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THE EFFECT OF TEMPERATURE ON THE BIODEGRADATION OF A NONYLPHENOL POLYETHOXYLATE IN RIVER WATER

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Abstract—In this paper a study is made of the biodegradability of a non-ionic surfactant, a nonylphenol polyethoxylate, in river water by means of monitoring the residual surfactant matter and the metabolites that may be generated. The influence of temperature on the extent of primary and ultimate biodegradation, and the kinetics of degradation are also determined.

The method used was the river die-away test, and the biodegradation process was monitored by normal and reversed phase high-performance liquid chromatography (HPLC). These results are supported by other indirect measurements and indicators of the existence of microbial degradation process, as well as the parameters for the control of the process.

The results obtained indicate that temperature has a strong influence on the period of acclimation of the microorganisms and on the rate of biodegradation. The percentages of primary biodegradation vary from 68% at 7°C to 96% at 25°C, and at all the temperatures studied, metabolites are generated during the biodegradation process which do not totally disappear at the end of the assay. The percentages of mineralization reached in the various assays, ranging from 30% at 7°C to 70% at 25°C, also show the great influence of temperature.

Finally, a kinetic study of the biodegradation process has been carried out, with excellent fit of the experimental data to the kinetic model of Quiroga and Sales.

Key words—biodegradation, temperature, nonylphenol polyethoxylate, river water, kinetic, intermediates, HPLC

NOMENCLATURE

APEO	alkylphenol polyethoxylates
COD	chemical oxygen demand
Empilan NP15	commercial name of the NP15EO used
h	maximum concentration of substrate which can be used to form biomass
HPC	standard heterotrophic plate count
HPLC	high-performance liquid chromatography
NPEO	nonylphenol polyethoxylate
NP15EO	nonylphenol polyethoxylate with an average of 15 ethoxylated units
NP1EC	nonylphenoxyacetic acid
NP2EC	nonylphenoxyethoxyacetic acid
NP2EO	nonylphenol diethoxylate
p	specific maximum velocity of microorganism growth
q	concentration of non-biodegradable substrate
S	substrate concentration
S_0	initial substrate concentration
TOC	total organic carbon

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INTRODUCTION

Alkylphenol polyethoxylates (APEO) have been an important group of surfactants since they were first synthesized in 1940 (Wang and Fingas, 1993). At present they are the most widely used at industrial level and third in the ranking for all types of application, with an annual volume of production of 370,000 ton (Raymond, 1996).

As a result of their wide field of application, their resistance to biodegradation at low temperatures and the generation during the degradation process of some persistent metabolites (Fig. 1) which are much more toxic than the original compound (Marcomini *et al.*, 1990; Schröder, 1993; Naylor, 1995), the use of these surfactants has been banned in domestic formulations in the United Kingdom, Germany and Switzerland. Their use is restricted to

industrial applications in which the specific nature of the properties required make it more difficult to substitute them.

Both primary and ultimate biodegradation of alkylphenol polyethoxylates depends to a large extent on environmental factors. Percentages of primary elimination can be found ranging from 63% (Okpokwasili and Olisa, 1991) to almost 100% (Specchia *et al.*, 1989; Patoczka and Pulliam, 1990), with mineralization varying from a very low percentage (Reinhard *et al.*, 1982; Patoczka and Pulliam, 1990) to over 90% (Gerike and Jasiak, 1984).

In this paper, a study has been made of the biodegradability of a nonylphenol polyethoxylate in river water by means of monitoring the residual surfactant matter and the metabolites that may be generated. An investigation was made of the influence of temperature on the extent of primary and total biodegradation, and on its degradation kinetics.

MATERIAL 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 2–22 ethoxylated units.

The water used in the assay 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. The most relevant physical and chemical characteristics of the water for this type of study are presented in Table 1.

The method chosen to carry out the biodegradation study was the "river die-away test". The reactors used for the assays had a capacity of 6 l and contained 4 l 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 a concentration of 5 ppm of NP15EO in each test. Finally, the reactors were covered with hydrophobic cottonwool and placed in the dark at temperatures of 7, 13, 21 and 25°C. The assays were carried out in duplicate. In addition test blanks were done to measure abiotic losses.

