ORIGINAL ARTICLE

Combined Use of Ozonation and Biodegradation of Anionic and Non-ionic Surfactants

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Abstract This study investigated the extent of primary and final biodegradation of anionic and non-ionic surfactants to evaluate the combined use of ozonation and biodegradation in surfactant removal. The surfactants used were alkylpolyglucosides and linear alkyl benzene sulfonates. The anionic surfactant containing a benzene ring on its structure was oxidized faster than was the non-ionic surfactant. Both surfactants showed poor mineralization due to ozonation indicating an ozone attack primarily on carbon bonds. The results indicate that the removal of surfactants and of the total organic carbon is increased by the consecutive use of ozonation and biodegradation.

Keywords Anionic surfactants · Biodegradation · Combined processes · Non-ionic surfactants · Ozonation

Introduction

Surfactants are one of the most important components used in the formulation of detergents [1], in both industrial and domestic applications, comprising from 15 to 40 % of the total detergent formulation [2]. However, the presence of surfactants in the water and wastewater promotes many undesirable phenomena, harming fauna and flora living in surface waters by causing eutrophication. Some detergents inhibit algal growth at the concentration of 1 mg/dm^3, and their toxic dosage for fish ranges from 2 to 8 mg/dm^3. For

humans, detergents have slight toxicity but can induce skin allergies. The above-mentioned facts make it necessary to use low-toxic and biodegradable surfactants. One of the most important criteria in ecotoxicological risk assessment is the biodegradability of chemicals [3]. This is of great importance in the biological treatment of all types of sewage.

The methods for removing surfactants can be divided into three groups: biological, mechanical, and chemical/physicochemical. Oxidation is one of the main methods of the chemical breakdown of detergents. Ozonation involves complex gas–liquid processes where mass transfer and chemical reactions can control the overall kinetics [4, 5]. Usually, different reaction pathways contribute to the ozonation processes simultaneously. Molecular ozone mainly attacks directly aromatic rings, alkenes and alkynes with a high selectivity and low reaction rate [1, 6]. Parallely, there are a large number of secondary reactions of the hydroxyl radicals resulting from the decomposition of ozone catalysed by the hydroxyl ion through a series of reactions including initiation and propagation [7–10]. The indirect hydroxyl radical reactions are non-selective and fast, and proceed more rapidly with increasing pH [6]. Ozonation processes do not lead to complete mineralization but rather partial oxidation of surfactants to organic acids, aldehydes, and ketones [7]. However the role of molecular ozone as ring destroyer could be consider as an important tool to improve biodegradability of surfactant solutions.

Compounds containing aromatic rings [11], such as surfactants, are known to be less susceptible to microbial breakdown than are analogous compounds lacking benzene rings. Thus, the use of ozonation, as a way of destroying aromatic rings, may have beneficial effects by increasing the biodegradability of surfactants present in aqueous

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media. Several papers examine the relationship between ozonation processes of surfactant solutions and their biodegradability and aquatic toxicity [1, 12–16]. However, to the best of our knowledge, there is a lack of research on the effects of ozonation on biodegradation. The present study investigates the extent of surfactant biodegradation in order to evaluate the combined use of ozonation and biodegradation in surfactant removal.

Experimental Procedures

Surfactants and Reagents

The linear alkyl benzene sulfonate LAS-R$_{11-14}$ was supplied by Petresa (Cádiz, Spain). The non-ionic surfactant alkylpolyglucoside was manufactured by Henkel-Cognis (Henkel KgaA, Henkelstrasse Dusseldorf, Germany) and supplied by Sigma. The alkylpolyglucoside used in this study is Glucopone 600 CS UP (APG-R$_{12-14}$DP$_{1.5}$). Table 1 shows the chemical structure, the abbreviation of the surfactants used in the tests and the critical micelle concentration (CMC) of the surfactants.

The rest of the reagents used were PA quality and supplied by Panreac. All the solutions used in the experiments were prepared with distilled water.

