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Aerobic Biodegradation of a Nonylphenol Polyethoxylate and Toxicity of the Biodegradation Metabolites

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Abstract In this paper a study was made of the biodegradation of a non-ionic surfactant, a nonylphenol polyethoxylate, in biodegradability tests by monitoring the residual surfactant matter. The influence of the concentration on the extent of primary biodegradation, the toxicity of biodegradation metabolites, and the kinetics of degradation were also determined. The primary biodegradation was studied at different initial concentrations: 5, 25 and 50 mg/L, (at sub-and supra-critical micelle concentration). The NPEO used in this study can be considered biodegradable since the primary biodegradation had already taken place (a biodegradation greater than 80% was found for the different initial concentration tested). The initial concentration affected the shape of the resulting curve, the mean biodegradation rate and the percentage of biodegradation reached (99% in less than 8 days at 5 mg/L, 98% in less than 13 days at 25 mg/L and 95% in 14 days at 50 mg/L). The kinetic model of Quiroga and Sales (1991) was applied to predict the biodegradation of the NPEO. The toxicity value was measured as EC_{20} and EC_{50}. In addition, during the biodegradation process of the surfactant a toxicity analysis was made of the evolution of metabolites generated, confirming that the subproducts of the biodegradation process were more toxic than the original.

Keywords Biodegradation · Nonylphenol polyethoxylate · Toxicity · Kinetics

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Currently, alkylphenol polyethoxylates (APEOs), including mainly nonylphenol ethoxylates (NPEOs) and octylphenol ethoxylates (OPEOs), are subject to use restrictions.

As a result of their field of application, their resistance to biodegradation at low temperatures and the generation during the degradation process of some persistent metabolites which are much more toxic than the original compound (Maguire 1999), the use of NPEOs has been banned in domestic formulations in some countries of the European Union (Germany, Spain, and the United Kingdom), (European Commission (EC) 2003) as well as Switzerland and Canada (Soares et al. 2006).

The use of these substances is restricted to industrial applications in which the specific nature of the properties required make it more difficult to replace them, as in the case of plant sprays and biocides. Since biodegradability is the main determinant of organic compatibility, it is important to establish simple ways to promote primary and ultimate biodegradation of NPEOs (Zhao et al. 2006). In this work a study was made of the biodegradability of a NPEO, at sub- and supra-critical micelle concentrations (CMC), in biodegradability tests. The influence of the concentration on the extent of primary biodegradation, the toxicity of biodegradation metabolites and the kinetics of degradation were also determined. In parallel, the biomass growth has been monitored, analysing the colony-forming units (CFU) formed during this process. The study of toxicity throughout the biodegradation process of the NPEO, together with the primary biodegradation profile can provide valuable information concerning the environmental risk of the compound. Finally, the kinetics analysis of the biodegradation curves enabled predictions on its behaviour in the environment.

Materials and Methods

The nonylphenol polyethoxylate, for which the average mole number of EO units is 9.5 and CMC is 34 mg/L, was purchased from Tokyo Chemical Industry (Tokyo, Japan).

The biodegradation tests were performed according to the OECD 301 E test for ready biodegradability (OECD 1993). A solution of the surfactant, representing the sole carbon source for the microorganisms, was tested in a mineral medium, inoculated and incubated under aerobic conditions in the dark. The procedure consists of introducing 1.2 L of surfactant solution (for which the biodegradability is to be determined) into a 2-L Erlenmeyer flask and inoculating the solution with 0.5 mL of water from a secondary treatment of a sewage-treatment plant (STP) that operates with active sludges. This water sample is a mixed aerobic culture of fecal microorganisms including, for the most part, total coliforms, fecal coliforms and enterococcus. The Erlenmeyer flask is plugged with a cotton stopper and left in darkness in a thermostatically controlled chamber at 25°C. The constant rotary speed of the orbital shaker (125 sweep/min) provides the necessary aeration. The surfactant solution is prepared by dissolving the desired quantity of surfactant in the nutrient solution.

