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Ecotoxicity and biodegradability of an alkyl ethoxysulphate surfactant in coastal waters

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ABSTRACT

Alkyl ethoxysulphates (AES) are anionic surfactants widely used in numerous commercial and industrial applications. In spite of the high AES volume consumption a few data concerning the occurrence, fate and effects of AES in marine environments are reported in literature. The objective of this study is to evaluate the biodegradability and toxicity of AES in pristine sea water. Ultimate biodegradation was studied according to the guideline 835.3160 "Biodegradability in sea water" proposed by the United States Environmental Protection Agency (USEPA). Acute toxicity of AES was studied to the microalgae *Nannochloropsis gaditana*, *Isochrysis galbana*, *Chaetoceros gracilis*, *Dunaliella salina* and *Tetraselmis chuii* and the invertebrate *Artemia franciscana*, using culture growth inhibition and death, respectively, as effect criteria. During the degradative process two different stages were observed, which were better described with the first order and logistic kinetic models, respectively. Lag times were 3.3 (stage A) and 26.5 (stage B) days whereas half-lives were 18.6 (stage A) and 49.8 (stage B) days. AES inhibited the microalgae growth, with 96-h EC_{50} values ranging from 4.68 g L^{-1} for *D. salina* to 24.02 mg L^{-1} for *I. galbana*. Mean 48- and 72-h LC_{50} values for *A. franciscana* were 38.30 and 23.92 mg L^{-1}, respectively. The results indicate an extensive biodegradability of AES in sea water, although at a very slow rate. Acute toxicity was highly dependent on the species tested, being the green alga *D. salina* the most affected organism. The present study provides relevant data concerning the biodegradability and adverse effects of an AES surfactant on marine organisms, which are useful to establish water quality criteria in a regulatory framework.

1. Introduction

Surfactants are a diverse group of chemicals widely used in many household cleaning detergents, personal care and consumer products (Utsunomiya et al., 1997; Van de Plassche et al., 1999; Sandbacka et al., 2000). Anionics constitute the earliest and most common surfactants (Liwarska-Bizukojc et al., 2005). Historically, linear alkylbenzene sulphonates (LAS) have been the most popularly used synthetic anionic surfactants (Temara et al., 2001; Ying, 2006). However, the importance and use of commercial alkyl ethoxysulphates (AES) have been increasing in the last years. As an example, the annual North American consumption volume of AES in 2003 was estimated to be 491,238 tons exceeding the annual volume of 317,513 tons estimated for LAS (Modler et al., 2004). The European consumption volume of AES surfactants on an active matter basic is estimated to be 276,000 tons/year of which 108,000 tons/year are used in household detergents and cleaning products (HERA, 2002).

AES, also known as alcohol ether sulphates and alcohol ethoxylate sulphates, consist of primary sulfate esters manufactured from the corresponding alcohol ethoxylates with a variable alkyl chain length (hydrophobic group) and a variable number of ethoxylated groups (hydrophilic groups). In addi-

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tion, typical commercial products are complex mixtures of homologues in variable proportion (Feijtel and Van de Plassche, 1995), containing a distribution of alkyl chain length from 12 to 18 carbons with one to five ethoxylated units (Fendinger et al., 1994).

Alkyl ethoxysulphates have been eventually discharged to the aquatic environment either directly or after some sort of wastewater treatment. However, data regarding the occurrence and fate in marine environments have not been reported in great detail. Data found in literature suggest that AES degrade well under aerobic conditions with a comparable primary and ultimate degradation rate to alcohol sulphates (AS) (Scott and Jones, 2000). However, very few data on the fate of AES under anaerobic conditions have been reported. The studies suggest that AES are also readily bioavailable in anaerobic conditions (Itoh et al., 1987; Painter, 1992).

As far as the freshwater environment, the toxicity of AES to freshwater organisms is well known (Yamane et al., 1984; Painter, 1992; BKH, 1994; Dyer et al., 2000; Singh et al., 2002); on the contrary, the effects of AES on marine organisms have practically not been investigated (Fisher et al., 1996; Hampel et al., 2001). In order to judge the significance of these compounds in the marine environment, it is important to collect data on their toxicology using organisms which are representative of the natural flora and fauna (Joy and Joseph, 1995). Typically the most adopted organisms in toxicity assessment are algae and crustacea. Algae constitute the first trophic level, being the basic suppliers of oxygen in the water basin and have been used in water quality assessments as *in situ* biomonitors (Schubert, 1984; Dixit et al., 1992). Furthermore, *Artemia sp.*, a small brine crustacean, has gained popularity in the assessment of the acute toxicity due its availability, easy and good handling and its comparable sensitivity with other planktonic organisms (Henke, 1987a,b; Sanchez-Fortun et al., 1995) and is considered suitable as test organism to assess and describe toxic effects of chemicals (Machera et al., 1996).