Analytical techniques for monitoring biodegradation

Analysis of the oligomeric composition of the surfactant. 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 detector, operating at 225 nm (excitation) and 304 nm (emission).

Table 1. Characteristics of the water used in the assays

Parameter	Value
NO_2^-	0.57 mg/l
NO_3^-	0
NH_4^+	0.15 mg/l
Total suspended solids	7 mg/l
SO_4^{2-}	33.74 mg/l
pH	8.2
Conductivity	503 μS/cm
Permanganate oxidability	2 mg O_2/l
Colony-forming units	84000 CFU/ml
APEO	0

A Hypersil APS-2,NH_2 column was used for the stationary phase, with a 5 μm particle size, an internal diameter of 4.6 mm and 250 mm in length. This analytical column was used in conjunction with a μBondapak NH_2 precolumn. The elution was carried out in the gradient mode, using a mixture of isopropanol/water (95/5) as eluent A, and hexane/isopropanol (98/2) as eluent B (Table 2).

The samples taken from the biodegradation experiments were pretreated before being injected into the chromatograph. They were dried at a temperature of 45–55°C, assisting evaporation with a nitrogen current. Once the dissolvent had evaporated, the dry residue was resuspended in 1 ml of hexane/isopropanol (80/20). The yield of this process of concentration and purification was 94%.

Identification was accomplished by injecting internal standard solution of known composition. In order to quantify 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).

Analysis of the acid biointermediates. The determination of the various acids resulting from the oxidation of the ethoxylated chain during the biodegradation process, NP1EC and NP2EC, was carried out by reversed phase high performance liquid chromatography, following the model proposed by Marcomini *et al.* (1989).

A gradient program was used with acetonitrile as eluent A and a water/acetonitrile mixture (75/25) containing 0.1 M $NaClO_4$ as eluent B. The gradient used is shown in Table 3.

For the stationary phase, a Lichrospher 100 RP 18 stainless steel column was used, with a particle size of 5 μm, an internal diameter of 4.6 mm and a length of 250 mm. This was used with a C-18 precolumn. The wavelengths of the fluorescence detector were 225 nm (excitation) and 295 nm (emission).

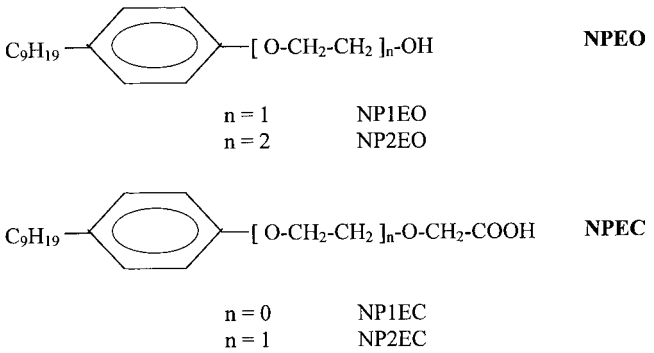

Fig. 1. Structures and acronyms of nonylphenol polyethoxylates and their metabolites.

Table 2. Gradient of the chromatographic method of analysis of the 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

Table 3. Gradient of the chromatographic method of analysis of the acidic intermediates of alkylphenol polyethoxylates (flow rate: 1 ml/min)

Time (min)	%A	%B
0	25	75
3	25	75
15	60	40
16	60	40
20	80	20
24	80	20
30	25	75
33	25	75

The identification of the peaks required the synthesis of the NP1EC, following the method described by Reinhard *et al.* (1982). The synthesis of the NP1EC permitted its quantification and that of the NP2EC, which has the same molar absorption coefficient as NP1EC (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 the number of live heterotrophic bacteria, 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

Evolution of NPEO content

Figure 2 shows the evolution of the percentage of residual surfactant during the biodegradation process.

