

Primary Biodegradation of Commercial Fatty-Alcohol Ethoxylate Surfactants: Characteristic Parameters

E. Jurado · M. Fernández-Serrano ·
J. Núñez-Olea · M. Lechuga

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Abstract This paper concerns the primary biodegradation of different commercial fatty-alcohol ethoxylate surfactants (FAEs), applying the OECD 301 E test for ready biodegradability. Changes were made both in the carbon-chain length of the surfactants as well as in the number of units of ethylene oxide (EO) in its molecule. The biodegradation were monitored, analysing the colony-forming units (CFU) formed during this process. From the biodegradation profiles drawn for the FAEs, parameters characteristic of the biodegradation process were defined: latency time (t_L), biodegradability at 50 h of assay (B), half-life ($t_{1/2}$), mean biodegradation rate until reaching biodegradability of 85% (V_M), and the residual concentration of the surfactant (S_R). The analysis of these parameters enabled the establishment of the influence of surfactant concentration and structure during the biodegradation process. The increase in the surfactant concentration lowered the rate of the biodegradation process and the biodegradability of the surfactant in addition to the half-life and residual concentration of the surfactant. The mean biodegradation rate, V_M , for fatty-alcohol ethoxylates increased with the number of EO units and molecular weight of the surfactant. At low initial test concentrations (less than 25 mg/L), the concentration of the residual surfactant rapidly diminished with biodegradation time. For higher concentrations, after an adaptation period of the microorganisms, the surfactant concentration declined

exponentially and the biodegradation rate became far slower for all the surfactants. The parameters characteristic of the growth curves: specific growth-rate, k , and the yield of biomass production per gram of surfactant, Y_{ap} , made possible the quantification and corroboration of the results during the biodegradation process.

Keywords Fatty-alcohol ethoxylates · Biodegradation · Surfactants

Introduction

Surfactants are widely used in household cleaning detergents, personal-care products, textiles, paints, polymers, pesticide formulations, pharmaceuticals, mining, oil recovery, and the pulp and paper industries. The world production of synthetic surfactants amounts to 7.2 million tonnes annually [2]. After use, surfactants as well as their products are mainly discharged into sewage-treatment plants and then dispersed into the environment by releasing effluents into surface waters and by sludge disposal on land. Surfactants have different behaviors and fates in the environment.

FAEs make up the second highest volume group of surfactant after LAS and the leading group within the family of non-ionic ethoxylate surfactants [3]. In Europe, 5×10^5 tonnes of these surfactants are sold yearly [4] representing some 40% of the total production of FAEs [5], most of these being used in detergent formulas. FAEs are easily biodegraded [3, 6, 7], although the massive dumping of these surfactants into sewage waters requires strict control to prevent the pollution of the aquatic environment, and generally waters are controlled by determining only the anionic surfactants. One key element

E. Jurado (✉) · M. Fernández-Serrano ·
J. Núñez-Olea · M. Lechuga
Department of Chemical Engineering, Faculty of Sciences,
University of Granada, Campus Fuentenueva s/n,
18071 Granada, Spain
e-mail: ejurado@ugr.es

in this process is the evaluation of the biodegradability and toxicity for guaranteeing the use of these surfactants in domestic and industrial formulations.

The FAEs used in this work belong to those called oleochemical—that is, they are from natural, renewable sources. FAEs are not pure as they are mixtures of molecules that differ in the size of the hydrocarbon and ethoxylate chain. The mixture of oligomers has mean lengths between 1 and 20 ethoxylate units and homologs with a number of carbon atoms in the alkyl chain between 12 and 18.

Due to the enormous economic importance of surfactants and their contribution to the deterioration of the environment when these persist in nature, numerous studies have sought to establish the structural characteristics that govern the susceptibility of these molecules to be degraded.

The factor that strongly affects the biodegradability of FAEs is the hydrophobic structure and in particular the linearity of the carbon backbone, which has a heavier influence than other factors such as length of the alkyl chain, the type of bond to the ethoxylate chain, and its length. The search for relationships between parameters in the biodegradation process and the structural properties of these surfactants would serve to characterize them with respect to an environmental standpoint.

The present work analyses the biodegradation of linear FAEs in relation to microorganism growth during this process. The study includes different FAEs in which the length of the carbon chain and the number of units of ethylene oxide has been modified, and the influence of the surfactant concentration has been analysed both in the biodegradation process as well as in the growth of the microorganisms.