Ozonation Process

The ozonation process was performed in a stirred tank reactor where gaseous ozone was bubbled (Fig. 1). Ozone was produced from pure oxygen by dielectric barrier discharge using an ozone generator Anseros COM-AD-01 (Anseros GmbH, Tübingen, Germany) with a maximum capacity of 4 g O$_3$/h. The gas mixture (air/ozone) at different flow rates was first passed through an ozone analyzer (Anseros Ozomat GM-6000), and then bubbled for 30 min into the stirred tank reactor (200 ml), for the surfactants ozonation study; and 15 min into the 2-L Erlenmeyer flask, for the ozonated screening biodegradation test, filled with the surfactant solution to be ozonated. The ozone concentration employed in the tests was 113 gN/m^3. The

unreacted ozone in the outlet gas was collected in two potassium iodide traps (5 % w/w) connected in series. A constant temperature (25 °C) was maintained in the reactor by circulating water in a thermostatic bath through the reactor jacket.
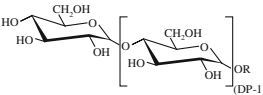
The primary biodegradation was monitored by means of the residual-surfactant concentration over time by the anthrone and methylene blue active substances (MBAS) method. The degree of mineralization achieved after the ozonation process was evaluated by measuring the total organic carbon before and after ozonization, using the TOC analyzer Shimadzu TOC-V CHS. The ozonated surfactant solution was used to perform the biodegradation tests. The initial pH of the test solution was 7.

Biodegradation Tests

Screening Biodegradation Tests

The static biodegradation tests were conducted according to the OECD 301 E test for ready biodegradability [17]. 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 for 28 days. The procedure consists of placing 1.2 L of surfactant solution (for which the biodegradability is to be determined) in 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 sludge. 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 is monitored by means of the residual-surfactant concentration over time. For the absorbance measurements, a double-beam spectrophotometer Spectronic Unicam UV-V is used. The absorbance is directly proportional to the surfactant concentration. The biodegradation was studied at different initial concentrations varying from 15 to 35 mg/L (below

Table 1 Description of the surfactants used in the tests

Surfactant	Family	Abbreviation	Structure	CMC (mg/L) (25 °C)
Linear alkyl benzene sulfonate	Anionic	LAS-R$_{11-14}$	CH$_3$(CH$_4$)CH$_4$(C$_6$H$_4$)SO$_3^-$	25.47
Alkylpolyglucoside	Non-ionic	APG-R$_{12-14}$DP$_{1.5}$		28.88

R alkyl chain length, DP average number of glucose units per alkyl radical
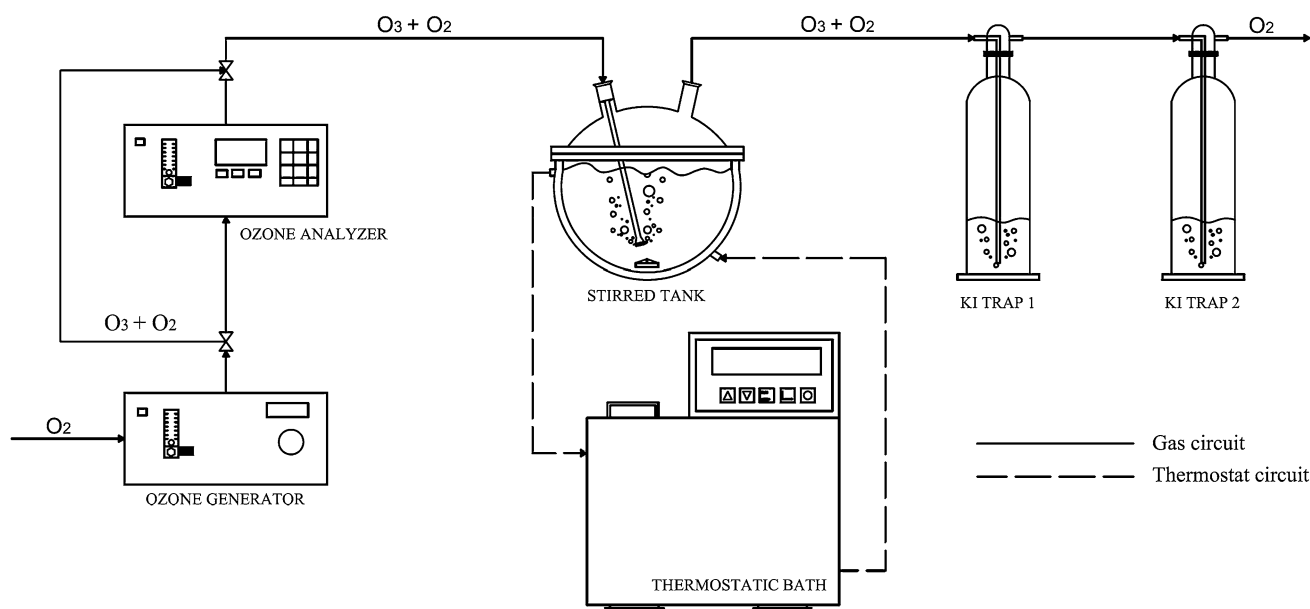
Fig. 1 Schematic diagram of the ozonation system

