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Sorption–desorption kinetics and toxic cell concentration in marine phytoplankton microalgae exposed to Linear Alkylbenzene Sulfonate

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ABSTRACT

Linear Alkylbenzene Sulfonates (LAS) are ubiquitous surfactants. Traces can be found in coastal environments. Sorption and toxicity of C_{12}-LAS congeners were studied in controlled conditions (2–3500 μg C_{12}LAS/L) in five marine phytoplanktonic species, using standardized methods. IC_{50} values ranged from 0.5 to 2 mg LAS/L. Sorption of $^{14}C_{12}$-6 LAS isomer was measured at environmentally relevant trace levels (4 μg/L) using liquid scintillation counting. Steady-state sorption on algae was reached within 5 h in the order dinoflagellate > diatoms > green algae. The sorption data, fitted a L-type Freundlich isotherm, indicating saturation. Desorption was rapid but a low LAS fraction was still sorbed after 24 h. Toxic cell concentration was 0.38 ± 0.09 mg/g for the studied species. LAS toxicity results from sorption on biological membranes leading to non-specific disturbance of algal growth. Results indicate that LAS concentrations in coastal environments do not represent a risk for these organisms.

1. Introduction

Linear Alkylbenzene Sulfonates (LAS) are anionic surfactants commonly used in household and industrial detergents with an annual global production of 4×10^6 tons (Zhu et al., 1998). They have replaced soap in detergents since the 1960s (Augier, 1992), with a per capita consumption of 1–2 kg/year in Western Europe and United States, and up to 3.5 kg in Japan (González-Mazo and Forja, 1998; Mackay et al., 1996). After use, most of LAS is disposed off with wastewater and is efficiently eliminated from the aqueous phase by biological treatment (typically between 95% and 99%) through biodegradation and adsorption on sludge (Mungray and Kumar, 2008). The remaining LAS in the treated effluents is further removed in receiving waters via biodegradation, sorption on suspended particles and deposition, limiting geographic dispersion to a few kilometers around urban effluent discharges (González-Mazo and Forja, 1998; Takada and Ogura, 1992). However, constant untreated effluent discharge along urbanized coasts can lead to chronic contamination of marine waters, sediments and organisms at the μg/L and μg/g levels (Lara-Martín et al., 2005; León et al., 2000; Sáez et al., 2000).

Marine unicellular algae are part of the suspended particulate organic matter found in the water column. They represent a key food source for the growth stages of bivalve molluscs, larval stages of some crustacean species and very early growth stages of some fish species (IPCS, 1996; Walsh, 1988). The hydrophobic nature of algal cell walls makes microalgae good candidates for LAS sorption through van der Waals interactions due to their alkyl chain (Sáez et al., 2001; Wang et al., 1997). Sorbed LAS can exert toxicity on cells through the denaturation and the binding of proteins in the cell wall and consequently the alteration of membrane permeability to nutrients and chemicals (Blasco et al., 1997; Hampel et al., 2001). Although LAS have a non-specific mode of action, the sensitivity of different algal species, as measured by toxicity tests in accordance with OECD guidelines (OECD, 1984, 2002), is highly variable. The measured toxicity values vary by three orders of magnitude depending on the homologues and isomers (Lewis, 1990).

In this study, five algal species *Tetraselmis levis* (green alga), *Dunaliella tertiolecta* (green alga), *Thalassiosira pseudonana* (diatom), *Skeletonema costatum* (diatom), and *Prorocentrum minimum* (dinoflagellate) present in the N-W Mediterranean coastal waters were chosen to assess LAS sorption and desorption kinetics and LAS toxicity. The aim of the study is to contribute to evaluate the potential risk of LAS to coastal phytoplankton.

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2. Materials and methods

2.1. Chemicals and organisms

A mixture of C_{12}-LAS isomers (five different congeners from C_{12}-2-LAS to C_{12}-6-LAS) was used in the toxicity experiments whereas a particular radiolabelled isomer: $^{14}C_{12}$-6-LAS was used in kinetics studies. The latter had a specific activity of 12.9 mCi/mmol. Stable and radiolabelled C_{12}-LAS were obtained from the Procter & Gamble Company. All other chemicals were HPLC grade purchased from Merck.

Five marine phytoplanktonic species were selected: the green algae *T. levis* (diameter: 5–7 μm) and *D. tertiolecta* (diameter: 5–7 μm), the diatoms *T. pseudonana* (diameter: 3.5–5 μm) and *S. costatum* (diameter: 4–5.5 μm) and the dinoflagellate *P. minimum* (diameter: 10–13 μm). Test algal species were selected from IAEA-MEL axenic culture collections, using standard methods from the APHA, AWWA and WPCF (Blasco et al., 2003), according to their ability to grow under similar conditions (Fisher et al., 1984; Lu et al., 2005). Their different cell wall structure (carbohydrate or silica) and shape but also the total surface area were taken into account to tentatively explain the differences in affinity of the algae to C_{12}-LAS. Algal cell dry weight was determined by filtering and drying algae from aliquots of culture of known concentration on glass-fiber filters (Zhu and Lee, 1997). Algal cell diameter was estimated by microscopic observation using a stage micrometer (S12-stage micrometer, Pyser-SGI with 2 μm divisions). Algal surface and volume were estimated from measured cell diameter, assuming a spherical shape.

