalene mineralization. These results have shown promising potential of these biodegradable PCL-surfactants in surfactant-enhanced remediation (SER) technology for removing residual organics from contaminated groundwater and soils.

Keywords:
Biodegradation - Bioremediation - Surfactants - Polycaprolactone - *Pseudomonas* - *Bacillus* - Indirect impedance technique - Naphthalene

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants that are formed during the incomplete combustion of fossil fuels. Due to atmospheric emissions of PAHs, low concentrations can usually be found everywhere. Highly polluted areas are associated with industrial activities (Brown et al., 1999a). Microbial degradation represents the major process responsible for the ecological recovery of PAH-contaminated sites (Cerniglia, 1992); however, the success of bioremediation projects has been limited by the failure to remove high-molecular-weight PAHs (Wilson and Jones, 1993).

Surfactant-enhanced remediation (SER) has been proposed as a promising technology for removing residual organics from contaminated soils and aquifers. These water soluble polymers may increase the solubilization of soil-bound compounds by increasing their desorption rate from soil, solubilizing them into the surfactant micelles (Brown et al., 1999a,b) and/or reducing the interfacial tension, resulting in a larger interfacial area (Liu et al., 1995).

Surfactants are amphiphilic molecules consisting of a hydrophilic part and a hydrophobic part (Banat et al., 2000). The effect of surfactants on pollutants biodegradation depends on a number of factors including the type of surfactant and the applied concentration, the type of contaminants and the identity of the microorganisms present in the medium (Makkar and Rockne, 2003).

Diverse groups of bacteria are able to utilize PAHs that contain less than four benzene rings in their structure, as their sole carbon and energy source. The species involved include *Rhodococcus* sp. (Walter et al., 1991), *Burkholderia cepacia* (Juhasz et al., 1997), *Pseudomonas* (Juhasz et al., 1996; Johnsen et al., 2005), *Stenotrophomonas maltophilia* (Boonchan et al., 1998), *Mycobacterium* sp. (Bouchez et al., 1995), *Alcaligenes denitrificans* (Weissenfels et al., 1990), and *Sphingomonas paucimobilis* (Mueller et al., 1990; Ye et al., 1996). Due to the efficiency of *S. paucimobilis* to biodegrade PAHs of 2–4 condensed benzene rings we have used this bacteria and naphthalene as contaminant, to evaluate the bioremediation in the presence of the new surfactants based on PCL.

Bioavailability of hydrophobic organic contaminants can be enhanced adding surfactants directly to soil *in situ* (Boopathy, 2002) or *ex situ* in bioreactors (Abbondanzi et al., 2006; Tiehn et al., 1997). In many cases, commercial surfactants could pass basically undegraded through wastewater-treatment plants. Hence, the

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biodegradability of surfactants is an important, additional criterion for evaluating these products. Insufficient biodegradation led to the accumulation of big masses of foam in streams and rivers. For example, the branched-chain alkylbenzenesulfonate (ABS) surfactants, which resisted biodegradation, were replaced by linear alkylbenzenesulfonate (LAS) surfactants, which are completely biodegradable (Ghazali, 2002; Kölbener et al., 1995). Also, biodegradable biosurfactants have been used and compared with synthetic surfactants with promising results (Makkar and Rockne, 2003) on bioremediation applications.

In the present work, the biodegradation by bacteria of new amphiphilic block copolymers, previously synthesized (San Miguel et al., 2008), and based on poly(ε-caprolactone) (PCL) was studied by indirect impedance technique. The new structures are ABA-type copolymers and are composed of two segments (A) of water soluble hydrochloride ammonium salt of poly(dimethylaminoethyl) methacrylate (PDMAEMA-H^+) and a central block of PCL (B). Polycaprolactone was chosen as hydrophobic and biodegradable segment since it is commercially available in large quantities and its biodegradation was studied in detail in terms of morphology and microbial variety (Benedict et al., 1983a,b). Their chemical structures and copolymer compositions are detailed in the experimental section. These structures at concentrations above their critical micelle concentration (CMC) can form "polymeric micelles" in water (Fig. 1), consisting of a core formed by the water insoluble part of the macromolecule surrounded by a shell of solvated "blocks".