and above CMC). The surfactant concentration was tested by the anthrone method for the alkylpolyglucosides and by MBAS method for linear alkylbenzene sulfonate, while the final biodegradation (mineralization) was analyzed by total organic-carbon determinations.

The tests were also performed with the surfactant previously ozonated for 15 min, adjusting the initial concentration to 15 and 35 mg/L.

Analytical Methods

Anthrone Analysis

The alkylpolyglucosides were quantified by a modification of the anthrone method proposed by Buschmann and Wodarczak [18]. Five milliliters of solution of 0.8 % (w/w) anthrone in concentrated sulfuric acid was dropped into 2 mL of degradation liquor. The mixture was hydrolyzed for 5 min in boiling water and then quickly cooled in cold water for about 10 min. The absorbency of this mixture at 622 nm was determined by a spectrophotometer. The alkylpolyglucosides were quantified from a calibration curve according to the absorbency at 622 nm. This method, simple and fast, can directly determine 1–100 mg/L of alkylpolyglucoside solution.

MBAS Analysis

The linear alkyl benzene sulfonate was quantified by a simplified spectrophotometric method for determining anionic surfactants, based on the formation of the ionic-pair anionic surfactant-methylene blue [19]. For spectrophotometric measurements, 5-mL samples placed in 10-mL glass vials were made alkaline to pH 10.0 by adding 200 µL 50 mM sodium tetraborate, pH 10.5, and then 100 µL of methylene blue 1 g/L stabilized were added. Finally, 4 mL of chloroform was added and, after stirring and a 5-min wait, the absorbance at 650 nm was measured against air or against a blank with chloroform.

TOC Analysis

The final biodegradation was analyzed by total organic-carbon determinations. In the TOC analyzer used, the organic compounds were first oxidized to carbon dioxide, and then the CO_2 released was measured quantitatively by an IR-detector. The oxidation method was high-temperature catalytic oxidation. The Shimadzu VCSH/CSH TOC analyzer equipped with an auto-sampler was used. Before the analyses, the analyzer was calibrated using standards prepared from potassium hydrogen phthalate, sodium hydrogen carbonate and sodium carbonate. A stock solution (total carbon concentration 1,000 mg/L and inorganic concentration 1,000 mg/L) was prepared by dissolving 2.12 g of potassium hydrogen phthalate (dried at 110 °C) in 1,000 mL water, and dissolving 3.5 g of sodium hydrogen carbonate and 4.41 g of sodium carbonate (dried at 280 °C) in 1,000 mL of water, respectively. Other standards were diluted from this solution. Samples were filtered through a 0.45-µm polyvinylidene fluoride filter (Millipore, SA) prior to TOC analysis.

Results and Discussion

Ozonation of Surfactants

The ozonation of aqueous solutions of anionic and non-ionic surfactants was analyzed using two selected commercial surfactants: LAS-R_{11-14} and APG-R_{12-14}-$DP_{1.5}$. The aqueous solution tested using these two products had an initial concentration of 80 mg/L before the ozonation process. The initial pH of the surfactant solution was neutral, although during the ozonation process, the acidity of the solution increased.