Experimental Procedures

Surfactants

The following commercial surfactants, fatty-alcohol ethoxylates with the general formula $R(-O-CH_2-CH_2)_n-OH$, were used in this study: FINDET 10/15, FINDET 10/18, FINDET 1214N/16, FINDET 1214N/23, FINDET 1618A/18 and FINDET 1618A/23 supplied by KAO CORPORATION S.A. (Tokyo, Japan). Table 1 shows the main characteristics of the surfactants analysed in this research.

Static-Biodegradation Assay

The static biodegradation tests were carried out according to the OECD 301 E test for ready biodegradability [1]. 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. 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 FAEs were determined by the iodine-iodide colorimetric

Table 1 Description and properties of the surfactants employed in the washing tests

Commercial name	Symbol	Structure ^a	HLB [8]	MW, (g/mol) [9]	CMC, (mg/L) [10]
FINDET 10/15	C ₁₀ E ₃	R: 10 EO: 3	9.6	272	163.20
FINDET 10/18	C ₁₀ E ₆	R: 10 EO: 6	12.7	387	348.30
FINDET 1214N/16	C _{12–14} E ₄	R:12(70%)-14(30%) EO: 4	9.5	370	20.35
FINDET 1214N/23	C _{12–14} E ₁₁	R:12(70%)-14(30%) EO: 11	14.4	630	88.20
FINDET 1618A/18	C _{16–18} E ₆	R:16–18 EO: 6	10.2	603	0.81
FINDET 1618A/23	C _{16–18} E ₁₁	R:16–18 EO: 11	13.2	1386	3.18

R size of the hydrocarbon chain; *EO* number of ethylene oxide units; *HLB* hydrophilic/lipophilic balance calculated according to Griffin; *MW* molecular weight

^a Manufacturer

method [11]. For the absorbance measurements, a double-beam spectrophotometer SPECTRONIC UNICAM UV-V was used. The absorbance was directly proportional to the surfactant concentration.

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. The initial concentration of the reference assay was 5 mg/L in all cases, and the mean biodegradability at 5 days was $97.28\% \pm 1.54$. According to the OECD [1], for the validity of the assay to be accepted, the percentage of the biodegradation of the soft standard after 5 days should be greater than 90%. It is known that sorption may significantly influence the resulting environmental effects of surfactants [12] and it has been studied by some authors [13] who have proposed expressions to predict the sorption onto activated sludge particles for alcohol ethoxylates. In the biodegradation assays presented here, the sorption can be considered negligible, given the scant biomass formation. To confirm this fact, abiotic assays were made in the presence of HgCl_2 , observing that 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 FAEs in the biodegradation tests made can be dismissed.

The biodegradation was studied at different initial concentrations varying from 5 to 50 mg/L: surfactant $\text{C}_{12-14}\text{E}_{11}$ was studied at concentrations of 5, 15, 20, 25, and 50 mg/L; the FAEs $\text{C}_{12-14}\text{E}_4$ and $\text{C}_{16-18}\text{E}_6$ at 5, 25, and 50 mg/L; while the surfactants C_{10}E_3 , C_{10}E_6 and $\text{C}_{16-18}\text{E}_{11}$ were studied at an initial concentration of 5 mg/L.

Biomass Growth During the Biodegradation Process

During the biodegradation assays, the number of viable microorganisms was measured by counting the heterotrophic microbes in a dish [14], expressing the result as colony-forming units (CFU) per mL. The culture medium, nutrient agar, enables the detection of a broad variety of microorganisms. With a sterile pipette, 1 mL of sample is taken from the culture, and a series of 1:10 dilutions are taken in CINa at 0.9% until reaching a suspension of microorganisms of between 30 and 80 viable cells per mL of test solution.

Each dilution was analysed in duplicate: 1 mL of sample to be analysed was placed on a 10-cm dish. Then 20 mL of previously sterilized culture medium was poured onto the dish, sterilized, melted and tempered at 60 °C, and gently stirred to complete the homogenization. The mixture was left to cool until complete solidification and then incubated at 25 °C for 72 h in darkness. The total number of microorganisms was determined by multiplying the number of CFU by the corresponding dilution factor.

Results and Discussion

The effect of the concentration of the FAEs on the primary biodegradation was studied for the commercial surfactants $\text{C}_{12-14}\text{E}_4$, $\text{C}_{12-14}\text{E}_{11}$ and $\text{C}_{16-18}\text{E}_6$. The biodegradation of surfactants C_{10}E_3 , C_{10}E_6 and $\text{C}_{16-18}\text{E}_{11}$ at 5 mg/L were also studied. Figure 1 presents the results of the primary biodegradation of these surfactants. The concentration is expressed as a percentage of residual surfactant and each point of the graphs represents the mean value of the two replicates.