The biodegradation process was monitored by measuring the residual-surfactant concentration over time. The NPEO was determined by the iodine–iodide colorimetric method (Jurado et al. 2002). Reference assays were made with an easily biodegradable surfactant (LAS) in order to determine the activity of the microbial population present in the test medium. During the biodegradation assays, the number of viable microorganisms was measured by a heterotrophic count in a dish (APHA et al. 1992), expressing the result as colony-forming units (CFU) per mL.

Sorption may significantly influence the environmental impact of surfactants (Belanger et al. 2006) and some authors have proposed expressions to predict sorption onto activated sludge particles for alcohol ethoxylates (Van Compernolle et al. 2006). In the biodegradation assays presented here, the sorption can be considered negligible, given the scant biomass formation. To test this assumption, abiotic assays were made in the presence of $HgCl_2$, and the values of the residual surfactant remained around 100% over the biodegradation period. These results indicate that the contribution of abiotic processes in the degradation of the NPEO in the biodegradation tests made can be dismissed.

In these experiments, the toxicity was measured with the system LumiStox® 300, which consists of an instrument for measuring bioluminescence and an incubation unit according to the UNE-EN ISO 11348-2 guideline (UNE EN ISO 11348-2 1999). The toxicity measurement was based on the luminous intensity of the marine bacteria of the strain *Vibrio fisheri* NRRL-B-11177 after a given exposure time to the NPEO.

Results and Discussion

The effect of the concentration on the primary biodegradation was analysed for NPEO under study. Figure 1 shows the results, where the concentration is expressed as a percentage of residual surfactant and each point of the curves represents the mean value of the two replicates.

In a preliminary analysis of the primary-biodegradation curves (Fig. 1), it was found that the concentration of the residual surfactant rapidly diminished with biodegradation time. The biodegradation curves found for 50 mg/L (at supra-critical micelle concentration) significantly differed from the rest of the concentrations studied, the shape of the curve was exponential, and, although the concentration did not significantly affect the latency time, the biodegradation process became slower. This profile suggests inhibition by substrate.

Biodegradation exceeded 99% in less than 8 days at 5 mg/L. For an initial concentration of the assay of 25 mg/L, the biodegradation reached was 98% in less than 13 days. When the initial concentration of the assay doubled at 50 mg/L, the biodegradation declined to 95% and the assay lasted 14 days, giving a residual surfactant concentration of 2.4 mg/L. Current legislation demands a minimum level of 80% of the primary biodegradation after 19 days, when surfactants are subjected to the OECD test (OECD 1993); thus, the NPEO used here can be considered biodegradable, since the primary biodegradation of NPEO was readily carried out – that is, biodegradation greater than 80% was found for the different initial concentration tested.

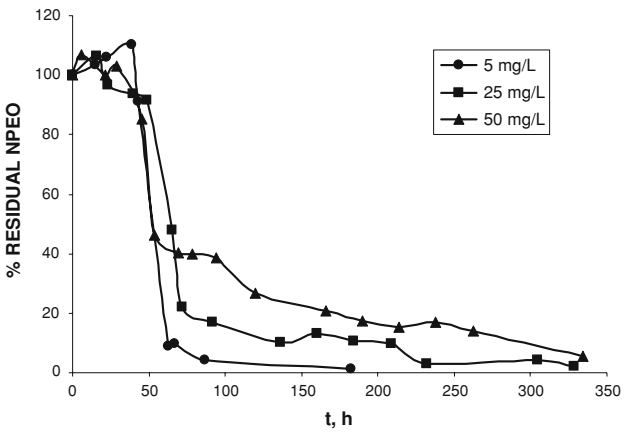

Fig. 1 Curves representing the extent of primary biodegradation of NPEO. Influence of the initial concentration

For the comparison and quantification of the different biodegradation assays, the parameters characteristic of the biodegradation profiles (Jurado et al. 2007) were evaluated: latency time (t_L), half-life time ($t_{1/2}$), mean biodegradation rate (V_M), residual-surfactant concentration (S_R) and surfactant biodegradability (B). Table 1 shows the parameters characteristic of the biodegradation profiles for NPEO at all the concentrations assayed. S_0 is the initial concentration of the biodegradation assay in mg/L and t_T is the total duration of the assay in hours.