We found that the concentration of the residual surfactant rapidly declined with ozonation time. The time needed to eliminate 50 % of the initial surfactant concentration depended on the tested surfactant. For APG-$R_{12-14}DP_{1.5}$, this time was 25.8 and 7.13 min for the case of LAS-R_{11-14}. For the surfactant LAS-R_{11-14} the surfactant concentration decreases exponentially with ozonation time while for APG-$R_{12-14}DP_{1.5}$ the surfactant concentration decreases linearly, except in the initial time. We conclude that the anionic surfactant containing a benzene ring in its structure was oxidized faster than the non-ionic surfactant. For both surfactants, the breakdown induced by ozone remained incomplete after 30 min.

The rate of surfactant destruction in aqueous solutions by ozone can be described by the following proposed equation, similar to that used by Hoigné et al. [20]:

$$-\frac{d[T]}{dt} = K_1 \cdot [O_3]^m \cdot [T]^n + K_2 \cdot [OH^\cdot]^k \cdot [T]^n + \sum_{i=3}^{N} K_i \cdot [X_i]^l \cdot [T]^n \tag{1}$$

where

  [T]: surfactant concentration
  [O_3]: ozone concentration
  [$OH^\cdot$]: free-radical concentration
  K_i: reaction-rate constants
  X_i: other radicals involved in the surfactant degradation

Considering the difficulty in determining most of the parameters involved in the intermediate ozonation reactions, as well as the kind and the amount of reactants acting over the initial surfactant, we assumed a simplified equation. This equation represents the global degradation of the initial surfactant:

$$-\frac{d[T]}{dt} = K_G \cdot [T]^n \tag{2}$$

where

  K_G: reaction rate global constant
  if $n = 1$

Table 2 Calculated model parameters and rate constants

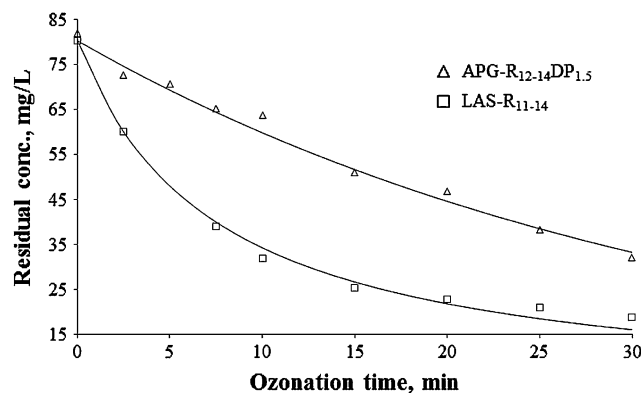| Surfactant	n	K_G	K_G error	R^2
APG-$R_{12-14}DP_{1.5}$	1	0.0287	9.31 E^{-4}	0.987
LAS-R_{11-14}	2	0.0017	6.57 E^{-5}	0.994

Fig. 2 Effect of ozonation on the breakdown of aqueous solutions of APG-$R_{12-14}DP_{1.5}$ and LAS-R_{11-14} as a function of ozonation time. Ozone flow 5 L/h, temperature 25 °C. Model based on Eqs. (3–4)

$$Ln\left(\frac{[T]}{[T_0]}\right) = K_G \cdot t \tag{3}$$

if $n \neq 1$

$$\frac{[T]^{1-n}}{n-1} = K_G \cdot t \frac{[T_0]^{1-n}}{n-1} \tag{4}$$

Experimental data were fit according to Eqs. 3 and 4. The calculated parameters are summarized in Table 2.

Figure 2 shows the effect of ozonation on the decomposition of aqueous solutions of APG-$R_{12-14}DP_{1.5}$ and LAS-R_{11-14} as a function of ozonation time. Theoretical curves based on Eqs. 3 and 4 agree with the experimental data.

Together with the primary biodegradation, the ultimate biodegradation of the surfactants was well established under aerobic conditions in the ozonation tests. Figure 3 shows the time course of the ultimate biodegradation of APG-$R_{12-14}DP_{1.5}$ and LAS-R_{11-14} over the ozonation period. The initial concentrations in the assays were 80 mg/L. The final mineralization reached at the end of the assay, min (%), was calculated with this expression:

$$Min\ (\%) = \frac{[TOC]_i - [TOC]_f}{[TOC]_i} \cdot 100 \tag{5}$$

The surfactants yielded 17.1 % min for the APG-$R_{12-14}DP_{1.5}$ and 12.94 % min for LAS-R_{11-14} within the 30-min test period.