Micellar aggregates in water can incorporate hydrophobic particles in their core. This behaviour becomes these copolymers as potential carriers of several compounds (San Miguel et al., 2008) and makes them suitable for application in bioremediation of organic compounds in waters. An important advantage of amphiphilic block copolymers is their very low CMCs (see data in Fig. 2). Consequently for the development of stable aggregates in water it is not necessary the use of high amounts of surfactants.

In this work, the biodegradation by bacteria of the new PCL-block copolymers was studied by determination of CO_2 generated using impedance technique and the method previously published (Abrusci et al., 2007). Different concentrations of surfactant, below and above CMC were employed to evaluate the influence of micelle formation on the surfactant biodegradation and the effect in bioremediation of naphthalene in water by *S. paucimobilis*.

2. Materials and methods

2.1. Materials

Polymeric surfactants based on poly(ε-caprolactone) were synthesized (San Miguel et al., 2008) by a "living"/controlled polymerization method (Atom Transfer Radical Polymerization, ATRP). These amphiphilic triblock copolymers ABA type were prepared with a central hydrophobic core of PCL and hydrochloride ammonium salt of poly(dimethylaminoethyl) methacrylate PDMAEMA-H^+, as terminal hydrophilic blocks of different lengths. Polymers will be named as $PCL_{Co}(x)$ where x denotes the percentage (w/w) of hydrophobic block in the copolymers. Also, the minimal concentrations for the microdomain formation, critical micelle concentration (CMC) of the copolymers were determined. As references, Poly(ε-caprolactone) diol (Aldrich, $M_n \sim 2000$ g/mol) and the commercial surfactant of low molecular weight, sodium dodecyl sulphate (SDS) (from Scharlau), were used as-received without further purification. Naphthalene from Aldrich was used as-received. The chemical structures, CMC values and experimental data of micelle diameter (D_m) determined by Dynamic Light Scattering (DLS) of the employed polymers (San Miguel et al., 2008) are shown in Fig. 2.

2.2. Bacterial inoculums and sample preparation

Bacterial inoculums used in all biodegradation experiences consisted of bacterial suspensions of Optical Density (O.D.) 1.0 at 550 nm from culture mediums in the exponential phase. Taking into account correlation between optical density and viable cells number, McFarland index (Murray et al., 1995) the number of cells at that O.D. = 1.0 corresponds to an initial bacterial concentration of 1.25×10^9 cells/ml, approximately. The microbial suspensions for inoculums were prepared from *S. paucimobilis* strain. Also, to confirm the biodegradation of the new surfactants, a mixture of *Pseudomonas aureginosa, Bacillus subtilis, Bacillus amyloliquefaciens* and *Bacillus megaterium,* all of them with identical proportion in the mixture, were used. All bacterial strains used were isolated and identified from cinematographic film in a previous work (Abrusci et al., 2005) except *P. aureginosa* that was obtained from *Colección Española de Cultivos Tipo*, CECT 111 (ATCC 9027).

All biodegradation experiences were carried out in saline solution (NaCl, 0.9% w/w) as solvent at 37 °C. Poly(caprolactone) diol and sodium dodecyl sulphate were used as references. Experiments were done at lower (0.05 g l^{-1}) and higher (0.5 g l^{-1}) concentration than CMC, with the exception of SDS, the employed concentrations were 0.05 g l^{-1} and 1.48 g l^{-1}, since its CMC value is equal to 0.92 g l^{-1}.

To study the biodegradation of PHA, water solutions of naphthalene 0.028 g l^{-1} were used; lower than naphthalene solubility in water 0.031 g l^{-1} (Mackay et al., 1992).

