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Effect of Concentration on the Biodegradation of a Nonylphenol Polyethoxylate in River Water

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Alkylphenol polyethoxylates (APEO) are an important group of surfactants since they were first synthesized in 1940. At present they are the most widely used at the industrial level and third in the ranking for all types of applications, with an annual volume of production of 370,000 T (Raymond 1996). As a result of their wide field of application, their resistance to biodegradation at low temperatures and the generation of some persistent metabolites during the degradation process, they have been identified in all kinds of natural water waste water and in drinking water for human consumption (Zoller 1989; Schröder 1993). The use of their metabolites as indicators of industrial contamination has been suggested (Ballarin et al. 1989).

Both primary and ultimate biodegradation of alkylphenol polyethoxylates depend to a large extent on environmental factors. Percentages of primary elimination can be found ranging from 63% (Okpokwasili and Olisa 1991) to almost 100% (Patoczka and Pulliam 1990) with mineralization varying from a very low percentage (Patoczka and Pulliam 1990; Reinhard et al. 1982) to over 90% (Fuka and Pitter 1980).

In this paper, a study has been made of the biodegradability of these substances in river water by means of monitoring the original surfactant matter and the generated metabolites. An investigation was made on the influence of concentration on the extent of primary and total biodegradation, and on its degradation kinetics.

MATERIALS AND METHODS

The surfactant used in all the assays was a nonylphenol polyethoxylate with an average of 15 ethoxylated units (NP15EO) and a wide range of oligomers of 2 to 22 ethoxylated units. The product was supplied by Albright & Wilson under the commercial name of Empilan NP15.

The water used in the assay, Table 1, came from the river Guadalete, in the South-West of the Iberian Peninsula, and was taken from a point near its source in order to ensure that it was as free of urban and industrial contamination as possible.

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Table 1. Characteristics of the water used in the assays.

PARAMETER	VALUE	PARAMETER	VALUE
NO_2^-	0.57 mg/L	pH	8.2
NO_3^-	< 0.1 mg/L	Conductivity	503 µS/cm
NH_4^+	0.15 mg/L	Permanganate oxidability	2 mg O_2/L
Total suspended solids	7 mg/L	Colony-forming units	84000 CFU/mL
$SO_4^=$	33.74 mg/L	APEO	< 10 ppb

The method chosen to carry out the biodegradation study was the "River die-away test" (Okpokwasili and Olisa 1991). The reactors used for the assays had a capacity of 6 L and contained 4L of river water, decanted and collected on the same day the tests began and the appropriate amount of a standard solution of NP15EO in order to reach the required concentration in each test (2.5, 5 and 10 ppm). Finally, the reactors were covered with hydrophobic cottonwool and placed in the dark at 21 °C. The assays were carried out in duplicate. In adition test blanks were done to measure abiotic losses.

The separation, identification and quantification of the oligomers contained in the commercial formula under study was carried out by means of normal phase HPLC with fluorescence detection at 225 nm (excitation) and 304 nm (emission). The gradient of the HPLC separation, Table 2, use a mixture of isopropanol/water (95/5) as eluent A, and hexane/isopropanol (98/2) as eluent B. A Hypersil APS-2, NH_2, 5 µm, 4.6 x 250 mm (Teknokroma) column was used in conjunction with a µBondapak NH_2 (Teknokroma) precolumn.

Table 2. Gradient of the HPLC separation of alkylphenol polyethoxylates.

Time (min.)	Flow (mL/min)	%A	% B
0	0.6	0	100
5	0.6	0	100
20	0.6	19	81
22	0.8	21	79
70	0.8	100	0

The 5 mL samples taken from the biodegradation experiments were dried at a temperature of 45-55°C assisting evaporation with a flow of nitrogen. Once the water had evaporated, the dry residue was resuspended in 1ml of hexane/isopropanol (80/20). The recovery of this process of concentration and purification was 94%. Identification was accomplished by injecting standard solution of known composition. In order to quantity the peaks registered and calculate the amount of NPEO in a sample taken from the assay, the concept of equality of the molar absorption coefficients of all oligomers was applied (Wang and Fingas 1993). This property can be used for simplifying the quantitation of NPEO. On the basis of the ratio amount/area obtained for the standard

of one NPEO oligomer (m_r/A_r), the other components can be quantitated by applying the following equation:

$$m_x = A_x/A_r \times m_r \times M_x/M_r$$

where m_x and m_r are the amount of the NPEO components corresponding to the unknown (x) and the reference (r) peak, A_x and A_r are the respective peak areas and M_x and M_r the molecular weights.