In order to assess the possible risks generated by the presence of chemicals in the marine environment the European Chemicals Bureau (ECB) developed the Technical Guidance Document (EC, 2003) in support of Commission Directive 93/67/EEC (EC, 1993), Commission Regulation (EC) No 1488/94 (EC, 1994) and Directive 98/8/EC (EC, 1998). In general, risk assessment is estimated by the systematic and tiered comparison of the predicted environmental concentration (PEC) against the predicted no-effect concentration (PNEC) for each environmental compartment. However, in practice there is rarely sufficient information to calculate these parameters in a detailed and rigorous manner. In absence of experimental data, estimates based on quantitative structure–activity relationships models (QSARs) are used in order to predict physico-chemical properties and toxicity of chemicals (Lipnick, 1995; Verhaar et al., 1995). In contrast, since QSAR is an estimation method and therefore there is a certain probability that the estimate is poor, these estimates should not be the only basis for a risk assessment of a substance. Therefore, the knowledge of experimental chemical, physical and toxicological characteristics of the compound seems to be necessary not only to improve QSARs estimations but also to provide a more complete understanding of the chemical behaviour in the environment (EC, 2003).

The first objective of this study is to investigate the rate and extent of ultimate biodegradation (mineralisation) of the anionic surfactant alkyl ethoxysulphate (AES) under aerobic conditions in pristine sea water. The second aim is to study the acute toxicity of the surfactant on marine organisms; microalgae *Nannochloropsis gaditana*, *Isochrysis galbana*, *Chaetoceros gracilis*, *Dunaliella salina* and *Tetraselmis chuii* and the invertebrate *Artemia franciscana*. Results obtained in the present study can be jointly used with QSARs estimations in order to refine the risk assessment of AES in marine environment.

2. Materials and methods

2.1. Chemicals

The alkyl ethoxysulphate surfactant Empicol® ESB 70/SP (CAS No. 68585-34-2) from Huntsman Surface Science Iberica S.L. (Barcelona, Spain) was tested. The surfactant consists of a mixture of homologues, with an alkyl chain length ranging from 10 to 16 carbons (predominantly C12–C14), two ethoxylated units (Fig. 1) and a reported purity of 70.0±1.0% of active substance. Sodium benzoate and chemicals used for the nutrients solutions were purchased from Fluka Chemie, A. G. (Barcelona, Spain).

2.2. Sampling

Sea water sample used for the biodegradation and toxicity tests was taken from the coastal area of Sancti Petri (Gulf of Cadiz, southwest of Iberian Peninsula) with a Ruttner oceanographic bottle at 0.5 m of depth. The sampling point was located in the external part of the mouth of Sancti Petri tidal channel; a 18-km long inflow–outflow channel which connects the inner part of the Bay of Cadiz with the outlet of the Atlantic ocean (Fig. 2).

2.3. Biodegradation tests

Ultimate biodegradation of the surfactant was studied following the guideline OPPTS (Office of Prevention, Pesticides and Toxic Substances) 835.3160 "Biodegradability in sea water", proposed by the United States Environmental Protection Agency (USEPA, 1998a). The shake flask method was employed in all the biodegradation tests. A positive result in the test (>70% DOC removal before 60 days) might indicate that there is a potential for the biodegradation in the marine environment. However, a negative result does not preclude

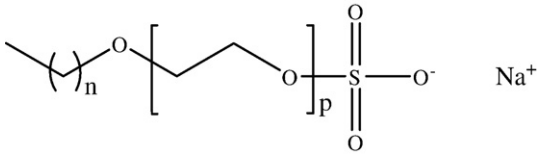

Fig. 1 – Chemical structure of the alkyl ethoxysulphate Empicol® ESB 70/SP (n=9–15; p=2).