2.3. Indirect impedance measurements

Indirect measurements of impedance were performed on a Micro-Trac 4100 (SY-LAB Geräte GmbH, Neupurkerdorf, Austria). The equipment description and general procedure was previously published (Abrusci et al., 2007). In this work, the bioreactors were filled with 2 ml of surfactant solutions and 0.02 ml of bacteria suspension. In such conditions the concentration was 1.25×10^7 cells/ml. All the experiments were duplicated to assure their reproducibility.

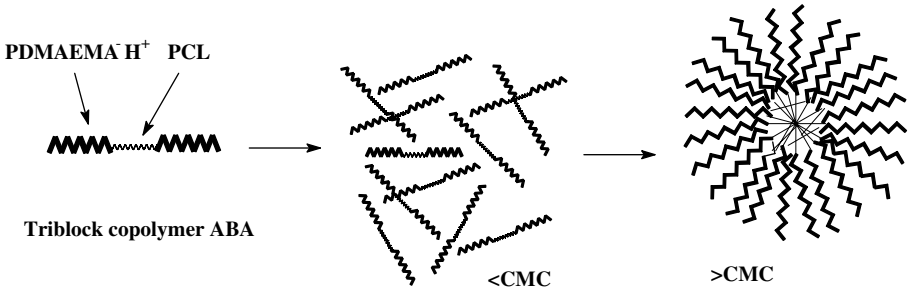
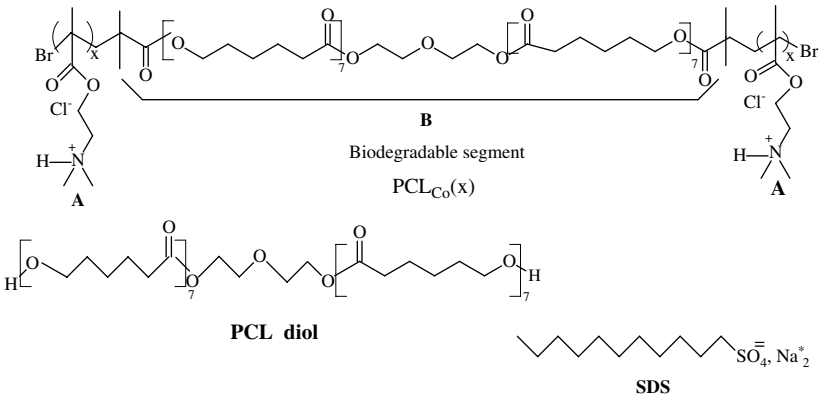


Fig. 1. Schematic formation of micelles in water from molecular solution of the triblock ABA polymeric surfactants; where B is the hydrophobic block (PCL), A is the polyelectrolyte hydrophilic block (PDMAEMA-H^+) and both are surrounded by an external shell constituted by Cl^- counterions.



Surfactant	% PCL	CMC[(a)] in water (g·l^{-1})	CMC[(a)] in 0.9% NaCl sol. (g·l^{-1})	M_n [(b)] (g· mol^{-1})	D_m [(c)] (nm)
SDS	-	0,92	n.d.	288	n.d.
PCL-diol	100	-	-	2.000	-
PCL$_{Co}$(10)	10	0.11	0,14	11.700	30
PCL$_{Co}$(20)	20	0.33	0,24	10.500	28
PCL$_{Co}$(30)	30	1.01	0,26	9.800	17

Data obtained from (San Miguel et al., 2008); (a) Determined by fluorescence spectroscopy, (b) Determined by GPC, (c) Determined by Dynamic Light Scattering (n.d. – not determined)

Fig. 2. Structures and data of the polymeric surfactants.

The device monitors the relative change (each 20 min) in the initial impedance value of KOH solution (0.2% w/w in water), $M_0 = 203\ \Omega^{-1}$, displayed as percentage variation of Media Impedance ($\Delta M\% = (M_0 - M_t) \times 100/M_0$) versus time.

Under our experimental conditions, by titration of the KOH solutions at different time intervals, a linear relationship was obtained between impedance variation and concentration of carbon dioxide.