The determination of the various acids resulting from the oxidation of the ethoxylated chain during the biodegradation process, 5-(p-nonylphenoxy) 3-oxa-pentanoic acid (NP1EC) and p-nonylphenoxy acetic acid (NP2EC) was carried out by HPLC in reversed phase, following the model proposed by Marcomini (1989). The gradient of the HPLC separation use *acetonitrilo* as eluent A and a *water/acetonitrilo* mixture (75/25) to which a 0.1 M concentration of *sodium perchloride* had been added as eluent B. The gradient used is shown in Table 3.

Table 3. Gradient of the HPLC separation of acidic intermediates of alkylphenol polyethoxylates.

Time (min.)	Flow (ml/min)	%A	Time (min.)	Flow (ml/min)	%A
0	1	25	20	1	80
3	1	25	24	1	80
15	1	60	30	1	25
16	1	60	33	1	25

For the stationary phase, a Lichrospher 100 RP18, 5 µm, 4,5 x 250 mm (Teknokroma) stainless steel column was used with a C-18 precolumn. The wavelengths of the fluorescence detector were 225 nm (excitation) and 295 nm (emission). The identification of the peaks required the synthesis of the NP1EC, following the method described by Yoshimura (1986). The synthesis of the NP1EC permitted its quantification and that of the NP2EC, as they have the same molar absorption coefficient (Marcomini et al. 1989).

In order to obtain additional information about the biodegradation process and to ensure that conditions were favorable, periodic tests were carried out on a number of other variables. These were *viable aerobic microorganisms*, using *the standard heterotrophic plate count* (HPC), *pH*, using a portable Crison 507 pH meter, and *dissolved oxygen* by means of a Crison Oxi 92 selective membrane electrode.

RESULTS AND DISCUSSION

Figure 1 shows the evolution of the percentage of residual surfactant throughout the assays with initial concentrations of 2.5, 5 and 10 ppm of NP15EO. Firstly, it can be observed that there was a lag-phase which has been observed by various investigators for different surfactants. These induction periods are, however, inferior to those found

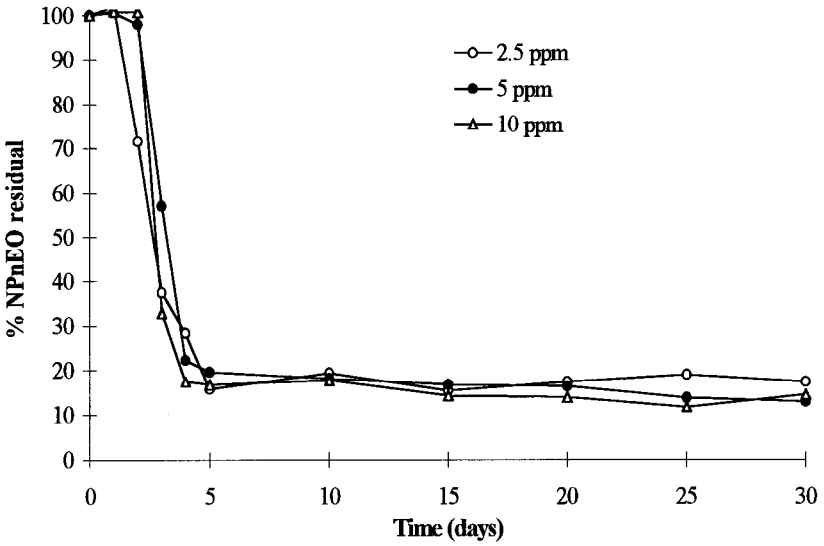

Figure 1. Evolution of the percentage of residual surfactant.

by other authors for surfactants such as LAS (Larson and Payne 1981). This may be because here, the active enzymes are fundamentally hydrolytic enzymes which are either already present in the medium or are synthesized relatively quickly, while in the case of LAS, the enzymes are more specific and take longer to be synthesized.

Moreover, it can be seen that this lag-phase is influenced by the initial concentration of the surfactant, as it becomes slightly longer when concentration is increased. At 2.5 ppm, the lag phase was between 1 and 2 days, whereas at 5 and 10 ppm, it was between 2 and 3 days. In all the cases, primary biodegradation, loss of the polyethoxylate structure, of approximately 85% was observed, while after 30 days, the NP15EO had not been completely eliminated in any of the tests. The explanation of the differences between the elimination percentages presented in this paper and those given in the bibliography 99.8% (Patoczka and Pulliam 1990) is that the methods used were not sensitive to short ethoxylated chain oligomers.