The decline in the TOC content was far slighter than the fall in the percentage of the starting surfactant concentration. These results show the poor mineralization due to
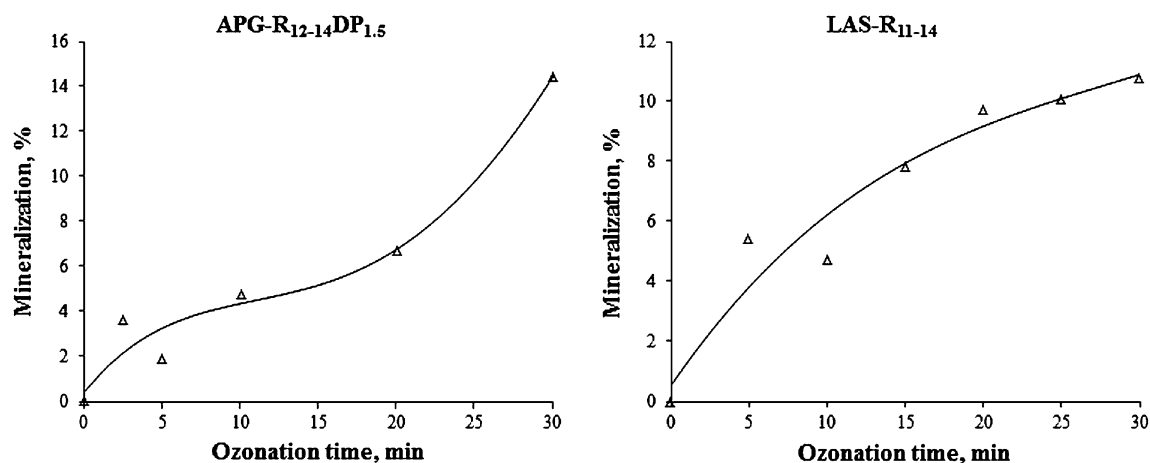
Fig. 3 Percentage of mineralization of aqueous solutions of APG-$R_{12–14}DP_{1.5}$ and LAS-$R_{11–14}$ as a function of ozonation time. Ozone flow 5 L/h, temperature 25 °C

ozonation, which indicates that ozone attacks primarily carbon bonds in the aromatic ring. The surfactant APG-$R_{12–14}DP_{1.5}$ has a mean reaction order of one while the surfactant LAS-$R_{11–14}$ has a mean reaction order of two (see Fig. 2). LAS-$R_{11–14}$ present a lower residual surfactant concentration (Fig. 2) and final mineralization values (Fig. 3) for the 30-min ozonation period compared with the APG-$R_{12–14}DP_{1.5}$ values. Molecular ozone, without taking into account the secondary surfactants produced by the breakdown of ozone, attacks primarily groups with double bonds, such as the present ones in the aromatic rings of LAS-$R_{11–14}$, this explaining the greater breakdown of this surfactant with respect to APG-$R_{12–14}DP_{1.5}$ and the difference in terms of the reaction orders of the two surfactants. These results highlight, the greater ozone selectivity by the aromatic rings as Ledakowicz reported [1], which may be the reason why they reach higher levels of surfactant breakdown in the case of surfactants with rings in comparison with ringless surfactants, due to a certain inhibition regarding radical reactions characterized by being faster and less selective [10].

Figure 4 depicts the change in surface tension as a function of ozonation time. We found a clear increasing trend in the surface tension as ozonation progressed. This appears to indicate that the breakdown products of the surfactants have, at least all jointly, weaker surface activity than did the starting surfactant. At 5 min, ozonation strengthened the surface tension, probably due to the mobilization of the surfactant towards the foam layer that formed at the beginning of the ozonation assay and also due to the initial breakup of the surfactant, which involved the formation of products having less surface activity. However, because the foam layer does not persist long enough to explain the high surface-tension values at around 10 min of reaction, this we conclude that the high surface-tension values are related to intermediate reaction products of less surface activity.

Combined Use of Ozonation and Biodegradation

Figure 5 shows surfactant removal by biodegradation and by biodegradation plus an initial ozonation as a time function. The concentration is expressed as a percentage of residual surfactant and each point of the graphs represents the mean value of two replicates. The initial concentrations in the biodegradation assays were 15 and 35 mg/L. These concentrations of the surfactants were chosen in such a way that they should be below and above CMC.