In light of legislation demanding a minimum level of 80% of the primary biodegradation after 19 days, when the surfactants are subjected to the OECD test [1] all the surfactants can be considered biodegradable, as shown in Fig. 1. Biodegradation exceeds 90% in less than 13 days (most in 4 days) except for $\text{C}_{12-14}\text{E}_4$ at 50 mg/L, which did not surpass 60% in this time period.

In a preliminary analysis of the primary-biodegradation profiles (Fig. 1), it was found that for the three surfactants for which the concentration had been modified, the concentration of the residual surfactant rapidly diminished with biodegradation time. For higher concentrations, after an adaptation period of the microorganisms, the surfactant concentration declined exponentially and the biodegradation rate became far slower for all the surfactants, this effect being more pronounced for the surfactant with the least number of ethylene oxide units, $\text{C}_{12-14}\text{E}_4$.

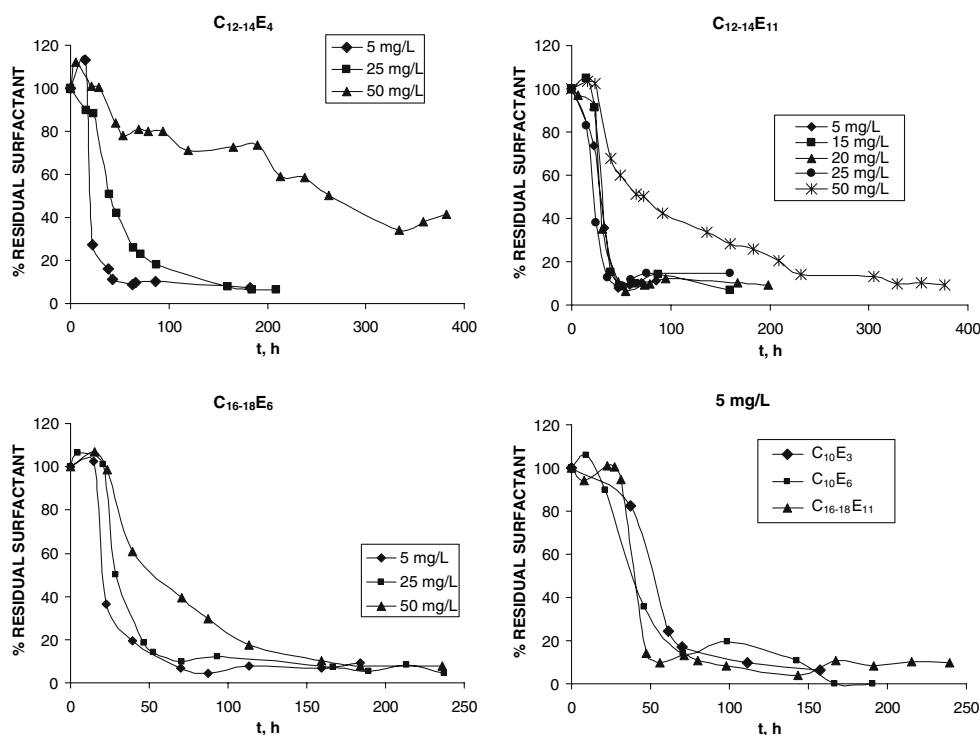
The biodegradation curves found for 50 mg/L significantly differed from the rest of the concentrations studied, the shape of the curve was exponential, and, although the concentration did not significantly affect the latency time, the biodegradation process became slower.

Parameters Characteristic of the Biodegradation Process

For the comparison and quantification of the different biodegradation assays, a number of parameters characteristic of the biodegradation profiles were defined and evaluated.

Latency time (t_L) is the time needed for the non-adapted microorganisms to acclimatize themselves to the new substrate. The latency or acclimation period prior to the biodegradation process of organic compounds in the aquatic environment can have several causes, such as a lack of nutrients, enzymatic induction, predation by protozoa, mutation of species, growth of a microbial population capable of metabolising the substrate, or simply the adaptation to the presence of toxic agents [15–17]. This time corresponds to the period during which a mild change occurs in the residual concentration. For each biodegradation assay, it was calculated by drawing two tangents to

Fig. 1 Biodegradation of the FAEs under the conditions of a ready biodegradability test (OECD 1993b). Influence of the initial concentration of the FAEs (a) $C_{12-14}E_4$ (b) $C_{12-14}E_{11}$ (c) $C_{16-18}E_6$ (d) Biodegradation of the surfactants $C_{10}E_3$, $C_{10}E_6$, and $C_{16-18}E_{11}$ at 5 mg/L



the adaptation and biodegradation stages (Fig. 2). The latency term was the cut-off point of both straight lines.