Firstly, it can be seen that a decrease in temperature causes a delay in the start of the process, which shows how important temperature is in the NPEO biological degradation process as it has a significant effect on the period of acclimation. Thus, for the assay at 7°C this phase lasts 15–20 days, while at 13°C the delay is 4–10 days. At 21°C, only 2–3 days are necessary, and less than a day at 25°C.

It can be seen that the grade of primary biodegradation achieved, the loss of ethoxylate structure, also depends on the temperature. The lower the temperature, the lesser the degree of degradation. At 7°C, there is 32% of residual surfactant, compared with 20% at 13°C, 13% at 21°C and 4% at 25°C. These results for elimination of nonylphenol polyethoxylates are in keeping with those obtained in the water treatment plant in the United Kingdom, namely 80% in summer and 20% in winter (Bergeron, 1974).

On the other hand, the data does not coincide with that obtained by the Water Pollution Research Laboratory (Rudling, 1972) in the United Kingdom, where such a clear dependence on temperature is not observed at an initial concentration of 5 ppm. In those assays, using a nonylphenol polyethoxylate called Nonidet P-40, 94% elimination was achieved at 15°C and 91% at 8°C. This was probably due to the fact that the determination of residual surfactant was carried out by means of methods which are not sensitive to ethoxylate chain oligomers of a length inferior to 4. This means that the results overestimate and do not distinguish between the states of degradation reached when the residual metabolites contain less than 4 ethoxylate units.

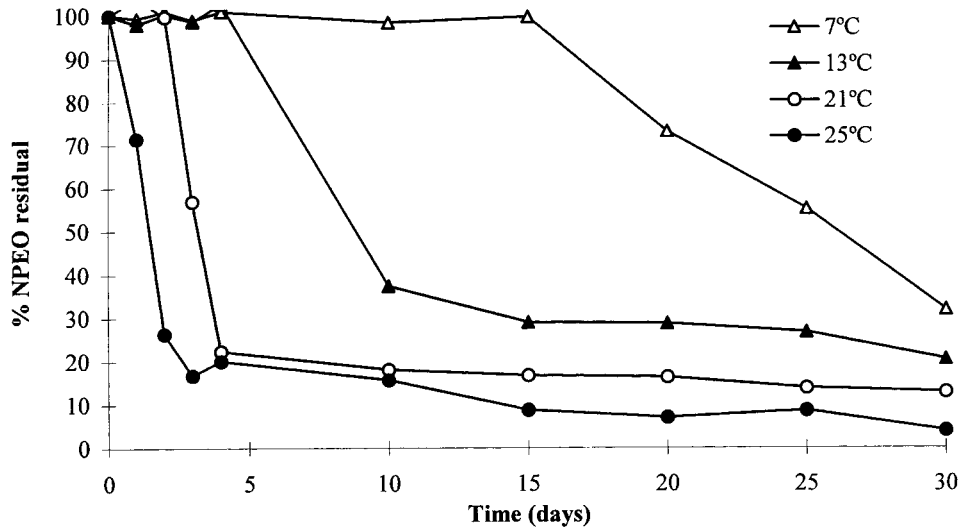

Fig. 2. Evolution of the percentage of residual surfactant.