The initial concentration affected the form of the resulting curve, the mean biodegradation rate (Table 1) and the percentage of biodegradation reached (Fig. 1). However, it did not significantly affect the acclimation time of the microorganisms, this varying between 38.48 h for the assay of 5 mg/L, and 42.56 h for the assay of 50 mg/L. In the assay at NPEO concentration higher than the CMC, two stages were distinguished. At the beginning times the biodegradation rate was very high, since the surfactant was forming micelles, and therefore there was less surfactant available. This trend changed on reaching approximately 50% biodegradation, this explaining the biodegradability values (B, %). However, once having surpassed 50% biodegradation, the process slowed down at the highest concentrations, as reflected by the fact that the total time needed to complete the biodegradation lengthened from 7.6 days (for 5 mg/L) to 9.7 and 13.9 days (for 25 and 50 mg/L, respectively), and the mean biodegradation rate fell from 13.0 $days^{-1}$ (for 5 mg/L) to 10.0 and 6.8 $days^{-1}$ (at 25 and 50 mg/L, respectively).

The standard methods used in the study of surfactant biodegradability (APHA et al. 1992) are useful to indicate whether a product is biodegradable or not but are not useful to predict the rate at which a given product biodegrades in the environment. In the literature, to analyse the biodegradation profiles corresponding to non-ionic surfactants, a first-order kinetics is used (Zhang et al. 1999). However, in the present study, the application of this model to the biodegradation profile of NPEO did not explain the experimental dependence observed, for two reasons: it did not consider the initial adaptation period of the microorganisms to the substrate; and the exponential function of the e^{-kt} type did not account for the decline in the concentration observed. The kinetic model of Quiroga and Sales (1991) was applied to predict the biodegradation of the NPEO. In this model, the degradation rate is given by a second-degree polynomial, exclusively as a function of substrate concentration. When integrated, the equation representing the model is:

$$s = \frac{h \cdot (S_0 - q) - q \cdot (S_0 - h) \cdot e^{p \cdot t}}{(S_0 - q) - (S_0 - h) \cdot e^{p \cdot t}} \quad (1)$$

where S_0 is initial substrate concentration; p, q and h are combinations of the coefficients of the second-degree polynomial which defines the substrate consumption rate, and t is time. Romero (1991) subsequently arrived at the same expression for dependence of substrate on time, but on a mechanistic basis, providing a physical meaning for the values of parameters p, q and h. Where p = maximum velocity of microorganism growth; h = maximum concentration of substrate which can be used to form biomass; and q = concentration of non-biodegradable substrate. The fitting of the experimental data to the model equation gave the values shown in Table 2 for the various kinetic parameters and the correlation coefficients for the different tests.

Figure 2 compares the experimental values and those calculated with the model. The values found in the tests for the parameter h were very similar to the initial NPEO concentrations used, confirming that the kinetic model was appropriate for using under the conditions being studied. The values for q, representing the concentration of non-biodegradable substrate, increased with concentration, this indicating that the levels of the remaining NPEO increased at higher concentrations. As regards p, it can be seen (Table 2) that the specific maximum growth decreased with initial concentration in the assay, suggesting a possible inhibition of the microorganisms with concentration.