2.2. Toxicity tests

C_{12}-LAS toxicity was assessed by measuring the growth inhibition of the five algal species using the methodology described in the OECD updated guideline 201 (2002) and according to Moreno-Garrido et al. (2000). Briefly, experimental double-glass bottles were filled with 150 ml of 0.2 μm-filtered seawater, enriched with F/2 medium lacking EDTA (Guillard and Ryther, 1962), inoculated with a specific cell density in order to reach an initial algal concentration close to 10^5 cell/ml and placed in a culture chamber at 20 ± 0.2 °C with a 12 h/12 h light cycle. Salinity was 38 p.s.u. and pH showed low variation (8.7 ± 0.4). Cells were exposed in triplicate to 0, 0.2, 0.4, 0.8, 1.5 and 3.5 mg/L for 72 h. Every 24 h, cell density was measured on a Fuchs Rosenthal haemocytometer (Paul Marienfeld GmbH & Co., Lauda-Königshofen, Germany).

Percentage of growth inhibition (f_x, %) was fitted to a sigmoid curve (Eq. (1)) as a function of the LAS concentration in water (C_w, mg/L). The model was fitted to the raw data using the non-linear estimation module of Statistica® 6.1 (StatSoft Inc., 1998) with the quasi-Newton method for calculating least squares. Parameters a, b and growth inhibition (IC_{50}, mg/L) were determined by iterative adjustments:

$$f_x = \frac{a}{1 + e^{\frac{-(C_w - IC_{50})}{b}}} \quad (1)$$

2.3. Sorption and desorption kinetics

Algal cells were filtered and suspended in 1 L double-glass bottles in order to reach similar initial density as previously described. The bottles were filled with 0.2 μm-filtered seawater enriched with F/2 medium lacking EDTA and spiked with 10 Bq/ml of $^{14}C_{12}$-6-LAS (equivalent to 4 μg/L). During the exposure period, cells were sampled in triplicate (3 × 50 ml) and filtered on 1 μm-polycarbonate filters (Osmonics), after 0, 1, 2, 3, 4, 6, 8 and 24 h. The ^{14}C activity of the samples was analysed using liquid scintillation counting. Briefly, the filters were placed in 20-ml glass vials, with 10 ml of scintillation cocktail (UltimaGold XR, Perkin Elmer) and counted using a liquid scintillation counting (LSC) analyzer (Tri-Carb 2900 TR, Perkin Elmer). LAS degradation was not expected during the 24 h period of experiment performed with filtered seawater (Terzic et al., 1992) and 100% of the measured ^{14}C activity was assumed to represent the parent compound, C_{12}-6-LAS. Basic parameters (temperature, salinity, pH, cell density) and ^{14}C radioactivity in aqueous phase were controlled at each sampling time. No variation in $^{14}C_{12}$-6-LAS concentration was observed in the water and sorption to the bottle wall was negligible. It is therefore assumed that sorption on phytoplankton cells was not limited by LAS concentrations.

A first-order one-compartment kinetic sorption model was used to describe the LAS concentration on cells, C_t (mg LAS/kg algae dry weight) over time t (h) (Eq. (2)), as a function of the exposure water concentration (Hamelink et al., 1971; Neely et al., 1974). The sorption coefficient at steady-state (K_{ss}, L/kg) and the desorption rate constant (k, h^{-1}) were calculated by iterative adjustments using Statistica® as previously described:

$$C_t = K_{ss}(1 - e^{-kt}) \times C_w \quad (2)$$

At the end of the exposure period, the cells were filtered and resuspended in 0.2 μm-filtered seawater enriched with F/2 medium lacking EDTA. The same protocol was used to measure desorption kinetics after 0, 1, 2, 3, 4, 6 and 24 h.

C_{12}-LAS desorption was expressed in terms of percentage of remaining activity, *i.e.* sorbed LAS concentration at time t divided by initial sorbed LAS concentration measured on the cells at the beginning of the decontamination period. The percentage of remaining activity was plotted against time. The kinetics were described by a two-component exponential model (Eq. (3)), where A_t and A_0 are the remaining activities (%) at time t (h) and 0, respectively, and k is the biological desorption rate constant (h^{-1}) that allows the calculation of the biological half-life ($T_{b1/2} = \ln(2)/k$). The 's' subscript refers to a short-lived component (s component) while the 'l' subscript refers to a long-lived component (l component) (Whicker and Schultz, 1982). The short lived-component is a model for the loss of the proportion of LAS pool that is weakly sorbed on cells, while the long-lived component is a model of the loss of the fraction of the LAS pool that is tightly bound on the cells:

$$A_t = A_{0s}e^{-k_s t} + A_{0l}e^{-k_l t} \quad (3)$$

2.4. Sorption isotherms

Experimental 250 mL double-glass bottles were used for cell incubation. The bottles were filled with 0.2 μm-filtered seawater, enriched with F/2 medium and inoculated with algae in order to reach similar initial algal density as previously described. After an adaptation period of 24 h, cells were counted on Fuchs Rosenthal haemocytometer and seawater was spiked with $^{14}C_{12}$-6-LAS (5 Bq/ml) and completed with non-radiolabelled C_{12}-LAS in order to reach 2.1, 2.7, 3.7, 7.5 or 13.5 μg/L. For three species (the green algae and *T. pseudonana*), the experiment was conducted with two additional exposure concentrations: 100 and 1000 μg C_{12}-LAS/L. Each exposure concentration was tested in triplicate during 10 h in order to reach steady-state adsorption on cells for all algal species. LAS concentration sorbed on cells was determined as previously described.Data was fitted to a linear Freundlich isotherm (Rico-Rico et al., 2009b; Sáez et al., 2001) using the following equation:

$$\log C_a = \log K + n \log C_w \quad (4)$$