Half-life time ($t_{1/2}$) is the time for which the substrate concentration diminishes to half from the beginning of the biodegradation process. Therefore, the latency time was also counted. As with the latency time, the half-life is calculated by graphic methods on the biodegradation curve (Fig. 2).

Mean biodegradation rate (V_M) has been defined as the mean velocity of biodegradation until achieving 85% biodegradation of the surfactant, and it has been calculated as

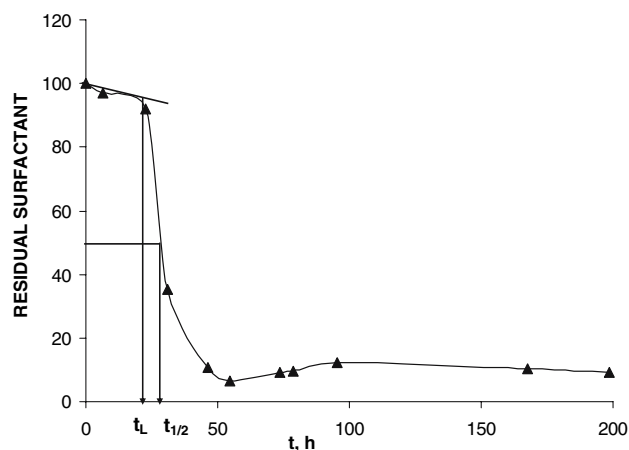


Fig. 2 Graphic determination of t_L and $t_{1/2}$ on the biodegradation curve

the quotient between the percentage of biodegradation reached and the time needed to reach this biodegradation value. This parameter provides the speed of the biodegradation process.

Residual-surfactant concentration (S_R) is the concentration which has not been degraded by the microorganisms and represents the concentration of the surfactant that is not metabolisable. It is calculated as the mean value of the surfactant concentration at the end of the assay when the surfactant remains practically constant over the biodegradation time.

Surfactant biodegradability (B) is defined as the percentage of surfactant that is biodegraded after 50 h of assay.

Table 2 shows the parameters characteristic of the biodegradation profiles for all the surfactants and all the concentrations assayed. S_0 is the initial concentration of the biodegradation assay in mg/L while t_T is the total duration of the assay in hours.

Figure 3 lists the characteristic parameters ($t_{1/2}$, V_M , B and S_R) of the biodegradation profiles as a function of the initial assay concentration for the surfactants $C_{12-14}E_4$, $C_{12-14}E_{11}$, and $C_{16-18}E_6$.

For the parameters $t_{1/2}$ and V_M , which are related to the rate of the biodegradation process, it is noteworthy firstly that $t_{1/2}$ slightly increased with the surfactant concentration to 25 mg/L, fundamentally for the surfactants with the highest number of ethylene oxide units, increasing notably for concentrations of 50 mg/L. Secondly, in a similar way,

Table 2 Parameters characteristic of the biodegradation profiles for the FAEs

S_0 (mg/L)	t_T (h)	B (%)	t_L (h)	$t_{1/2}$ (h)	V_M (%/h)	S_R (mg/L)
C₁₀E₃						
5	157.00	77	37.00	50.43	1.49	0.17
C₁₀E₆						
5	190.50	83	9.00	19.14	1.63	0.30
C_{12–14}E₄						
5	182.50	91	14.50	19.29	2.23	0.33
25	209.50	56	23.79	40.91	0.73	1.83
50	381.75	26	27.21	55.43	0.20	19.29
C_{12–14}E₁₁						
5	85.00	94	22.51	26.20	2.07	0.50
15	159.00	92	22.28	30.75	2.17	0.96
20	167.50	92	22.52	–	2.11	1.76
25	160.00	91	–	19.71	1.99	3.52
50	376.50	44	24.28	54.02	1.37	4.45
C_{16–18}E₆						
5	183.91	87.5	14.74	21.13	1.93	0.40
25	237.50	85	20.37	28.54	1.66	1.08
50	236.00	54	21.64	54.92	0.78	3.46
C_{16–18}E₁₁						
5	239.50	88	29.71	39.42	1.80	0.45

the mean biodegradation rate slowed with the initial surfactant concentration. This indicates that, as the concentration increased, biodegradation slowed down. This slowing in the mean biodegradation rate may have been due to an auto-inhibition triggered by a bacteriostatic effect of the substrate.