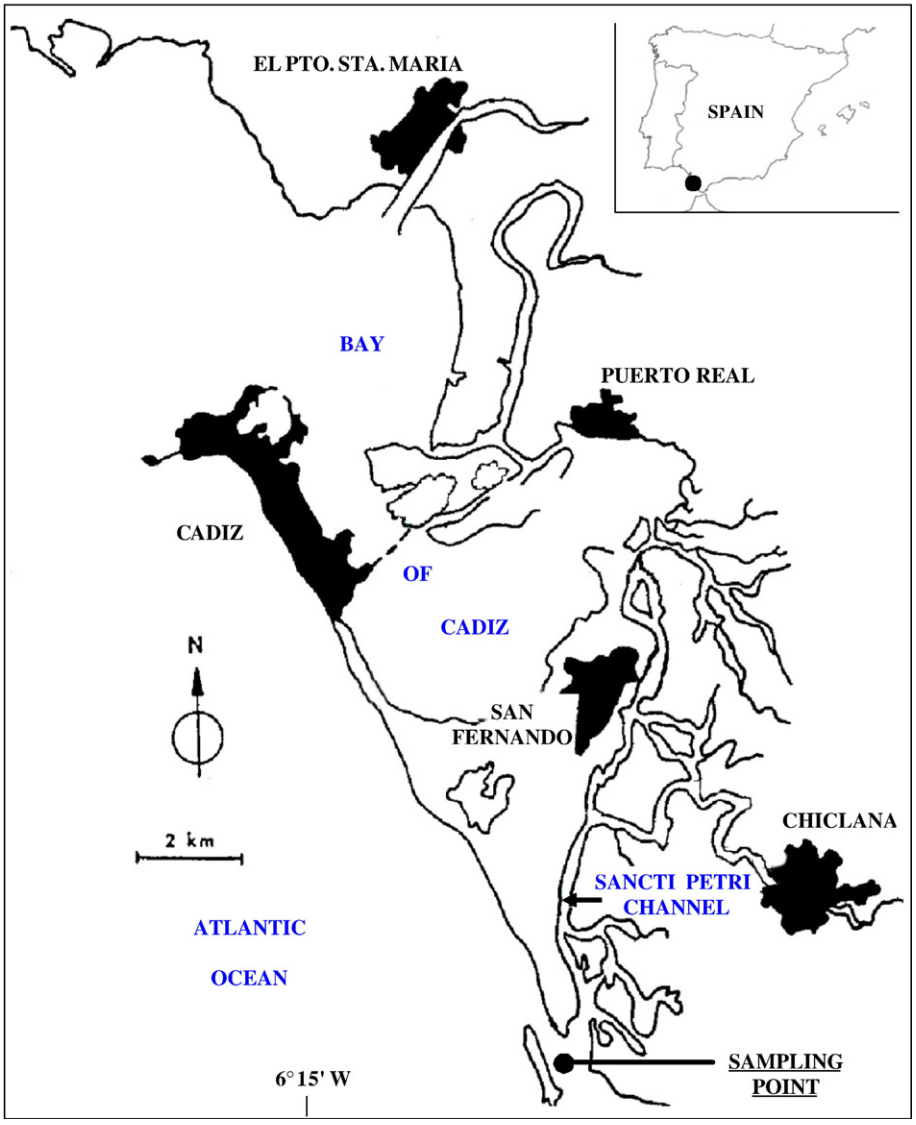

Fig. 2 – Geographic location of the selected sampling point.

such potential but indicates that a further study is necessary (USEPA, 1998a).

Sea water was filtered through 1 μm glass fiber filters, enriched with nutrient solutions (USEPA, 1998a) and acclimated during 24-h in darkness at 20±1 °C. Substrate concentration in the biodegradation tests was determined by means of dissolved organic carbon (DOC) measurements using a Model TOC-5050 Analyzer (Shimadzu, Kyoto). Samples were filtered through a 0.22 μm polyvinylidene fluoride filter (Millipore S.A.) prior to analysis of DOC. The experiment was constituted by (a) a control test, composed only by pre-treated sea water (filtered, enriched with nutrients and acclimated during 24-h), (b) a reference test, formed by pre-treated sea water containing 15 mg DOC L^{-1} sodium benzoate, (c) an abiotic test, composed by pre-treated sea water containing 100 mg L^{-1} of mercury chloride and a surfactant concentration of around 18 mg DOC L^{-1} and (d) a triplicate of surfactant tests, containing pre-treated sea water and an initial surfactant concentration of 18 mg DOC L^{-1}, approximately. The reference test was used to check the microbial activity of the sea water sample whereas the objective of the abiotic test was to ensure that no other removal processes (photo-degradation, adsorption, precipitation, etc) were occurring. All tests were run in 2.5-L amber borosilicate bottles maintained in darkness at constant temperature (20±1 °C) and filled with 1.5-L of test medium. Control parameters (temperature, pH and oxygen concentration) were periodically measured to ensure no limiting conditions for the degradative process.

2.4. *Toxicity tests*

2.4.1. *Algal tests*

The uniculture of the marine microalgae *N. gaditana*, *I. galbana*, *C. gracilis*, *D. salina* and *T. chuii* were obtained from the Andalusian Institute of Marine Sciences, ICMAN-CSIC (Cadiz, Spain). Growth inhibition tests were performed according to standard methods proposed by USEPA (USEPA, 1992, 1996, 1998b, 2002a,b), APHA-AWWA-WPCF (APHA et al., 1992),

Organisation for Economic Co-operation and Development (OECD, 1998) and several authors (Rand, 1995; Kooijman et al., 1996). Inocula were cultivated at 20±1 °C and 24-h light in synthetic sea water (USEPA, 2002a) enriched with a supply of nutrients and vitamins according to the f/2 medium (Guillard and Ryther, 1962) modified with double nitrate and phosphate concentrations (Huertas et al., 2000) and silicate (250 μg/L SiO_2). Cell density was estimated by the optical density of the culture at 690 nm (OD 690 nm). An initial absorbance between 0.200 and 0.300 for both control and test samples was used in order to ensure exponential algal growth. Toxicity tests were performed in 10-mL glass vials containing 2 mL algal inoculum and 2 mL surfactant solution, both prepared in natural sea water and enriched with modified f/2 medium. All vials were incubated at 20±1 °C and exposed under 11,000 Lux light and 24-h photoperiod. After 24, 48, 72 and 96 h the algal density was determined. Ten surfactant concentrations and one control were performed in triplicate for every organism tested.