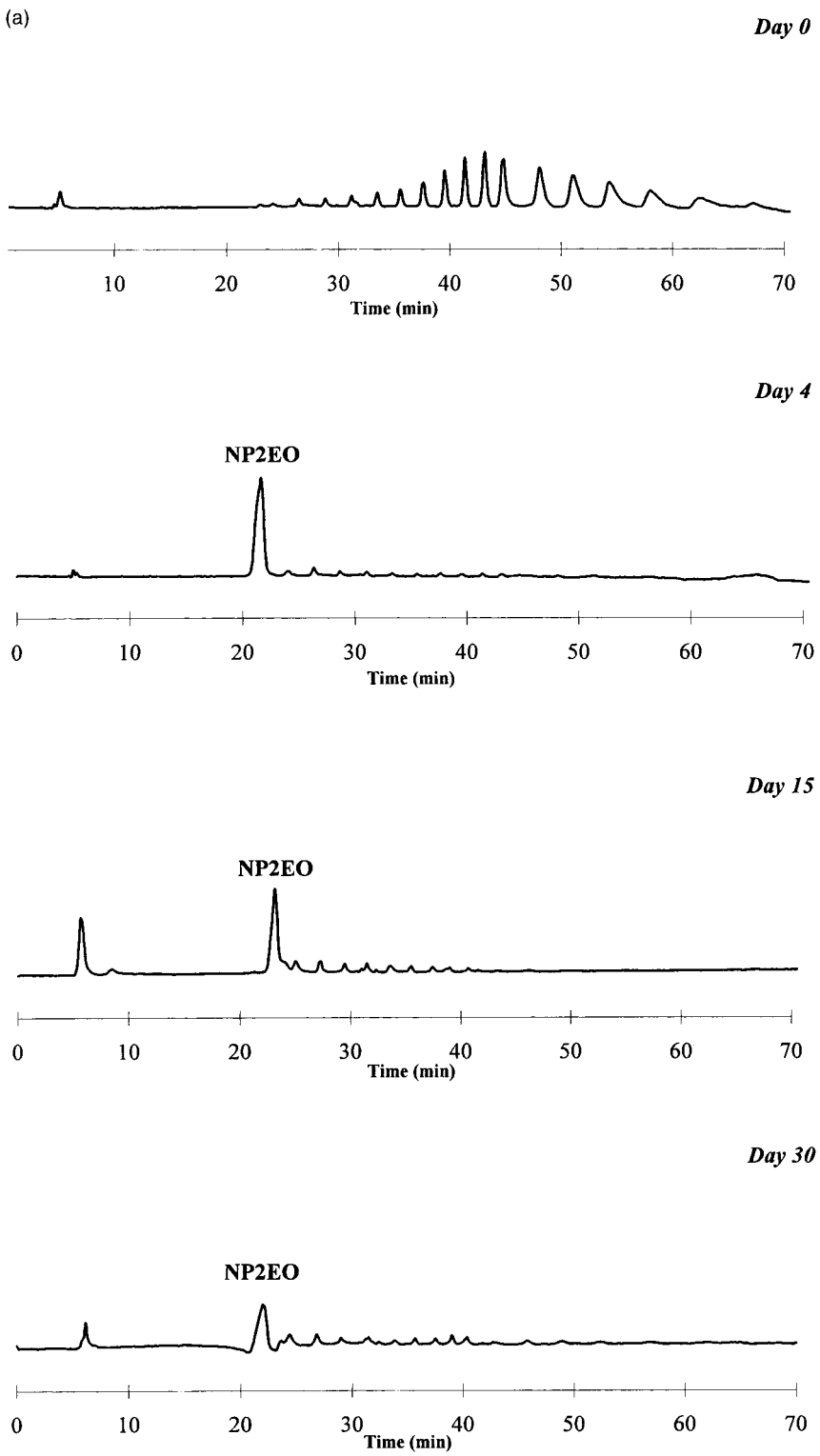
(a)
Day 0
10
20
30
40
50
60
70
Time (min)
Day 4
NP2EO
0
10
20
30
40
50
60
70
Time (min)
Day 15
NP2EO
0
10
20
30
40
50
60
70
Time (min)
Day 30
NP2EO
0
10
20
30
40
50
60
70
Time (min)

Fig. 3(a).