The percentage of biodegradation of the sample was calculated as a percentage of the ratio between the accumulated amount of CO_2 produced in the biodegradation at time t and the theoretical amount of carbon dioxide (%Biodegradation=$[CO_2]_t \times 100/[CO_2]_{Theo.}$). The theoretical amounts of carbon dioxide ($[CO_2]_{Theo.}$) evolved from the biodegradation of naphthalene and PCL were calculated taking in account their mineralization equations (Andrady, 2000) and their corresponding chemical composition.

2.4. Naphthalene biodegradation in liquid cultures: extraction and gas chromatograph analysis

The conversion of naphthalene to products versus biodegradation time was studied using 50-ml flasks on a shaker and containing 15 ml of the same samples in solution detailed before. Such sealed bioreactors were 3D-shaken with the speed of 24 rpm and incubated at 37 ± 0.1 °C during 8 days. To assure aerobic conditions the flasks were open and closed every 2 days. At different time intervals, 0.5 ml of bacterial cultures was taken from each bioreactor and one equal volume of dichloromethane (DCM) was added to the extracted and vigorously shaken during 1 min. After that, 20 μL of stock dichloromethane-solution of phenanthrene (0.7 g l^{-1}) was added to the mixture as internal standard. To separate the emulsion, the mixture was held at room temperature for 2 h before freezing overnight at −20 °C. Under such conditions the organic phase, containing naphthalene and phenanthrene, was separated and collected for analysis by gas chromatography.

The efficiency of naphthalene extraction was evaluated by comparing the amount of naphthalene and phenanthrene standard recovered from dichloromethane extractions of known amounts of naphthalene added to liquid medium without and with killed bacterial biomass in the employed range of concentrations. The followed extraction procedure was efficient and reproducible in agreement with the results obtained by other authors (Boonchan et al., 2000).

Concentration of naphthalene in dichloromethane solution was determined on a Agilent Technologies 6890 N Gas Chromatograph, equipped with a 5973 quadrupole mass selective detector (Agilent Technologies). A HP-5ms fused-silica capillary column (30 m by 0.25 mm i.d., 0.25 μm) film thickness of 5% poly(-phenylmethylsiloxane) was employed for PHA separation. Helium was the carrier gas, with a gas flow velocity of 0.8 ml/min. The split ratio was 42:1. Injector temperature was 250 °C. Oven temperature programming consisted of a initial temperature of 80 °C held for 2 min, an increase in temperature of 6 °C per minute until 130 °C, and a hold time of 4 min at 130 °C. The mass spectrometer detector was tuned by maximum sensitive autotune. Under these experimental conditions, the peak areas of both internal standards, phenanthrene ($t = 9.30$ min) and naphthalene ($t = 4.39$ min) were used to calculate peak area ratios. For the biodegradation experiments, the naphthalene calibration curve ($A_{sample}/A_{phenantherene}$ versus $C_{naphthalene}$) was linear in the concentration range of 0.2 to 30×10^{-3} g l^{-1} used in this work. In a control experiment without bacteria, the initial naphthalene concentration determined by GC remained constant indicating that naphthalene volatilization could be discarded during the experimental procedure.

3. Results and discussion

3.1. Biodegradation of PCL-surfactants by bacteria

The biodegradation rates of the PCL-block copolymers, of varying composition, were determined onto a period of two weeks

by indirect impedance technique. The biodegradation of the water soluble homopolymer PDMAEMA-H, (A block in the copolymers, Fig. 2), was not observed after three weeks with the bacteria employed in this work. This hydrophilic segment however, could be biodegraded by other microorganisms present in a municipal sewage treatment plant, since it is a member of the family of surfactants based on alkyl dimethyl ammonium halides (Nishiyama et al., 1995). It is believed that their biodegradation begins initially by *N*-dealkylation, followed by *N*-demethylation. In this case, the polymeric nature of the ammonium salt inhibited the biodegradation process, since the chain length of the ammonium salt not only determines the properties of the surfactant but also plays an important role in the aerobic biodegradation (Swisher, 1987). This result confirms that in the biodegradation of the new surfactants, bacteria use the PCL block (B in the copolymers, Fig. 2) as a source of carbon.