2.4.2. *Tests with invertebrate A. franciscana*

Acute toxicity tests were conducted according to the standard methods proposed by USEPA (USEPA, 1992, 1996, 1998b, 2002a,b), APHA-AWWA-WPCF (APHA et al., 1992), Organisation for Economic Co-operation and Development (OECD, 1998), American Society for Testing and Materials (ASTM, 2004) and Rand (1995). *A. franciscana* cysts were purchased by the Andalusian Institute of Marine Sciences, ICMAN-CSIC (Cadiz, Spain). Cysts were hatched in 100-mL synthetic sea water (USEPA, 2002a) at 20±1 °C under 11,000 Lux light intensity and slight aeration during 24 h, approximately. The hatched nauplii were separated from their shells and remaining cysts using a Pasteur pipette and transferred to fresh sea water. Ten organisms, contained in less than 50 μL, were pipetted into a glass Petri dish (55 mm diameter). Subsequently, 8-mL of a surfactant solution prepared in natural sea water was added. The tests were carried out in darkness at 20±1 °C. After 48 and 72 h, the number of alive and dead individuals was recounted. Five replicates for each test concentration and the control were performed.

2.5. *Statistical analysis*

Data from biodegradation tests were analyzed using nonlinear regression procedures. In order to determine the most appropriate model the experimental data were fitted to the first-order and logistic models described by Simkins and Alexander (1984) and the biodegradation kinetic model proposed by Quiroga et al. (1999). The best-fit model was selected according to the coefficient of determination (R^2), the biological meaning of the kinetic parameters and the analysis of χ^2. Lag time (t_L), half-life ($t_{1/2}$) and the time starting from the end of the lag phase needed to reach 50% of biodegradation (t_{50}) (USEPA, 1998a) were calculated according to the equations proposed by Perales et al. (2007).

The analysis of χ^2 is based on the calculation of the parameter Q, the computed probability that χ^2 should exceed a particular value by chance, which gives a quantitative measure for the goodness of fit of the model. Low Q values indicate that the apparent discrepancies are unlikely to be chance fluctuations, so the model must be rejected. Likewise, Q values too close to the unity indicate an excellent fit of the model; literally too good to be true (Press et al., 1986). Often, its cause is an overestimation of the measurement errors. In general, a good value of χ^2 for a moderately good fit is $\chi^2 \cong \nu$, where ν is the number of degrees of freedom ($\nu = N - M$, N is the number of data points and M the number of parameters to be fitted) (Press et al., 1986).

The endpoint of toxicity tests using marine algae and invertebrates were based on cell growth and lethal effects, respectively. 96-h EC50 values were calculated by means of point estimation techniques using the ICpin software (Norberg-King, 1988, 1993). Acute mortality data for *A. franciscana* were analysed by the Trimmed Spearman–Karber analysis and expressed as 48- and 72-h LC_{50}. Also 95% confidence intervals were estimated. Experimental no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) values for algae and invertebrates were obtained using a one-way analysis with a hypothesis testing approach such as Dunnett's procedure or Steel's Many-one Rank Test (USEPA, 2002a). Previously, normality and homogeneity of variance were formally tested using the Shapiro–Wilk's Test and Bartlett's Test, respectively. The statistical calculations were conducted using ToxStat software (West and Gulley, 1996).

3. Results and discussion

3.1. *Test medium characteristics*

A summary of the characteristics of the sea water used in this study is presented in Table 1. As can be observed, nitrites, ammonia and phosphate levels were under detection limits. In addition, no presence of faecal streptococcus, faecal coliforms and enterococcus was observed and the concentration of dissolved organic carbon was in the same order of

Table 1 – Selected chemical, biological and physical characteristics of the sea water used in the biodegradation and toxicity tests

Parameter	Units	Value
Dissolved oxygen	% saturation	92.7±1.85
Temperature	°C	12.5±0.3
Conductivity	mS cm^{-1}	50.9±0.25
pH	–	8.14±0.04
Salinity	–	38.5±0.47
Total carbon	mg L^{-1} C	26.9±0.53
Inorganic carbon	mg L^{-1} C	26.4±0.52
Organic carbon	mg L^{-1} C	0.5±0.01
Nitrites	mg L^{-1} NO_2^-	<0.015
Nitrates	mg L^{-1} NO_3^-	0.033±0.009
Ammonia	mg L^{-1} NH_4^+	<0.007
Phosphate	mg L^{-1} PO_4^{3-}	<0.015
Silicates	mg L^{-1} Si	0.021±0.003
Total hardness	mg L^{-1} Ca^{2+}	418±13.9
Chlorophyll a	mg m^{-3} Cl a	0.73±0.12
Faecal streptococcus	CFU 100 mL^{-1}	ND[a]
Faecal coliforms	CFU 100 mL^{-1}	ND[a]
Enterococcus	CFU 100 mL^{-1}	ND[a]

All values are expressed as mean±standard deviation (n=3).
[a] ND=Not detected.