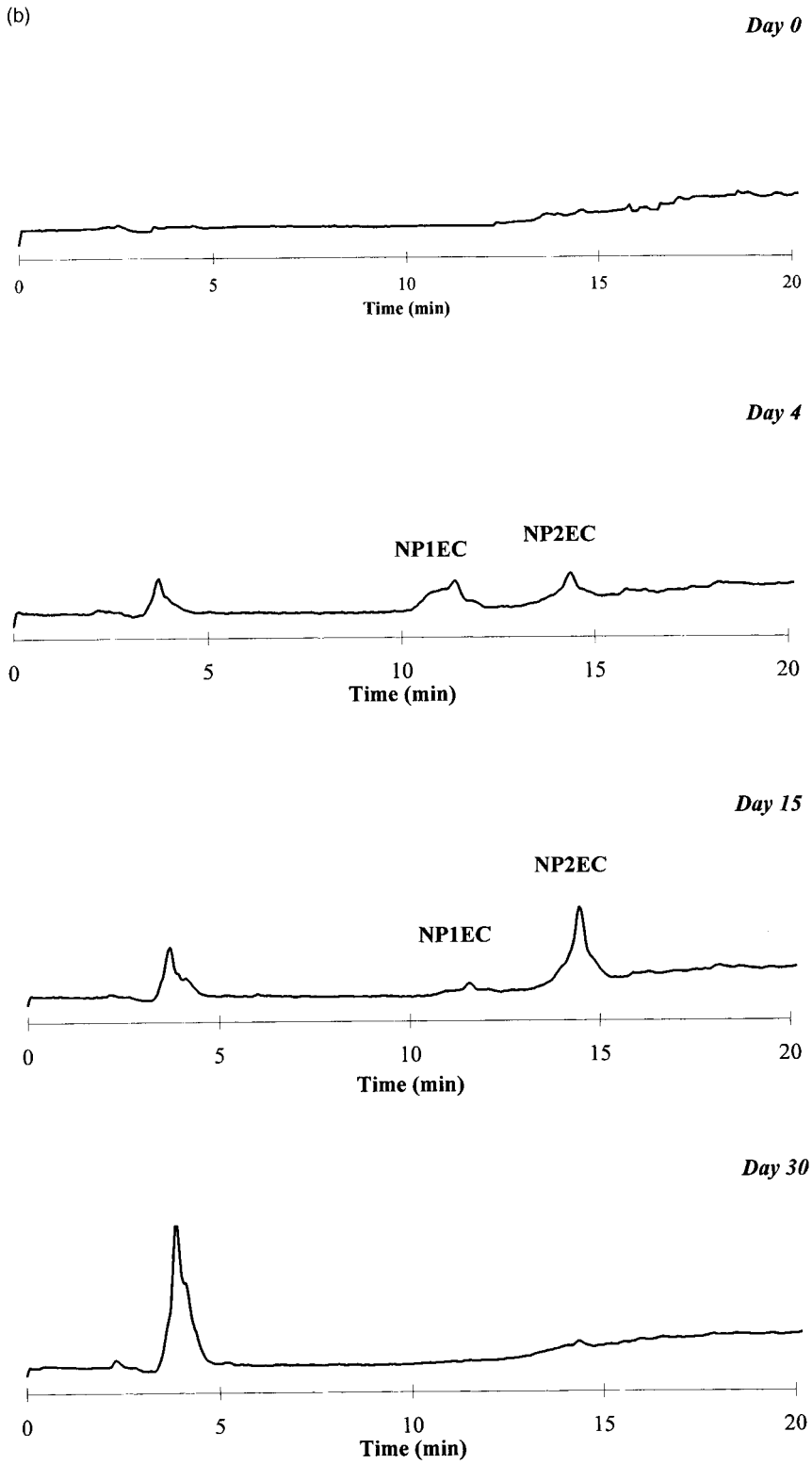

Fig. 3. (a) Normal and (b) reversed phase chromatograms for days 0, 4, 15 and 30 of the assay with 5 ppm of Empilan NP15 at 21°C.

Table 4. Grade mineralization achieved in the assays

Concentration (ppm)	5	5	5	5
Temperature (°C)	7	13	21	25
% min. mineralization	31.94	61.21	66.73	70.51
% max. mineralization	33.89	66.37	85.35	96.01

Evolution of the metabolites generated during the biodegradation process

Figure 3 presents the chromatograms showing all the metabolites generated during the biodegradation process for the assay at 21°C. 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 NP2EO, with the corresponding liberation of ethyleneglycol. From this point on, there is no further shortening of the ethoxylate chain, as is proved by the fact that nonylphenol monoethoxylate 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 coincides with the period 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 of the 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 affinity 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 of the ethoxylate chain and the aromatic ring described by Hughes *et al.* (1996).