The biodegradation study of the surfactants was carried out using not only *S. paucimobilis*, but also by a mixture of bacteria (*P. aureginosa, B. subtilis, B. amyloliquefaciens* and *B. megaterium*) as inoculums due to their ubiquitous presence in aqueous media and activated sludges (Pike and Curds, 1971).

In all the experiments, a linear rate of production of carbon dioxide was observed after a short lag phase of two days. After this period, different linear rates of PCL-biodegradation were observed up to two weeks; the obtained data are summarized in Table 1.

The results revealed that the new surfactants were biodegraded efficiently by *S. paucimobilis* and by the mixture of bacteria. SDS was biodegraded efficiently at concentrations below its CMC with a mineralization rate significantly higher than that observed in a micellar phase. It has been shown that several bacteria, especially from the genera *Pseudomonas*, are able to biodegrade SDS through desulfation and further assimilation (Thomas and White, 1989; Kahnert and Kertesz, 2000). The important decrease in biodegradation rate for SDS, at higher concentration (1.41 g l^{-1}) than its CMC, is in agreement with the results obtained by other authors (Abboud et al., 2007).

From the results (Table 1), biodegradation rates reveal that the microbial degradation of the new PCL-surfactants takes place efficiently when the surfactant concentration exceeds the critical micellar concentration. The production of carbon dioxide when surfactant molecules form micelles is in the order of that observed with the water soluble model PCL-diol and similar to the biodegradation rates obtained at concentrations below aqueous CMC. As expected, the most outstanding features of these results is the confirmation that bacteria, which are present in waters, are capable of biodegrading efficiently the PCL segment of the new block copolymers in micellar solution. This result is important from the applied point of view since the autoassemble of these surfactants can be destroyed by bacteria. The non-biodegraded segment (PDMAEMA-H^+) could be easily separated from the medium by basic pH control and filtration.

As was found in solution, biodegradation of solid PCL in soil, compost and aqueous environment is very efficient; for example, films of 75 μm thickness were biodegraded in soil at ambient temperature and in compost at 60 °C, 100% weight loss in 10 and 2.5 weeks respectively (Müller, 2005). PCL is considered readily biodegradable in such environments and it has been recently proposed as a standard test material (Funabashi et al., 2007).

Table 1
Biodegradation data of PCL-block copolymers by bacteria in saline solution at 37 °C.

Bacteria	Surfactant	Biodegradation rate (mg CO_2/day)	Biodegradation rate (mg CO_2/day)
		[surfactant] = 0.05 g l^{-1} < CMC	[surfactant] = 0.5 g l^{-1} > CMC[a]
S. paucimobilis	SDS	0.020	0.008
	PCL-diol	0.030	0.034
	PCL_{Co}(10)	0.025	0.030
	PCL_{Co}(20)	0.030	0.036
	PCL_{Co}(30)	0.024	0.036
P. aureginosa	PCL-diol	0.050	0.030
B. subtilis	PCL_{Co}(10)	0.032	0.026
B.amyloliquefaciens	PCL_{Co}(20)	0.029	0.026
B. megaterium	PCL_{Co}(30)	0.029	0.029

CMC - critical micelle concentration.
[a] except for SDS, employed concentration 1.48 g l^{-1}, CMC = 0.92 g l^{-1}.

3.2. Biodegradation of naphthalene by S. paucimobilis

Biodegradation of naphthalene was study by gas chromatography and, in parallel, also by an indirect impedance technique to measure the mineralization reaction. Due to the efficient biodegradation of the new PCL-surfactants, the competitive biodegradation of naphthalene and the water soluble PCL-diol was studied first. The results obtained by the indirect impedance technique are plotted in Fig. 3.