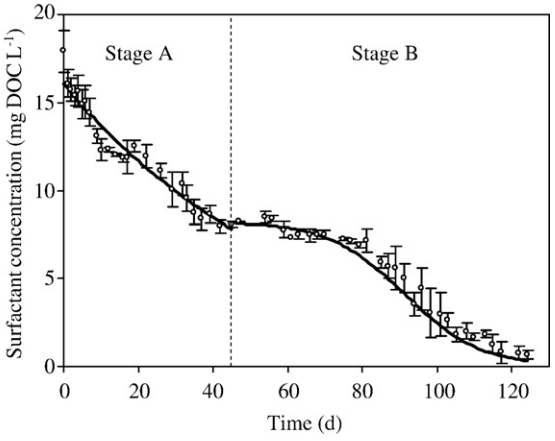

Fig. 3 – Evolution of Empicol® ESB 70/SP concentration (mg DOC L^{-1}) in the biodegradation tests. Experimental data are expressed as mean ± SD (n=3). Solid line represents the fitted curve according to a first order and logistic model for the stages A and B, respectively.

magnitude as those reported for open oceans (USEPA, 1998a). The results obtained from the physico-chemical and biological analyses demonstrate the low human influence of the sampling area.

3.2. *Biodegradation of AES*

Temperature, pH and dissolved oxygen values (mean ± standard deviation, SD) during the biodegradation experiment were within 19.83 ± 0.11 °C, 7.95 ± 0.01 and 81.8 ± 0.85% of saturation, respectively. Sodium benzoate (reference substance) reached biodegradation percentages >50% in 6 days and >90% by 9th day, indicating that the microbial activity of the tested sea water was appropriate (Nyholm and Kristensen, 1992). The mineralisation percentage of the abiotic test was 1.82 ± 3.32%, showing that the contribution of abiotic processes to the surfactant removal seems to be negligible in the biodegradation tests conducted.

Fig. 3 shows the ultimate biodegradation (mineralisation) of the anionic surfactant Empicol® ESB 70/SP at an initial concentration of around 18 mg DOC L^{-1}. Initial substrate concentration decreased by 25% and 60% in 9 and 60 days, respectively. According to the guideline OPPTS 835.3160 (USEPA, 1998a) this result (<70% DOC removal after 60 days) does not preclude that there exists a potential for its biodegradation in the marine environment although it suggests that further studies should be carried out. However, biodegradation percentages >96.5% were observed after 124 days, demonstrating the high extent of ultimate biodegradation of the AES in the test medium although at a very slow rate.

Two different stages were observed during the biodegradation process (Fig. 3). The first stage (stage A) ranged from the initial day to 45th day and no acclimation of the microorganisms responsible of the degradative process was observed. Likewise, the second stage (stage B) ranged between 46th and 124th day and the presence of a significant lag phase was observed. Studies on the AES degradation suggest that the breakdown mechanism consists of a sequential process with the cleavage of an ether bond (hydrolytic reaction) as the most frequent starting step, producing a fatty alcohol or an alcohol ethoxylated and ethylene glycol sulphate of various lengths (Steber and Berger, 1995). Subsequently, the resulting alcohol is degraded by ω- and β-oxidations to the corresponding fatty acid, whereas the ethylene glycol sulphate is degraded stepwise by oxidation and cleavage of two carbon units along with a desulphation (Steber and Berger, 1995). Furthermore, a very short acclimation phase has been described for the nonionic surfactants nonylphenol ethoxylates in pristine water, where the main starting breakdown mechanism is the hydrolysis of the ethoxylated chain (Manzano et al., 1998). In contrast, high lag time values (lag time = 6.67 ± 0.6 days) have been reported for the anionic surfactant linear alkyl benzene sulphonates (LAS) in pristine sea water from the same geographic area than the present study (Perales et al., 2007). In this case, the breakdown mechanism of LAS starts with ω- and β-oxidation reactions catalyzed by oxidative enzymes (Schöberl, 1989; Scott and Jones, 2000). Considering these assumptions, the results from the present study suggest that the first stage of the AES degradation in sea water (Fig. 3) may correspond to the cleavage of ether bonds (hydrolytic reactions). Afterwards (stage B), the oxidative process (ω- and β-oxidation) of the alcohol and ethylene glycol sulphate resulting from the first step may mainly occur.

The experimental data were fitted with the first order (stage A) and logistic (stage B) kinetic models, which are shown as the solid lines in Fig. 3. The kinetic and the associated statistical parameters obtained from both models

Table 2 – Values of the best-fit models parameters and the associated statistical parameters obtained from a nonlinear fit to the kinetic first order (stage A) and logistic model (stage B) for the anionic surfactant Empicol® ESB 70/SP

Model	Kinetic parameter [a]				Statistical parameter			
	S_0 (mg DOC L^{-1})	K_1 (d^{-1})	B_0 (mg DOC L^{-1})	K_{Lg} (mg DOC L^{-1} d^{-1})	R^2	χ^2	ν	Q
First order (stage A)	16.05 ± 1.48	0.016 ± 0.005	–	–	0.9267	36.65	21	0.050
Logistic (stage B)	8.23 ± 1.46	–	0.001 ± 0.004	0.012 ± 0.009	0.9511	24.03	26	0.574

S_0, the initial concentration of surfactant.
K_1, the first-order kinetic constant.
B_0, the concentration of substrate required to produce the initial microbial concentration.
K_{Lg}, the logistic kinetic constant.
[a] Data presented as mean ± SD.

are summarized in Table 2. In the first step (stage A), lag time, t_{50} and half-life estimated were 3.3, 15.3 and 18.6 days, respectively. In addition, lag time, t_{50} and half-life in the second step (stage B) were 26.5, 23.3 and 49.8 days, respectively.