Growth curves of the microorganisms constitute an alternative method to evaluate the total or final biodegradability of the surfactants when these are the only carbon source in the culture medium. Figure 3 shows the growth curves together with the corresponding primary-biodegradation profiles for NPEO. There was a coupling between the biodegradation curves and the growth curves of the microorganisms. During the exponential growth phase of the microorganisms, a linear decline occurred in the residual concentration of the surfactant, and, when the surfactant

Table 1 Parameters characteristic of the biodegradation profiles for NPEO

S_0 (mg/L)	t_T (h)	B (%)	t_L(h)	$t_{1/2}$(h)	V_M (%/h)	S_R (mg/L)
5	182.50	58	38.489	52.740	1.41	0.053
25	328.50	17.5	38.757	64.498	0.89	0.528
50	334.25	49	42.563	60.763	0.44	2.439

Table 2 Kinetic parameters derived from the Quiroga–Sales model

S_0 (mg/L)	h (mg/L)	q (mg/L)	p ($days^{-1}$)	r
5	5.0001	0.3342	4.0319	0.9956
25	23.0735	1.9300	3.6103	0.9947
50	43.2195	8.1625	2.4130	0.9794

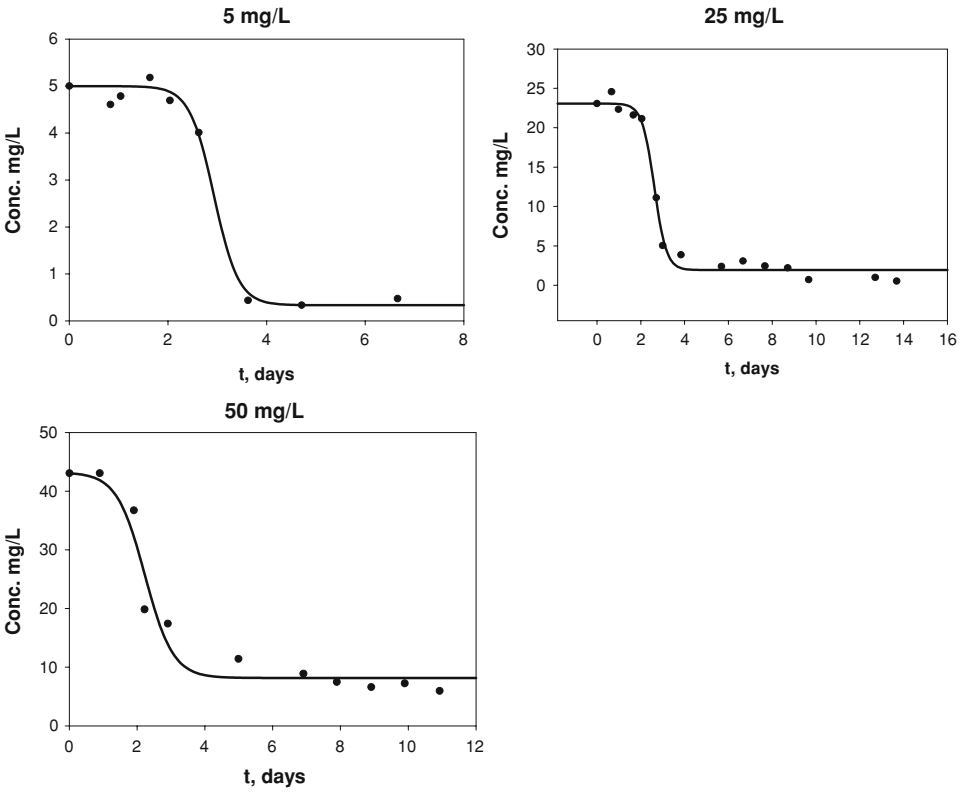

Fig. 2 Comparison of the experimental results and those found applying the Quiroga–Sales model at different initial concentrations