In the case of the parameters S_R and B , as the initial surfactant concentration increased in the assay, the residual concentration rose and the surfactant biodegradability increased, as might be expected (Fig. 3).

In an effort to establish relationships between the parameters found in the biodegradation process on the one hand, and the structural characteristics of the surfactants on the other, Fig. 4 presents the mean biodegradation rate against the number of EO units of the different surfactants assayed. It was found that for high assay concentrations the biodegradation rate increased with the size of the ethoxylate chain. For 5 mg/L, the effect of the number of EO units on the mean biodegradation rate was less pronounced (Fig. 4).

Several authors [5] have demonstrated that the primary degradation step is a central fission mechanism for which the intermediates are polyethyleneglycol (PEG) and fatty alcohols (Fig. 5). Steber and Wierich [18] have also studied the ultimate biodegradation of PEG.

The biodegradation rate appears to be greater the higher number of EO units of the ethoxylate chain

(Fig. 4) for surfactant concentrations above 5 mg/L. Furthermore, this effect is more pronounced the higher the initial surfactant concentration. The results suggest that the longer the ethoxylate chains released in the biodegradation process, the less acute is the inhibitory effect exerted on the microorganisms responsible for the biodegradation process.

The biodegradability of surfactants is influenced simultaneously by several parameters: HLB, CMC, carbon chainlength (Fig. 6), etc., and it is difficult to find a prominent parameter. However, there seems to be a positive correlation between the biodegradation and the HLB (Table 3).

The FAE C_{12–14}E₁₁, the surfactant with the greatest HLB, presents the greatest biodegradability. For the set of the surfactant tested, the FAEs which show a greater HLB have a lower CMC (Table 1). For the same surfactant concentration lower CMC surfactants have less free surfactant. Thus we can relate the high biodegradability to the lower presence of free surfactant which have an inhibitory effect in the medium.

In general, for all the concentrations assayed, the results appear to confirm that the biodegradation was preferential towards the surfactant with the longest alkyl chain (Fig. 6) and the greatest degree of ethoxylation (Fig. 4), this effect may be due to the lower CMC of these FAEs. If the biodegradation rate is considered as the slope of the biodegradation curve, the rate can be seen to be greater (at the three concentrations studied) for the surfactant with the longest carbon chain, C_{16–18}E₆; that is, the surfactant with the longest carbon and ethoxylate chain degraded to a greater extent and more rapidly than did the rest of the surfactants tested. This fact was more discernible when high surfactant concentrations were assayed (Fig. 1).

The literature provides examples of increase, decrease, and random values in the biodegradation rate in relation to the longest alkyl chain lengths [19]. For biodegradation assays in river water, it was found that the biodegradation rate increased for C₈–C₁₂ chain lengths and decreased for C₁₂–C₁₈. In other assays, the biodegradation rate diminished on going from the C₁₈ to C₈ chain length, but with different ethoxylate chain lengths. However, when only the hydrophobic part of the surfactant was taken into account, there was evidence of higher rates of primary biodegradation for homologs with longer alkyl chains [20]. A possible explanation to this fact may be that the CMC of the surfactant depends on its hydrophilic group (type and size) and on its lipophilic group (length and branching). In an aqueous medium, the CMC decreases when the number of C atoms in the alkyl chain of the surfactant increases and the surfactant can exert a minor inhibitory effect.

Fig. 3 Variation in the parameters characteristic of the biodegradation profiles for the FAEs with the initial concentration of surfactant material