The ultimate biodegradation of AES has been well established under aerobic conditions in OECD 301 test for ready biodegradability. For example, for $C_{12-14}AE_2S$ and C_{12-15}oxo-AE_3S ultimate biodegradation percentages of 58–100% and 96–100% after 28 days have been reported (Schöberl et al., 1988). Moreover, in a closed bottle test a complete ultimate biodegradation (100% ThOD removal) was observed for $C_{12-18}AE_{8.5}S$ (Steber and Berger, 1995). Results obtained from the present study also show an extensive ultimate biodegradation although at a significantly slower rate than in inoculated mineral medium.

3.3. *Toxicity of AES on marine organisms*

The toxicity of the anionic surfactant AES varies considerably among the organisms tested. Fig. 4 shows the algal growth (OD_n, net optical density) exposed to different concentrations of Empicol® ESB 70/SP. A significant inhibition growth was observed for all the algae cultures after a 24-h exposure. After 96-h, the inhibitory effects were much higher, especially for the algae *N. gaditana*, *C. gracilis* and *D. salina*. Furthermore, the

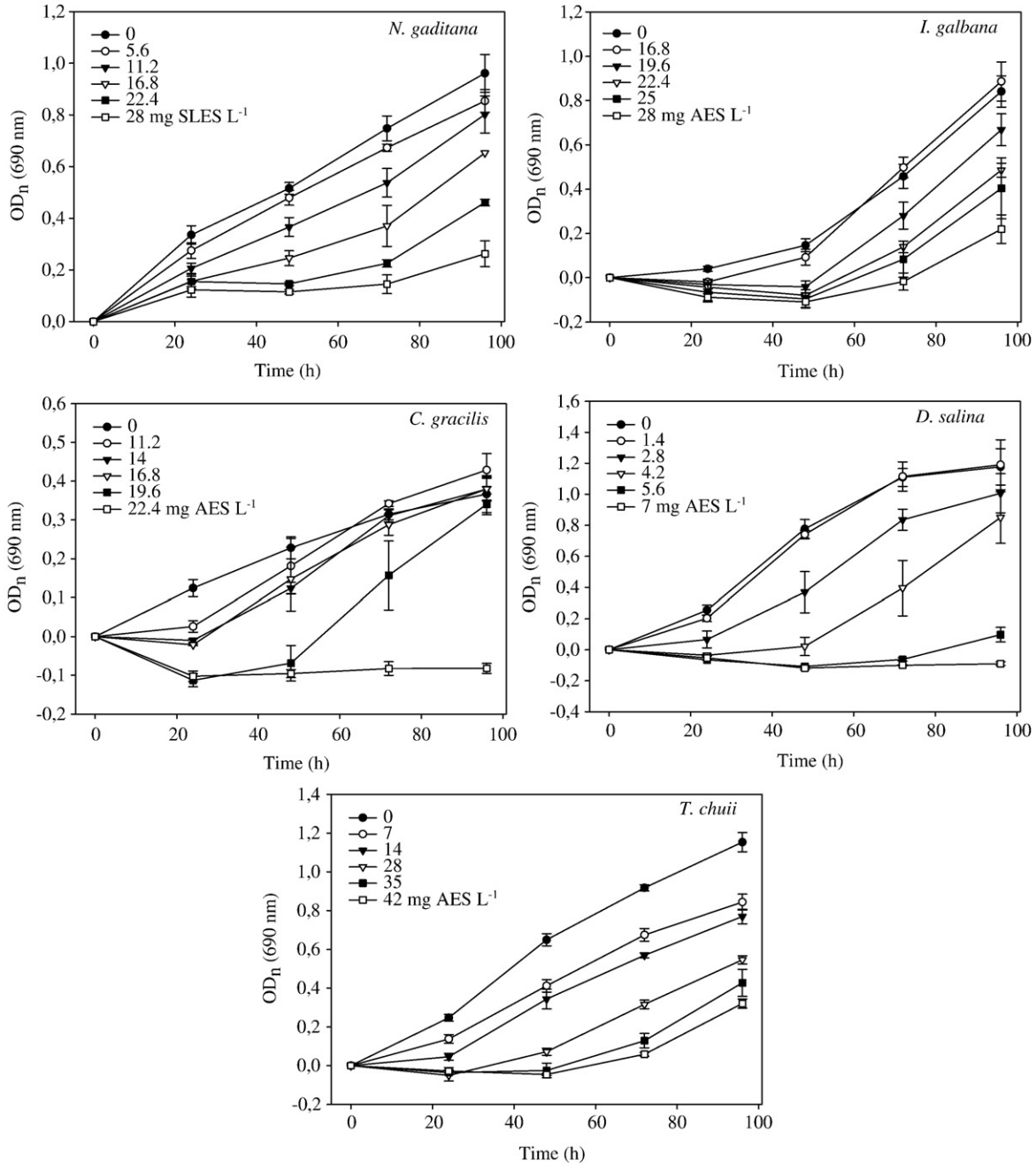

Fig. 4 – Algal growth curves (OD_n, net optical density) exposed to some of the Empicol® ESB 70/SP concentrations tested (mg L^{-1}). Error bars denote standard deviations between 3 replicates.