Figure 2 presents the chromatographs showing all the metabolites generated during the biodegradation process for the assay with a initial concentration of 5 ppm of Empilan NP15EO. It can be observed that, to begin with, the dominant mechanism is the shortening of the ethoxylate chain until practically all the initial nonylphenol polyethoxylate has been transformed into nonylphenol diethoylate (NP2EO), with the corresponding liberation of ethylenglycol. From this point on, there is no further shortening of the ethoxylate chain as is proved by the fact that nonylphenol monoethoxylate (NP1EO) was not detected in any of the assays. Once the ethoxylate chain has been shortened to two units, the main mechanism becomes the oxidation of the terminal ethoxylate unit of the NP2EO, producing NP2EC which then forms NP1EC. A slight acidification of the medium (0.3 pH units) coincides with the period

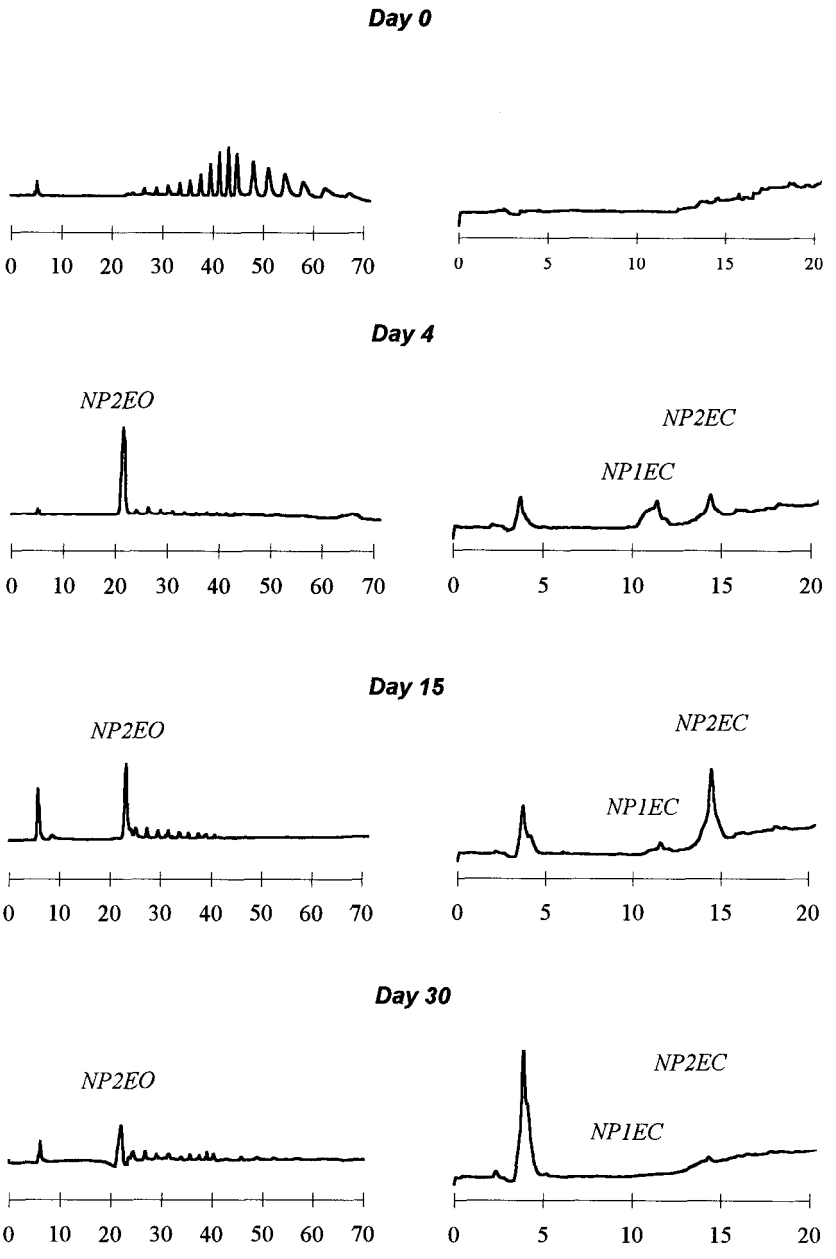

Figure 2. Normal (left column) and Reverse-phase cromatogrammes (right column) for days 0, 4, 15 and 30 of the assay with 5 ppm of Empilan NP15 at 21°C. X-Y axes units are minutes and volts respectively

of maximum concentration of these metabolites. In addition, it can be observed that as the biodegradation process takes place, the intensity of the peak that appears to the left in the reverse-phase chromatograms increases.This signal represents a metabolite which is not identified as a nonylphenol, a nonylphenol polyethoxylate or any of its acidic derivatives, suggesting the possibility that oxidation of the alkylic chain of the nonylphenol group gives rise to acids whose afinity for the column is lower and which therefore eluate first. This would agree with the studies carried out by Schobert et al. (1981). From the discussion so far, it can be deduced that the nonylphenol polyethoxylates do not completely disappear in any of the conditions studied, as residues and non-degraded metabolites are left. This contrasts with the total elimination in soils of the ethoxylate chain and the aromatic ring described by Hughes et al. (1996).