In a preliminary analysis of the primary-biodegradation profiles, it was found that for the two surfactants, the concentration of the residual surfactant rapidly diminished with the duration of the test. The surfactant concentration declined exponentially and the adaptation period was negligible for all the surfactants.

For the comparison and quantification of the assays, some parameters characteristic of the biodegradation profiles [21] were evaluated: latency time (t_L), half-life time ($t_{1/2}$), mean biodegradation rate until reaching biodegradability of 85 % (V_M), and the biodegradability at 50 h of assay (B). Table 3 shows the characteristic parameters of the biodegradation profiles during biodegradation and ozonation tests. 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.

In general, an increase in the initial concentration of the tests did not significantly affect the latency time. The parameters $t_{1/2}$ and V_M are related to the rate of the surfactant removal; $t_{1/2}$ slightly decreased, the mean biodegradation rate increased with the combination of biodegradation and ozonation for surfactant concentrations below and above the CMC. This indicates that biodegradation is higher with the combined use of ozonation and biodegradation.

For the surfactant APG-$R_{12–14}DP_{1.5}$, the biodegradability at 50 h of assay increased with a higher initial
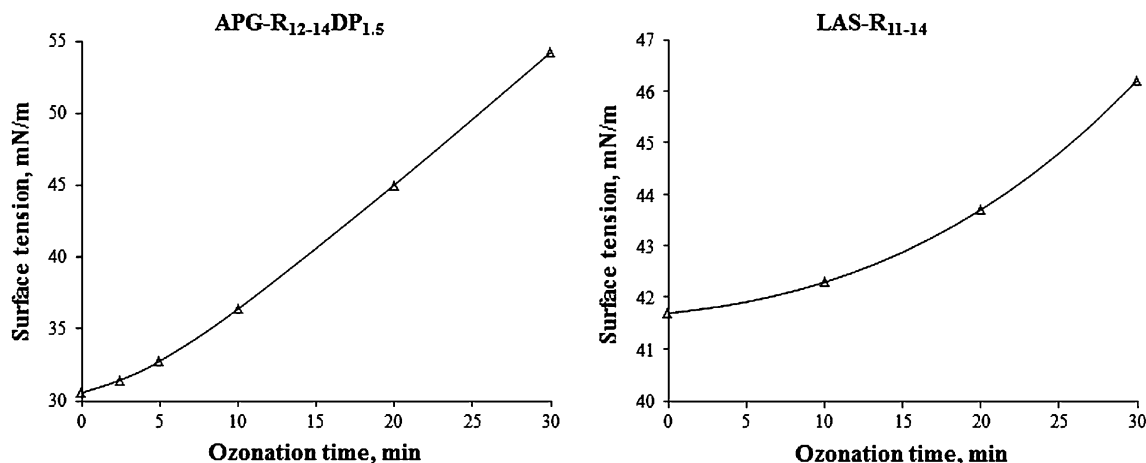
Fig. 4 Evolution of surface tension during ozonation. Ozone flow 5 L/h, temperature 25 °C
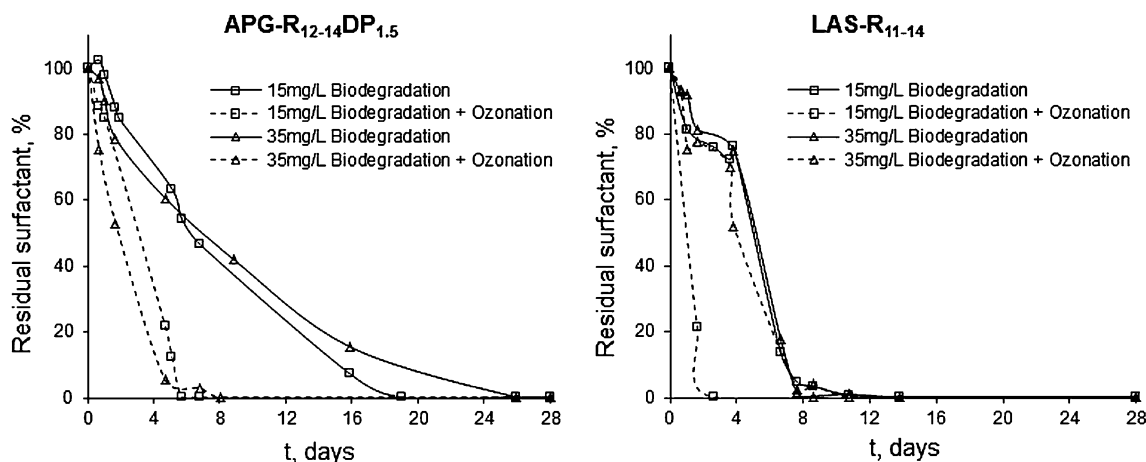