S. paucimobilis mineralized naphthalene as a sole carbon and energy source in water (80% of biodegradation in three weeks) and the presence of PCL-diol do not change substantially the rate of carbon dioxide production (0.010 mg of CO_2 per day) after few days (4–7 days) of inhibition time. In contrast the biodegradation of the model product PCL-diol in solution exhibited a higher rate of carbon dioxide production (0.030 mg per day), with only 2 days of inhibition time. All the measurements of naphthalene mineralization showed an inhibition time of 4–7 days before a linear rate of CO_2 production was observed. This delay time is in agreement with the biochemical pathways for the bacterial biodegradation of PAHs, in particular for naphthalene, under aerobic conditions (Eaton and Chapman, 1992). In Fig. 4, a general scheme of the oxidative cleavage of naphthalene induced by bacteria is shown.

Mineralization of naphthalene requires few metabolic steps to produce salicylic acid through dihydroxylated intermediates and following this carbon dioxide production takes place. This oxidative pathway delays the detection of carbon dioxide by the indirect impedance technique. In contrast, a decrease in naphthalene concentration from the medium occurred immediately after inoculum with *S. paucimobilis* as observed from gas chromatographic analysis. In Fig. 5, the evolution of naphthalene concentration is plotted versus incubation time.

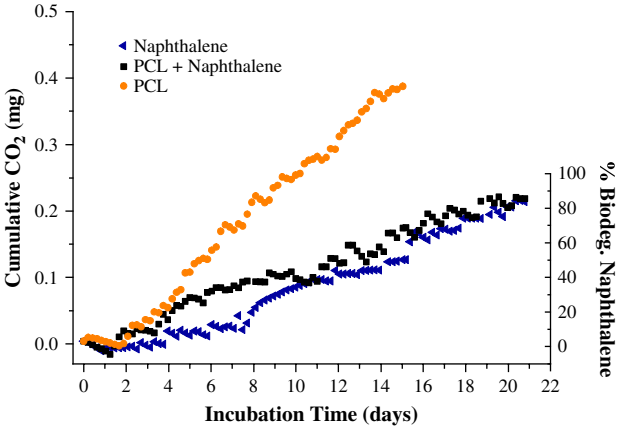


Fig. 3. Accumulated amount of CO_2 produced (left axis) and percentage of naphthalene biodegradation (right axis) in saline solution at 37 °C by *S. paucimobilis*. (Naphthalene, 0.028 g l^{-1} and PCL-diol, 0.05 g l^{-1}).

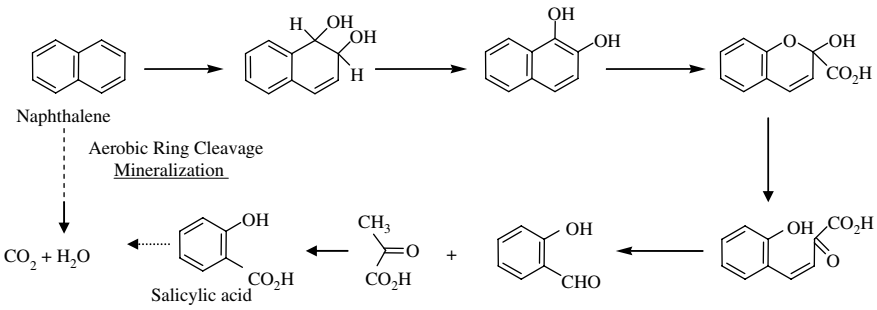


Fig. 4. Scheme of the oxidative mechanism of the aerobic mineralization of naphthalene by the metabolism of bacteria.

The data confirmed that naphthalene is biodegraded efficiently immediately after inoculum and after 140 h of incubation time, while, the remaining naphthalene concentration is about 10% of the initial concentration, except in the case of the SDS. The latter showed a slow down in the naphthalene degradation, and this was especially important at higher concentrations (Fig. 5b) in agreement with other results (Tiehm, 1994).

The GC analysis carried out with the samples containing naphthalene and the new PCL-copolymers exhibited similar decay profiles with slight differences with respect to the naphthalene biodegradation curve in the absence of surfactant. This fact confirms that the presence of the block copolymers in solution and micellar phase does not interfere with the naphthalene biodegradation.

In parallel experiments, the biodegradation of naphthalene was studied by measuring with the indirect impedance technique the accumulated amount of carbon dioxide produced versus incubation time (Fig. 6).