The percentage of mineralization reached has been calculated using two limits, the maximum and minimum. The inferior extreme would be the percentage of the mass represented by the lost ethoxylate units. When the ethoxylate chain is shortened, ethylenglycol is released, a substance which is easily mineralized, producing CO_2 and H_2O (Swisher 1987). From the calculation of the amount of ethylenglycol released, the minimum percentage of mineralization which has taken place can be calculated, i.e. that which is due exclusively to the shortening of the hydrophylic chain. The amount of ethylenglycol liberated in the above-mentioned process can be calculated as the difference between the number of moles of ethoxylate units at the beginning and at the end of the assay. As for the upper limit of mineralization produced in the assays being described, this can be calculated by the difference between the amount of Empilan NP15 used at the beginning of the experiment and the amount of metabolites generated, NP2EO, NP2EC and NP1EC, as well as the residual oligomers with a ethoxylated chain length of between 3 and 22 units which have not been biodegraded by the last day of the assay. The results obtained were as follows:

Table 4. Average of the grade mineralization achieved in the assays.

Concentration (ppm)	2.5	5	10
Temperature (°C)	21	21	21
% min.mineralization	64.14	66.42	66.73
% max. mineralization	73.25	85.35	69.73

It can be observed that the minimum percentages of biodegradation achieved by the nonylphenol polyethoxylate tested were around 65%. The great difference between the percentage of maximum and minimum mineralization in the assay with 5 ppm of Empilan NP15 is probably due to the fact that the concentration of the non-identified metabolite, therefore not quantified, that appears to the left in the reverse phase chromatograms should have also been subtracted in the maximum grade of mineralization that can be achieved. It is precisely in this test where it is most important to take into account the non-identified metabolite as its signal is strongest here. It should be pointed out that the percentages of mineralization calculated in this way coincide with those described in the bibliography by other authors (Patoczka and

Pulliam 1990), who obtain percentages of total biodegradation in the order of 70-75%, calculated by means of COD (chemical oxygen demand) and TOC (total organic carbon).

Quiroga and Sales (1991) have developed a kinetic model in which the rate of degradation is given by a second-grade polynome, exclusively in function of substrate concentration. When integrated, the equation which represents the model is obtained:

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

Where S_0 is initial substrate concentration; p, q and h are combinations of the coefficients of the second grade polynome 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, obtaining a physical meaning for the values of parameters p, q and h.

p= maximum velocity of microorganism growth.

h= maximum concentration of substrate which can be used to form biomass.

q= concentration of non-biodegradable substrate.

By fitting the experimental data to the model equation, the values shown in Table 5 for the various kinetic parameters and the correlation coefficients for the different tests are obtained. It can be seen that the correlation coefficients shown in Table 5 are very close to one, which implies that the theoretical values predicted by the kinetic model proposed by these authors are very close to the experimental results obtained for all the concentrations studied. As far as the value of the parameters in the various tests is concerned, several interpretations can be made.

Table 5. Kinetic parameters obtained from the Quiroga-Sales model.

Test	1	2	3
Conc.(ppm)	2.5	5	10
Temp.(°C)	21	21	21
h (mg/L)	2.522	5.021	10.063
q (mg/L)	0.466	0.856	1.525
p ($days^{-1}$)	1.876	5.596	7.032
r^2	0.991	0.995	0.998

The values obtained for parameter *"h"* in the tests are very similar to the initial concentrations of NPEO employed, which confirms that the kinetic model is appropriate for use under the conditions being studied. The values for *"q"*, which represent the concentration of non-biodegradable substrate, increases at higher concentrations, with 0.46 ppm in Test 1 and 1.52 ppm in Test 3, which indicates that the levels of remaining NPEO increase at higher concentrations. As regards the values

for *"p"*, it can be seen in Table 5 that at higher concentrations, the degradation rate increases, as is shown by the fact that the value for *"p"*, in Test 1 at the lowest concentration, is 1.876, while in Test 3, at the highest concentration, it is 7.03. These figures agree with the data for the percentage of residual surfactant material.

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