Fig. 5 Surfactant removal by both biodegradation and by biodegradation plus ozonation as a function of time for the surfactants APG-$R_{12-14}DP_{1.5}$ and LAS-R_{11-14}

Table 3 Characteristic parameters of the biodegradation profiles during biodegradation and ozonation

	S_0 (mg/L)	t_T (h)	t_L (h)	$t_{1/2}$ (h)	V_M (%/h)	B (%)	Final mineralization (%)
APG-$R_{12-14}DP_{1.5}$							
Biodegradation	15	672	23	142	0.35	15	85.97
	35	672	16	108	0.46	34	65.47
Biodegradation + ozonation	15	672	–	72	0.69	29	82.27
	35	672	–	43	1.16	55	87.27
LAS-R_{11-14}							
Biodegradation	15	672	86	125	0.40	22	43.34
	35	672	53	130	0.38	15	43.17
Biodegradation + ozonation	15	672	–	24	2.08	90	77.43
	35	672	58	99	0.50	23	45.73

S_0 initial surfactant concentration; t_T total assay time; t_L latency time of microorganisms; $t_{1/2}$ half-life time; V_M mean biodegradation rate until reaching biodegradability of 85 %; B biodegradability at 50 h of assay

surfactant concentration. For the two surfactants used, B increased with the combined use of ozonation and biodegradation.

For the surfactant APG-$R_{12-14}DP_{1.5}$ the primary biodegradability is greater for higher CMC test concentrations. For the surfactant LAS-R_{11-14} the contrary effect was observed.
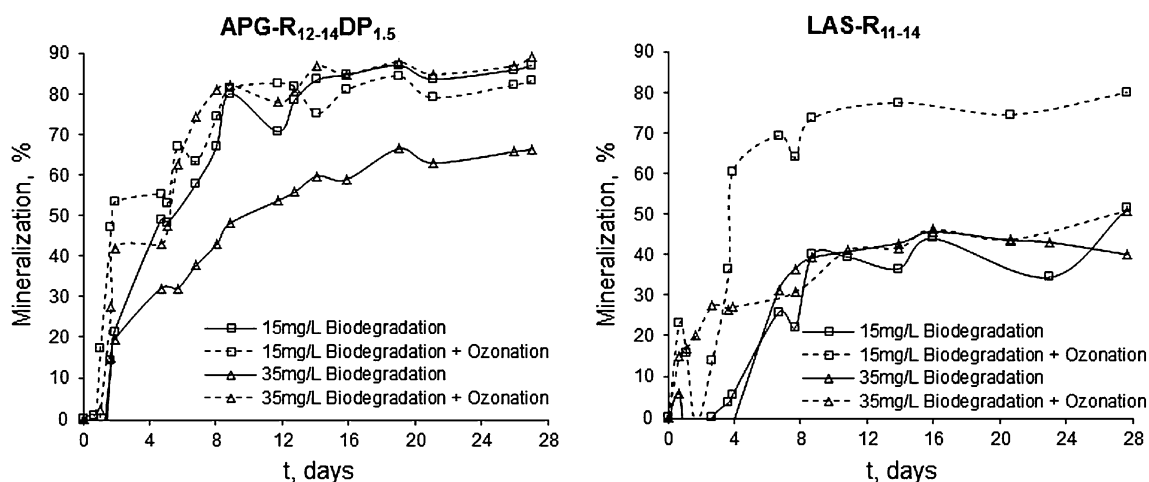
Fig. 6 Mineralization of surfactants by both biodegradation and by ozonation plus biodegradation as a function of time for the surfactants LAS-R_{11-14} and LAS-R_{11-14}

Table 3 shows the mineralization values during the biodegradation and ozonation tests and Fig. 6 presents the percentage of final mineralization plotted for biodegradation as well as ozonation plus biodegradation as a function of time. The mineralization curves present three well-differentiated phases: the acclimation phase of the microorganisms, rapid decrease in the concentration due to the metabolization on the part of the microorganisms and the period in which the residual concentration remained practically constant. For both surfactants, an increase in the initial concentration of the tests diminished the mineralization.