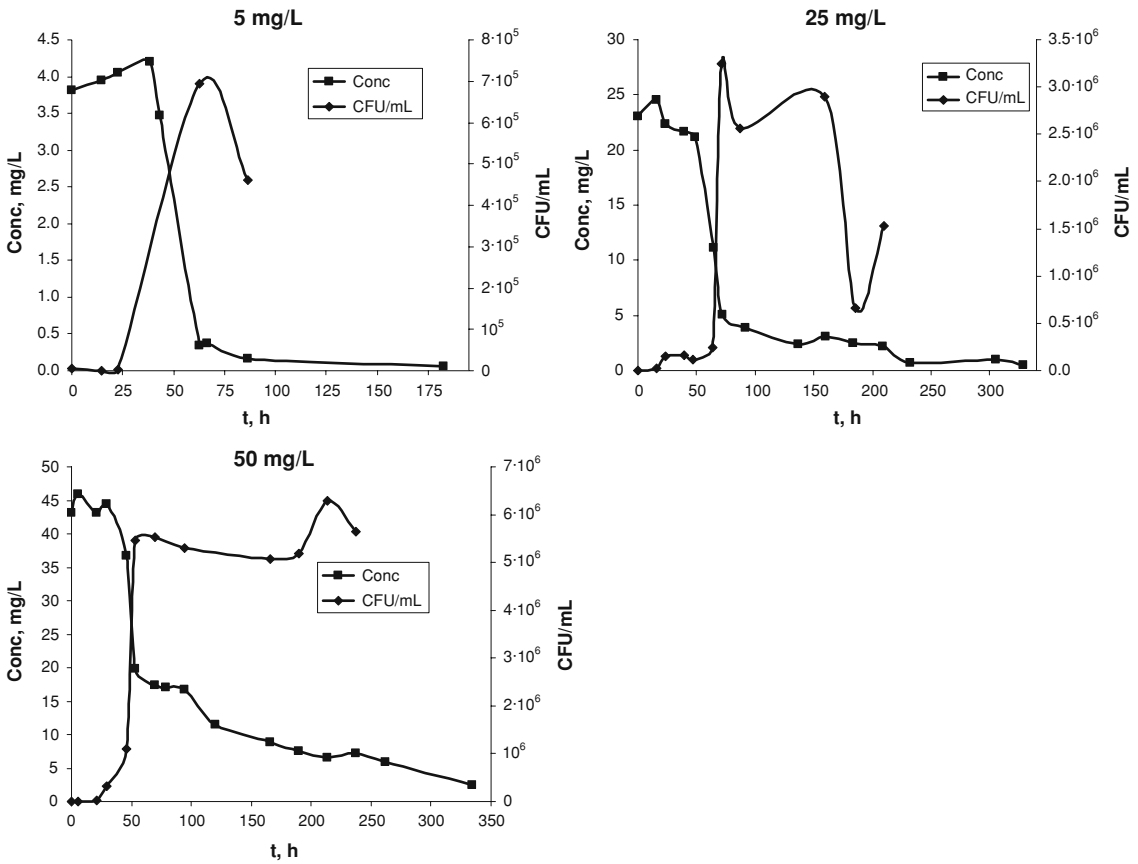

Fig. 3 Growth curves and biodegradation profiles for NPEO at 5, 25 and 50 mg/L

concentration remained constant, the microorganisms registered no growth. Finally, the bimodal growth curves resulted (two peaks), suggesting the inhibition of the microorganisms existing initially in the assay and the growth of new populations due to the biodegradation of the metabolites.

Microbial growth during the primary phase of the exponential growth can be described by Monod's equation (Monod 1949) according to the expression:

$$\frac{dX}{dt} = \mu \cdot X \tag{2}$$

or in its integrated form:

$$X = X_0 \cdot \exp(\mu \cdot t) \tag{3}$$

where μ represents the specific growth rate in h^{-1}, X the biomass concentration at each time expressed as CFU/mL, and X_0 the biomass at the beginning of the assay. For a comparison of the different assays of microorganism growth, the parameters characteristic of these curves were determined. By non-linear regression to Eq. 3, the specific growth rate was determined for the NPEO (μ); X_0, is the initial value of CFU/mL found in the assay. Table 3 shows the parameters characteristic of the growth curves during the biodegradation process.