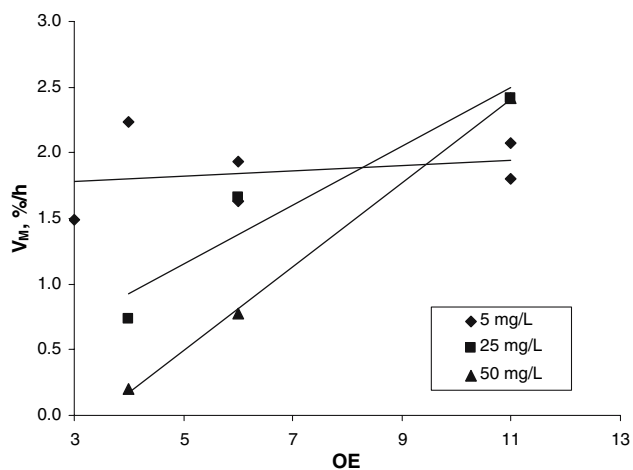
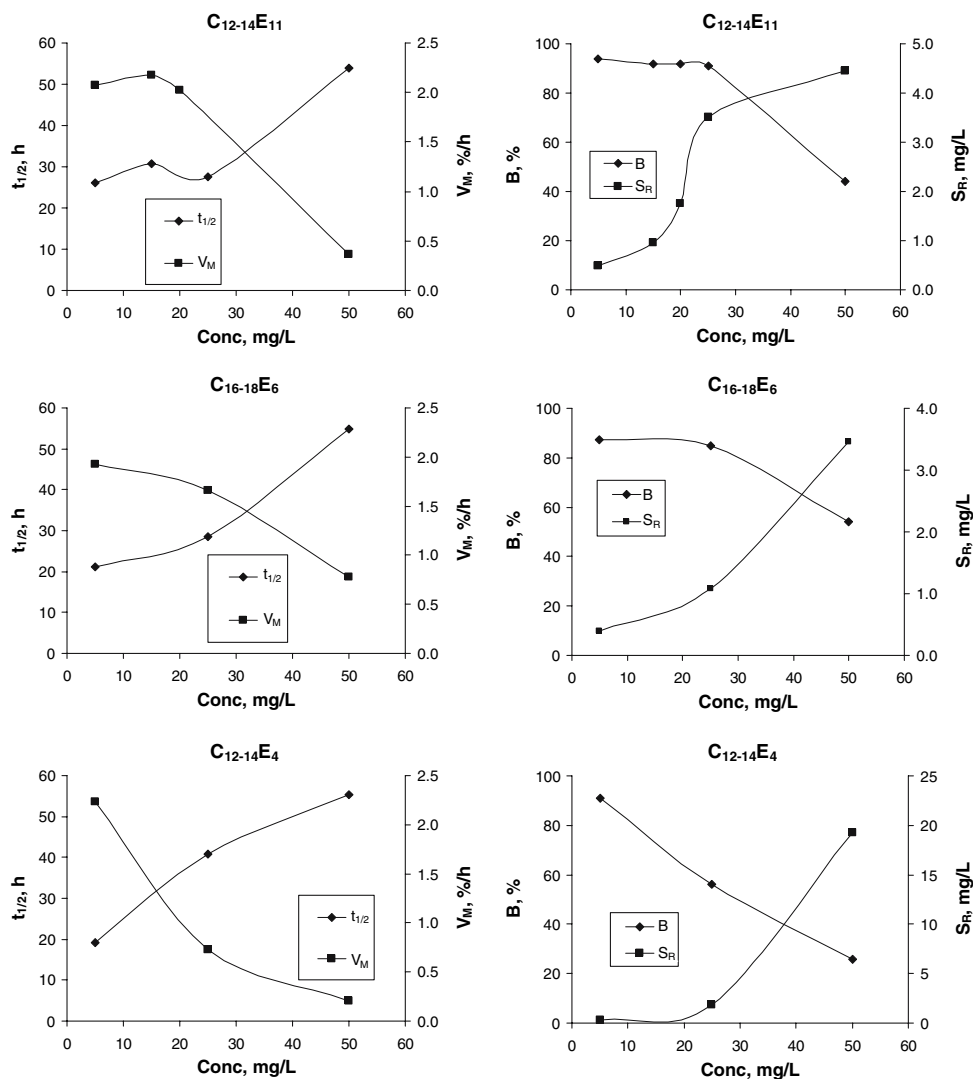
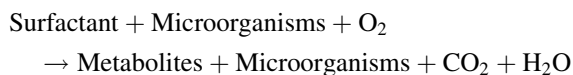


Fig. 4 Variation in the mean biodegradation rate against the number of EO units at different initial concentrations

Microbial Growth During Primary Biodegradation

During the biodegradation process, microorganisms consume the surfactant, which was the only carbon source present in the medium, and they transform it according to the following reaction:



That is, the microorganisms consume the surfactant molecule, break it down and transform it into biomass and into subproducts of degradation with the release of CO_2 . 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.