Table 3 – Acute toxicity data of Empicol® ESB 70/SP on marine algae and invertebrate

Group	Test organism	Endpoint	Concentration (mg L^{-1})
Marine algae	*N. gaditana*	96-h EC_{50}	22.05 (21.10–28.66)[a]
		96-h NOEC	8.40[b]
		96-h LOEC	11.20[b]
	I. galbana	96-h EC_{50}	24.02 (21.39–27.73)[a]
		96-h NOEC	16.80[b]
		96-h LOEC	19.60[b]
	C. gracilis	96-h EC_{50}	20.83 (20.56–21.07)[a]
		96-h NOEC	16.80[b]
		96-h LOEC	19.40[b]
	D. salina	96-h EC_{50}	4.68 (4.09–4.93)[a]
		96-h NOEC	2.80[b]
		96-h LOEC	4.20[b]
	T. chuii	96-h EC_{50}	23.10 (20.46–24.55)[a]
		96-h NOEC	7.00[b]
		96-h LOEC	14.00[b]
Marine invertebrate	*A. franciscana*	48-h LC_{50}	38.30 (34.15–42.45)[a]
		72-h LC_{50}	23.92 (19.59–28.26)[a]
		48-h NOEC	4.90[b]
		48-h LOEC	9.80[b]
		72-h NOEC	4.90[b]
		72-h LOEC	9.80[b]

[a] Concentration, mg L^{-1} (95% CI).
[b] Effect concentration based on tested concentrations.

mortality of the culture was detected for all algae tested after a 24-h exposure, except for *N. gaditana*. However, after a 48-h exposure a growth recovery of the cultures was appreciated. In the case of *I. galbana* and *T. chuii* the growth recovery was observed for all the surfactant concentration tested. On the contrary, for *C. gracilis* and *D. salina* the growth recovery occurred only at surfactant concentrations equal or lower than 22.4 and 7 mg L^{-1}, respectively.

The acute toxicities of Empicol® ESB 70/SP to marine algae and invertebrate are summarized in Table 3. The 96-h EC_{50} value estimated for *D. salina* was notably lower than for the other species tested. Similar mean 96-h EC_{50} values can be noticed among the other microalgae (*N. gaditana*, *I. galbana*, *C. gracilis* and *T. chuii*). Mean 96-h NOEC values ranged from 2.80 mg L^{-1} for *D. salina* to 16.80 mg L^{-1} for *I. galbana* and *C. gracilis* whereas 96-h LOEC values ranged from 4.20 mg L^{-1} for *D. salina* to 19.60 mg L^{-1} for *I. galbana* and *C. gracilis*. Considering the EC_{50} values obtained, the following surfactant tolerance can be established: *N. gaditana*≈*I. galbana*≈*C. gracilis*≈*T. chuii*>*D. salina*, being the green alga *D. salina* the most sensitive to the surfactant. In the case of the marine invertebrate *A. franciscana*, the toxic effect (LC_{50}) was significantly higher after 72- than 48-h of exposure (48-h LC_{50}=38.30 mg L^{-1}; 72-h LC_{50}=23.92 mg L^{-1}). However, equal 48- and 72-h NOEC and LOEC values were obtained (NOEC=4.90 mg L^{-1}; LOEC=9.80 mg L^{-1}).

A large database is available on the short-term effects of AES on several taxonomic groups: algae, diatoms, crustaceans and fish (mainly freshwater organisms) (Table 4). Typical mean EC_{50} values describing the toxicity of AES towards algae vary between 0.5 and 65 mg L^{-1} AES. In the case of invertebrates, mean lethal concentrations (LC_{50}) range from 0.78 to 167.3 mg L^{-1} AES, whereas values ranging from 0.8 to 250 mg L^{-1} AES have been reported to freshwater fishes. Furthermore, an intraspecies variability can be also observed, especially in invertebrates (Table 4). For example, for *D. magna*, L(E)C_{50} values are between 4.2 and 72 mg L^{-1} (BKH, 1994). Results obtained from the present study (Table 3) also show a large inter- and intraspecies variability. Thus, in the case of algae, the acute toxicity (96-h EC_{50}) varies interspecies from

Table 4 – Summary of reported acute toxicity data for AES to freshwater and marine organisms