Again, the results indicated that the biodegradation of naphthalene took place efficiently and that 75% of naphthalene mineralization was achieved in three weeks by *S. paucimobilis* in the absence of a surfactant. The production rates of CO_2 in the biodegradation of naphthalene in the presence of the surfactants in solution phase (Fig. 6a), at concentration below their CMC, was similar to that of naphthalene without surfactants (0.010–0.019 mg CO_2 per day), except for copolymer $PCL_{Co}(20)$ of 20% of PCL-content which exhibited a higher CO_2 production rate (0.030 mg of CO_2 per day). This higher rate is probably due to the contribution of the PCL block biodegradation since the accumulated amount of CO_2 was higher than that expected at 100% biodegradation of the naphthalene in the sample.

The most interesting feature was the mineralization of naphthalene in the micellar phase at concentrations higher than their CMC (Fig. 6b), where inhibition times of 2–7 days with different behaviours depending on the content of PCL in the surfactant were observed. Again, the highest mineralization rate (0.031 mg CO_2 per day) has been observed for the surfactant $PCL_{Co}(20)$ confirming the biodegradation of the PCL segment. The addition of a higher concentration of PCL-diol (0.5 g l^{-1}) to the medium increases the mineralization rate (0.026 mg of CO_2 per day) respect to rate at lower concentration (Fig. 6a), indicating that there is a contribution of PCL biodegradation in the CO_2 production rate. SDS in micellar phase also enhanced the mineralization rate of naphthalene, 0.023 mg of CO_2 per day, but only a 50% of naphthalene biodegradation was reached and after 10 days the mineralization was stopped. This behaviour is in accordance with the decrease in naphthalene biodegradation rate observed by GC analysis (Fig. 5b). Similar inhibition was observed at higher concentrations of SDS but under higher biomass levels (Abboud et al., 2007) and explained by the increase in permeability (Lamaza et al., 1991) and alteration of the hydrophobicity (Marchesi et al., 1994) of the membrane cell that induce anionic surfactants at higher concentration. These changes seem to correlate with their biodegradation capacity.

The mineralization rate of naphthalene in the presence of the new PCL surfactants in solution and micelle phase was not affected and similar rate values were obtained to those for naphthalene without surfactants. In accordance with these results, there are different cases in the literature where the use of surfactants, mainly non-ionic, above the critical micelle concentration (CMC) enhanced the biodegradation efficiency (Makkar and Rockne, 2003; Volkering et al., 1995; Boonchan et al., 1998) or just diminished the extent of their biodegradation (Guha et al., 1998). In other cases, surfactants in the micellar phase inhibited the biodegradation of organic contaminants (Laha and Luthy, 1992).

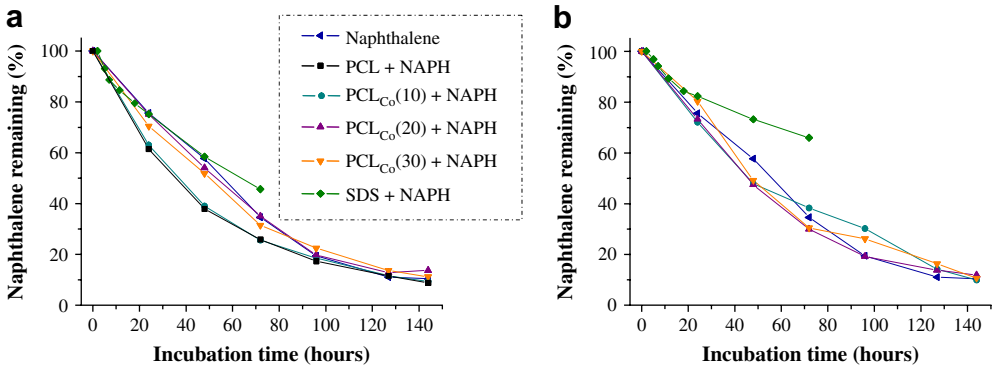


Fig. 5. Decay of naphthalene concentration in percentage versus incubation time with *S. paucimobilis*. ([naphthalene] $_{initial} = 0.028$ g/l). (a) [surfactant] = 0.05 g l^{-1} < CMC, (b) [surfactant] = 0.5 g l^{-1} and [SDS] = 1.48 g l^{-1} > CMC.