Fig. 5 Simplified mechanism of FAEs biodegradation

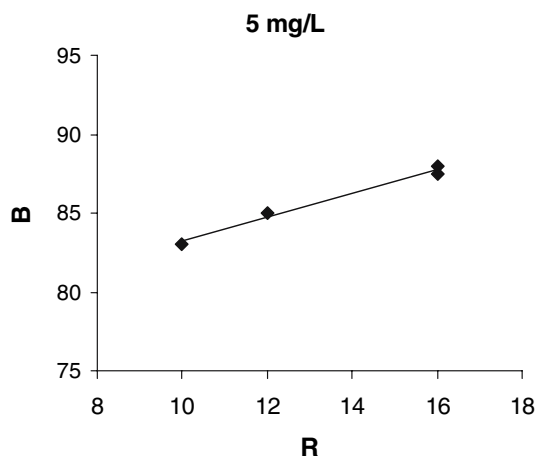
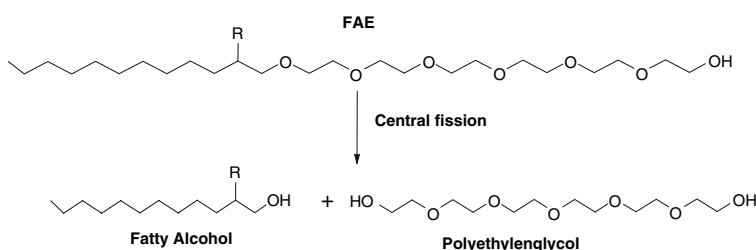


Fig. 6 Variation in the biodegradability against the length of the alkyl chain

Figure 7 presents, as an example, the growth curves together with the corresponding primary-biodegradation profiles for the surfactant C₁₂₋₁₄E₄. It can be seen that the biodegradation curves were adjusted by 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 concentration remained constant, the microorganisms registered no growth. Finally, for substrate concentrations greater than 5 mg/L, 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. This may be due to the mechanism of biodegradation of FAEs with fission of the central ether bond to yield the corresponding fatty alcohol (FA) and the (poly)ethylene oxide chain (PEO) which may be better substrates for the culture as compared to the original surfactant.

Microbial growth during the primary phase of the exponential growth can be described by Monod's equation [21] according to the expression

$$\frac{dX}{dt} = k \cdot X \tag{1}$$

or in its integrated form

$$X = X_0 \cdot \exp(k \cdot t) \tag{2}$$

where *k* 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. (2), the specific growth rate was determined for the FAEs assayed (*k*); *X*₀ is the initial value of CFU/mL found in the assay. Table 4 shows the parameters characteristic of the growth curves during the biodegradation process.

For each surfactant, *k* diminishes with the initial concentration of the assay, though for C₁₂₋₁₄E₄ there was no increase in growth rate when 25 was changed to 50 mg/L. These results indicate a possible inhibition of the microorganisms with concentration. Table 4 shows that at the

Table 3 Biodegradability of the FAEs against the HLB of the different initial concentrations

C ₁₀ E ₃ HLB = 9.6		C ₁₀ E ₆ HLB = 12.7		C ₁₂₋₁₄ E ₄ HLB = 9.5		C ₁₂₋₁₄ E ₁₁ HLB = 14.4		C ₁₆₋₁₈ E ₆ HLB = 10.2		C ₁₆₋₁₈ E ₁₁ HLB = 13.2	
S ₀ (mg/L)	B (%)	S ₀ (mg/L)	B (%)	S ₀ (mg/L)	B (%)	S ₀ (mg/L)	B (%)	S ₀ (mg/L)	B (%)	S ₀ (mg/L)	B (%)
5	77	5	83	5	91	5	94	5	87.5	5	88
–	–	–	–	25	56	15	92	25	85	–	–
–	–	–	–	50	26	20	92	50	54	–	–
–	–	–	–	–	–	25	91	–	–	–	–
–	–	–	–	–	–	50	44	–	–	–	–

Fig. 7 Growth curves and biodegradation profiles for the surfactant $C_{12-14}E_4$ at 5, 25 and 50 mg/L