The specific growth rate (μ) diminished with the initial concentration of the assay, these results indicating a possible inhibition of the microorganisms with concentration. Also, the parameter Y_{ap} was calculated, this representing the biomass yield – that is, the proportion of the original substrate converted into biomass, which was assumed to be constant over the biodegradation process. Y_{ap} was calculated as $\Delta X/\Delta S$ quotient, where ΔX is the quantity of microorganisms formed during the exponential growth phase and ΔS the substrate consumed during the same time period.

For the completion of the ecotoxicity study of this surfactant, it is necessary to measure biodegradability and toxicity. The toxicity value was measured as EC_{50} or EC_{20}, which are, respectively, the surfactant concentrations that inhibit 50 and 20% after 15 and 30 min of exposure, respectively (UNE-EN ISO 11348-2). In addition, during the biodegradation process of the surfactant, a toxicity analysis was made of the evolution of the metabolites generated. In this case, the results could not be expressed in terms of concentration, as the samples were of unknown

Table 3 Parameters characteristic of the growth curves for NPEO

S_0 (mg/L)	X_0 (CFU/mL)	μ (h^{-1})	Y_{ap} (CFU/g substrate)
5	$5.80 \cdot 10^3$	0.196	$1.92 \cdot 10^5$
25	$4.50 \cdot 10^2$	0.190	$1.95 \cdot 10^5$
50	$1.75 \cdot 10^3$	0.144	$2.15 \cdot 10^5$

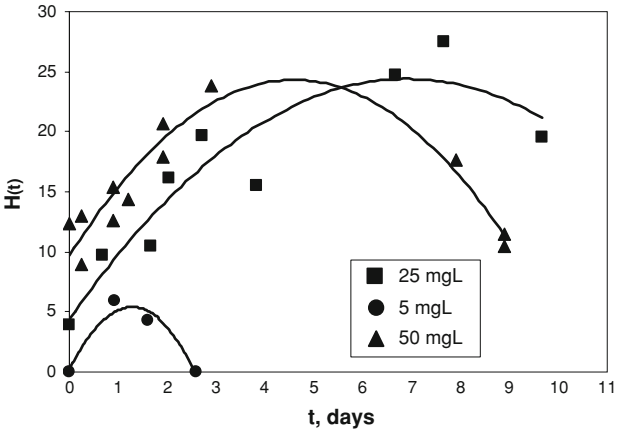

Fig. 4 Variation in the percentage of inhibition during the biodegradation process

composition. Therefore, to evaluate the toxicity during the biodegradation process the toxicity value of the sample was expressed as a percentage of inhibition, and the variation in toxicity during the biodegradation assay as the variation of the inhibition percentage. The percentage of inhibition (inhibitory effect) was calculated by the expression:

$$H_t = \frac{(I_{0t}(c) - I_t(c))}{I_{0t}(c)} \cdot 100 \tag{4}$$

where:

$$I_{0t}(c) = \bar{f}_k \cdot I_0(c) \tag{5}$$

with $\bar{f}_k$ being the average correction factor of the control samples, $I_0(c)$ and $I_t(c)$ being readings of luminous intensity in the well containing concentration c at time 0 and t. The inhibitory effect of the samples assayed was calculated for an incubation time of 15 min.

To eliminate any doubt concerning the environmental lability of NPEO, a study was made of the evolution of the toxicity over the biodegradation process for the initial assay concentrations of 5, 25 and 50 mg/L (Fig. 4). In this way, it can be confirmed whether the subproducts of biodegradation were more toxic than the original. It was found that during the biodegradation process, a maximum was reached, confirming that the biodegradation metabolites were more toxic than the beginning compound. On the other hand, the highest value of the inhibition percentage was found for high surfactant concentrations.

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