Group	Species	AES	Endpoint	Toxicity (mg L^{-1})	Reference
Marine diatoms	*Phaeodactylum tricornutum*	$C_{12}AES$	72-h EC_{50}	0.50	Pavlic et al., 2005
	Skeletonema costatum	$C_{12}AES$	72-h EC_{50}	0.37	Pavlic et al., 2005
Freshwater algae	*Selenastrum capricornutum*	$C_{10-15}AE_3S$	48-h EC_{50}	65	Yamane et al., 1984
	Selenastrum capricornutum	$C_{12-14}AES$	72-h EC_{50}	32	Verge et al., 1996
	Selenastrum capricornutum	C_xAE_9S	EC_{50}	4–8	Painter, 1992
	Selenastrum capricornutum	AES	L(E)C_{50}	3.5–10	BKH, 1994
	Pseudokirchneriella subcapitata	$C_{12}AES$	72-h EC_{50}	3.5	Pavlic et al., 2005
	Scenedesmus subspicatus	$C_{12}AES$	72-h EC_{50}	0.50	Pavlic et al., 2005
Marine invertebrate	*Artemia salina*	AES	24-h LC_{50}	11.97	Liwarska-Bizukojc et al., 2005
Freshwater invertebrate	*Daphnia magna*	$C_{13.67}AE_{2.25}S$	96-h EC50	1.17	Maki, 1979
	Daphnia magna	AES	L(E)C_{50}	4.2–72	BKH, 1994
	Daphnia pulex	AES	L(E)C_{50}	20.2	BKH, 1994
	Ceriodaphnia dubia	$C_{12-15}AE_{1-8}S$	48-h LC_{50}	0.78–167.31	Dyer et al., 2000
Freshwater fish	*Salmo gairdneri*	$C_{12}AES$	48-h IC_{50}	10.84	Singh et al., 2002
	Gambusia affinis	$C_{12}AES$	48-h IC_{50}	13.64	Singh et al., 2002
	Carassius auratus	$C_{12}AES$	48-h IC_{50}	12.35	Singh et al., 2002
	Cirrhina mrigala	$C_{12}AES$	48-h IC_{50}	7.20	Singh et al., 2002
	Pimephales promelas	$C_{11-18}AE_{2-6}S$	24-h LC_{50}	0.8–80	Painter, 1992
	Oncorhynchus mykiss	$C_{9-15}AE_{2-3}S$	96-h LC_{50}	8.9–250	Painter, 1992
	Salmo trutta	$C_{12-15}AE_3S$	96-h LC_{50}	1.0–2.5	Reiff et al., 1979
	Rasbora heteromorpha	$C_{12-15}AE_3S$	48-h LC_{50}	3.9	Reiff et al., 1979
	Idus idus melanotus	$C_{12-15}AE_3S$	48-h LC_{50}	3.95	Reiff et al., 1979
Aquatic amphibian	*Xenopus laevis*	AES	72-h LC_{50}	6750	Cardellini and Ometto, 2001

4.68 mg L^{-1} for *D. salina* to 24.02 mg L^{-1} for *I. galbana*. In addition, the toxicity data for the crustacean *A. franciscana* vary greatly. As an example 72-h LC_{50} values ranged between 19.59 and 28.26 mg L^{-1} AES (95% CI) between replicates (intraspecies variability).

Available information concerning the concentration of AES in the environment is almost absent. Studies conducted in EEUU reported surface water total AS/AES concentrations ranged from 10 to 172 ng L^{-1} up- and down-stream of the wastewater treatment plants (Sanderson et al., 2006). In addition, effluent wastewater concentrations ranged from 0.24 to 2.85 mg L^{-1}, respectively. Moreover, concentrations of $C_{12–15}$AES ranging between 0.003 and 0.012 mg L^{-1} (with an average value of 0.0065 mg L^{-1}) have been detected in the effluent of seven representative municipal wastewater treatment plants in the Netherlands (Matthijs et al., 1999). Considering the AES levels reported by Matthijs et al. (1999) and Sanderson et al. (2006), NOEC and LOEC values obtained in this study for *N. gaditana*, *I. galbana*, *C. gracilis*, *D. salina*, *T. chuii* and *A. franciscana* were notably upper this range (Table 3). It means that no acute effects would be expected on the marine algae and crustacea populations. However, either an inappropriate application or uncontrolled discharge of AES would cause damages to natural populations in the marine environments.

Although formal environmental risk assessments of AES have been published in freshwater environments (HERA, 2002), the information concerning the risk of adverse effects on marine organisms is very scarce or absent. The present study provides relevant data concerning the toxicity of AES in two different taxonomic groups: marine plankton and invertebrates, which may be useful to establish water quality criteria and safety recommendations in a regulatory framework. Six different species have been included as surrogate species to derive the sensitivity of the marine environmental communities. Based on NOEC values obtained in this study, the microalga *D. salina* can be judge as the most sensitive organism (96-h NOEC=2.80 mg L^{-1}) to the AES surfactant and appears to be the most suitable for monitoring large increases in AES concentrations as its NOEC value was a few orders of magnitude higher than measured ambient AES concentrations.

4. Conclusions

- Experimental results obtained demonstrate the extensive biodegradability of the alcohol ethoxysulphate surfactant Empicol® ESB 70/SP in sea water although at a slower rate than those reported for AES in inoculated mineral medium.
- Two different steps were observed during the degradative process, which were better described by a first order and logistic models. In the first step, the breakdown of the surfactant seems to be carried out mainly by means of hydrolytic reactions which imply the cleavage of ether bond. The second step may be characterized by the final degradation via ω- and β-oxidations of the intermediates resulting from the first step.
- Regarding the acute toxicity, a large intra- and interspecies variability was observed for the organisms tested. *D. salina* was the most affected organism whereas no significant differences were found among the other microalgae.
- NOEC values estimated for the marine microalgae and the invertebrate were notably upper than the reported AES environmental concentrations, which means that no acute effects would be expected on algae and crustacea populations.

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