For the surfactant APG-$R_{12-14}DP_{1.5}$, the final biodegradation percentage reached exceeded 60 %. Existing legislation [22] requires that a minimum level of 60 % be reached in the final biodegradation before 28 days elapse, when one of the methods listed in Appendix III of the EU regulation n° 648/2004 is applied. If this condition is satisfied, the surfactant can be considered biodegradable. For both surfactants, the final biodegradation of the ozonated samples increased initially. This increase was more pronounced for the assay conducted below the CMC more than for the assay made above the CMC, with respect to the non-ozonated samples. However, for APG-$R_{12-14}DP_{1.5}$, at times for more than 10 days show a reverse trend by which the sample ozonated above the CMC presents a greater increase in the final biodegradation with respect to the non-ozonated sample.

For the surfactant LAS-R_{11-14}, the trend to increase the final mineralization persisted more for the ozonated sample below the CMC than the sample above the CMC, with respect to the non-ozonated samples.

For both surfactants, combined use of biodegradation and ozonation promotes faster decreases in the residual surfactant concentration than those for biodegradation alone. By using biodegradation and ozonation the surfactant concentration becomes negligible after approximately 8 days. At this moment the increase in the mineralization seems to be due mainly to the species with lower molecular weight resulted from the ozone attack. Around this time, there is also a change in the trend of mineralization data, which becomes slower, as Fig. 6 indicates. However when no ozone is applied the above-mentioned change in the trend of mineralization curve is not observed.

If the residual surfactant concentration and mineralization decrease over time (Figs. 5, 6, respectively) are considered jointly, the results seem to indicate that the removal of surfactants and the total organic carbon is increased by the consecutive use of ozonation and biodegradation. Ozonation, by destroying aromatic rings, generates molecules with lower molecular weight, favoring metabolism of the organic species present by the microorganisms and their removal from the medium [23]. Therefore, ozonation can facilitate the subsequent treatment of the washing wastewaters in a conventional biological plant.

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Author Biographies

Manuela Lechuga Graduated in chemical engineering from the University of Granada (Spain) and then worked on an investigation project entitled "Formulation of Liquid Detergents Specifically for the Industrial Agrofood and Hotel Sector" directed by Professor Encarnacion Jurado. She received her Ph.D. in 2005 and is currently an Associate Professor at the University of Granada. Her research interests include surfactants, their applications and the study of ecotoxicological properties.

Alejandro Fernández-Arteaga Graduated in chemical engineering of the University of Granada in 2002. He earned his Ph.D. in 2006 from the same University. He is currently working there as an Assistant Lecturer in the Chemical Engineering Department, after a post-doctoral stay with Dr. Conxita Solans, at the Colloidal & Interfacial Chemistry Research Center in Barcelona. He is conducting research on properties of surfactants and synthesis of bioemulsifiers.

Mercedes Fernández-Serrano 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 and the study of ecotoxicological properties of surfactants as biodegradability and toxicity. At present, she is head of the Chemical Engineering Department at the University of Granada.

Encarnacion Jurado 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 1996. Her main research areas are enzymes, kinetics of enzymatic reactions, surfactant biodegradation, emulsions, and the physical chemistry and applications of surfactants.

Alejandro Burgos Graduated in chemical engineering at the University of Granada in 2011. He is currently working for his Ph.D. dissertation on a project entitled "Ozonation Impact on Biodegradation and Toxicity of Anionic and Non-Ionic Surfactants."

Francisco Ríos Graduated in chemical engineering at the University of Granada in 2009. He is currently working for his Ph.D. dissertation on a project entitled "Environmental Impact of commercial surfactants".