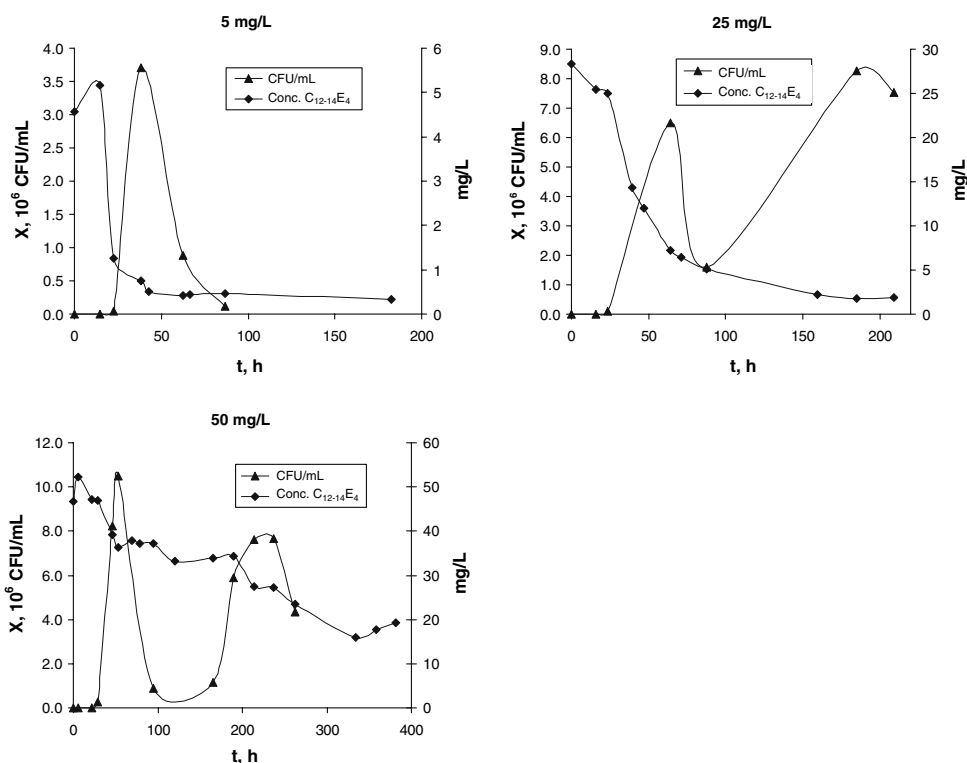


Table 4 Parameters characteristic of the growth curves for the FAEs

S_0 (mg/L)	X_0 (CFU/mL)	k (h^{-1})	Y_{ap} (CFU/g substrate)
$C_{12-14}E_4$			
5	2.05×10^3	0.271	9.01×10^5
25	2.65×10^2	0.155	5.43×10^5
50	2.95×10^2	0.162	8.84×10^5
$C_{12-14}E_{11}$			
15	4.30×10^3	0.207	6.13×10^5
20	4.98×10^2	0.116	9.71×10^5
50	8.02×10^4	0.052	1.11×10^5
$C_{16-18}E_6$			
5	1.75×10^3	0.076	9.82×10^3
25	1.50×10^4	0.020	6.25×10^4

two concentrations studied, the surfactant $C_{16-18}E_6$ (with lower CMC) had the lower growth rate. Also, the parameter Y_{ap} has been calculated, this representing the yield of the biomass production—that is, the proportion of the original substrate converted into biomass, which is 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.

When the test concentration is higher than the CMC, (for the surfactant $C_{12-14}E_4$) a decrease in the yield (Y_{ap})

can be seen, possibly due to the formation of micelles and to the lower presence of free surfactant in the medium.

For the same concentration of surfactant (5 mg/L), the yield of the biomass production is lower for the surfactant with lower CMC ($C_{16-18}E_6$) because of the formation of micelles, hence to the lower presence of free surfactant in the test medium.

The results of this study demonstrate that the biodegradation process is strongly related to the concentration of the initial surfactant and to the CMC of the surfactant. The concentration of the surfactant can exert an inhibitory effect whereas the quantity of the free surfactant present in the medium (C source for the microorganisms responsible for the process) depends on the CMC.

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Author Biographies

Dr. E. Jurado was born in 1951 and graduated from the University of Granada in 1975. She received her Ph.D. in 1980 and became full professor of Chemical Engineering in the Science Faculty at the same University in 1966. Her main research areas are: enzymes, kinetic enzymatic, biodegradation of surfactants, emulsions, and the physical chemistry and applications of surfactants. At present, she is head of the Chemical Engineering Department at the University of Granada. She has published over 60 papers in different fields.

Dr. M. Fernández-Serrano was born in 1967 and studied chemistry at the University of Granada. She was awarded a 4-year fellowship at the same University and received her Ph.D. in 1995. She became an associate professor at the University of Granada in 1999. Her research activities include searching for new groups of biodegradable surfactants.

Dr. J. Núñez-Olea was born in 1966 and studied chemistry at the University of Granada, where she received her Ph.D. in 1994. Currently, she is working for a water-supply and -treatment company (EMASAGRA) and is also an associate professor (part time) at the University of Granada. Her research interests include environmental surfactants and investigation of their properties.

Dr. M. Lechuga was born in 1976. She graduated from the University of Granada in Chemical Engineering and then worked on an investigation project entitled “Formulation of Liquid Detergents Specifically for the Industrial Agrofood and Hotel Sector” directed by E. Jurado. She received her Ph.D. in 2005 and is currently an associate professor at the University of Granada. Her research interest includes surfactants and their applications.