Mineralization reached

Until now, the term biodegradation or elimination of NPEO has been used to refer to primary biodegradation or loss of the polyethoxylate structure. This, however, is far from what is known as ultimate biodegradation or mineralization, i.e. the breakdown of a compound into inorganic substances or compounds making up the cellular biomass. 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, ethyleneglycol is released, a substance which is easily mineralized, producing CO_2 and H_2O (Swisher, 1987). From the calculation of the amount of ethyleneglycol released, the minimum percentage of mineralization which has taken place can be worked out, i.e. that which is due exclusively to the shortening of the hydrophylic chain. The amount of ethyleneglycol liberated in the above-mentioned process can be worked out by calculating 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):

It can be observed that the minimum percentages of biodegradation achieved by the nonylphenol polyethoxylate tested were around 65%, except in the cases where extreme temperatures were reached. In these cases, an additional 5% was registered at 25°C, whereas at 7°C, the figure fell to 32%.

The great difference between the percentage of maximum and minimum mineralization in the assays at 21°C and 25°C is probably due to the fact that the concentration of the non-identified metabolite 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 these two tests where it is most important to take into account the non-identified metabolite as its signal is strongest here.

Table 5. Kinetic parameters obtained from the Quiroga–Sales model (1991)

Conc. (ppm)	5	5	5	5
Temp. (°C)	7	13	21	25
h (mg/l)	5.003	5.107	5.021	5.178
q (mg/l)	1.603	0.997	0.856	0.565
p (days^{-1})	0.381	1.901	5.596	8.936
r^2	0.999	0.998	0.995	0.998

It should be pointed out that the percentages of mineralization calculated in this way coincide with those described in the bibliography by other authors, who obtain percentages of total biodegradation in the order of 70–75%, calculated by means of COD and TOC (Patoczka and Pulliam, 1990).

Biodegradation kinetics. The Quiroga–Sales model

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, an equation which represents the model is obtained,

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

where S_0 is initial substrate concentration and p, q and h are combinations of the coefficients of the second grade polynome which defines the substrate consumption rate.

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 = specific 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 unit, which implies that the theoretical values predicted by the kinetic model proposed by these authors are very close to the experimental results obtained at all temperatures and concentrations studied, even in those cases in which there were prolonged induction periods (7 and 13°C).

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 represents the concentration of non-biodegradable substrate, increase as the temperature falls, ranging from 0.56 ppm at 25°C to 1.60 ppm at 7°C. This indicates that at lower temperatures, there was a greater amount of remaining NPEO.

As regards p, it can be seen that the specific maximum growth rate increases in line with temperature. Thus, in test 4 at 7°C, p is 0.381, whereas at 13 and 21°C it is 1.901 and 5.596 respectively, increasing to 8.936 at 25°C, the maximum temperature studied.

CONCLUSIONS

1. The levels of primary elimination of the surfactant under study fell as temperature decreased. Thus, at 25°C there was 96% primary biodegradation, while at 21°C this had fallen to 87%, at 13°C to 80% and at 7°C to 68%.
2. The degradation rate of the nonylphenol polyethoxylates increased with temperature. Temperature plays a primary role in the degradation of nonylphenol polyethoxylates, with significant increases in the rate of the process brought about by rises in temperature.
3. Temperature also plays an important role in the acclimation period, with decreases in temperature resulting in increasingly higher values.
4. The shortening of the ethoxylate chain to produce a nonylphenol diethoxylate is confirmed as the principal mechanism in the biodegradaton of nonylphenol polyethoxylates in river water, as reflected in the bibliography on waste water treatment plants. The main mechanism of the reaction then becomes the oxidation of the terminal ethoxylate unit of the above-mentioned metabolite, producing the NP2EC and, from this, the NP1EC.
5. Total elimination or mineralization of the nonylphenol polyethoxylate was not observed in any of the test conditions over the duration of the assays, due to the persistence of the degradation intermediates in the medium under all the test conditions studied.
6. The Quiroga–Sales kinetic model offers a very accurate description of all the tests carried out.

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