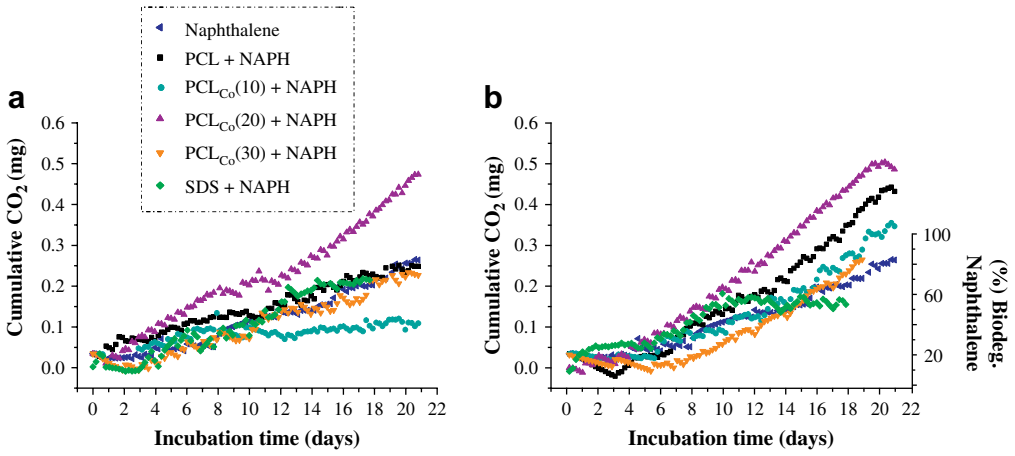


Fig. 6. Accumulated amount of CO_2 produced (left axis) and percentage of naphthalene biodegradation (right axis) on the biodegradation of the mixture naphthalene-surfactant in saline solution at 37 °C by *S. paucimobilis*. ([naphthalene] $_{initial} = 0.028\ g\ l^{-1}$). (a) [surfactant] = 0.05 g l^{-1} < CMC, (b) [surfactant] = 0.5 g l^{-1} and [SDS] = 1.48 g l^{-1} > CMC.

4. Conclusions

The present study represents a systematic investigation of the effects of new surfactant molecular structure on the biodegradation of naphthalene. Two techniques, gas chromatography (contaminant analysis) and indirect impedance technique (CO_2 determination), were used. Our studies have demonstrated that *S. paucimobilis* has the ability to degrade naphthalene. Therefore, this organism may be useful for enhancing the microbial degradation of this contaminant and other PAH at sites contaminated with these chemicals. In bioremediation, toxic concentrations should be avoided, and the new ABA block copolymers based on PCL require a very low concentration to reach their CMC. The central segment of PCL in the new surfactants was biodegraded efficiently by *S. paucimobilis* and by the mixture of bacteria (*P. aureginosa*, *B. subtilis*, *B. amyloliquefaciens* and *B. megaterium*).

Naphthalene in the presence of the new surfactants was biodegraded efficiently by *S. paucimobilis* and the conversion to products started immediately after the inoculum as observed by GC-analysis. After 140 h of incubation time, the remaining naphthalene concentration was about 10% of the initial concentration, except in the case of the SDS that showed a slow down in the naphthalene degradation.

S. paucimobilis mineralized naphthalene as a sole carbon and energy source in water and a value of 75% of naphthalene mineralization degree was achieved in three weeks. Again, the results obtained by indirect impedance technique confirmed that the presence of PCL-surfactants, in solution and in micellar phase, did not interfere in the naphthalene mineralization.

This behaviour in the bioremediation of naphthalene becomes the new biodegradable surfactants based on PCL copolymers as potential carriers of several compounds and makes them suitable for application in bioremediation of organic compounds in waters.

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