
Aerobic Biodegradation of Surfactants

Encarnación Jurado, Mercedes Fernández-Serrano,
Francisco Ríos and Manuela Lechuga

Additional information is available at the end of the chapter

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1. Introduction

Surfactants are a wide group of chemical compounds which have a large number of applications due to their solubility properties, detergency, endurance of water hardness, as well as emulsifying, dispersing, and wetting properties. Surfactants have a characteristic structure, with one or several hydrocarbon chains that form the lipophilic part of the molecule (or the hydrophobic part of the molecule) and one or several polar groups that form the hydrophilic part. These compounds, also called surface-active agents, can have different lengths and degrees of unsaturation in the hydrocarbon chains, as well as in the polar groups, giving rise to a wide variety of surfactants with different properties.

Surfactants can be classified as ionic or non-ionic, depending on the nature of the hydrophilic group. The ionic surfactants are disassociated in water, forming ions. Notable within this group are organic acids, and their salts are anionic surfactants, while bases—amines of different degrees of replacement— and their salts are cationic surfactants. Some surfactants contain both acid and basic groups. These surfactants may be anionic or cationic and are therefore called amphoteric, or ampholytic.

Surfactants constitute a group of substances in which the main characteristic is their accumulation in the interfaces, solid-liquid or liquid-gas, weakening the surface tension of the liquid. This property enables the formation of foams and the penetration of solids as a wetting agent, leading to wide and varied applications of these compounds [1].

These substances are widely used in household cleaning detergents, personal-care products, textiles, paints, polymers, pesticides, pharmaceuticals, mining, oil recovery, and the pulp and paper industries. Detergents and cosmetics involve the mayor use of these compounds. After use, residual surfactants and their degradation products are discharged to sewage-treatment plants or directly to surface waters. Several of these compounds are not biologically degradable

and, depending on their concentration, may be harmful to fauna and flora in surface waters. Surfactants can also produce waste which can react with some water components and generate toxic products harmful to human health. For example, endometriosis or decreased sperm quality appear to be consequences (though unconfirmed) associated with the presence of surfactants in the environment.

Due to the enormous economic importance of surfactants and their contribution to the deterioration of the environment when these persist in nature, it is necessary to establish the structural characteristics that govern the susceptibility of these molecules to be degraded. The massive worldwide use of surfactants requires them to be as innocuous as possible for the environment, i.e.: low toxicity and easily biodegradability [2].

Balson and Felix [3] described biodegradation as the destruction of a chemical by the metabolic activity of microorganisms. Degradation of surfactants through microbial activity is the primary transformation occurring in the environment and an important process to treat surfactants in raw waste in sewage-treatment plants. During biodegradation, microorganisms can either utilize surfactants as substrates for energy and nutrients or co-metabolize surfactants by microbial metabolic reactions [4].

The biodegradation process of organic compounds is affected by many factors, the most important of which are the physiochemical characteristics of the compounds (solubility, concentration, structure, etc.), the physiochemical conditions of the environmental media (dissolved oxygen, temperature, pH, light, nutrient concentration, etc.) and the microorganisms present in the aquatic environment. Most surfactants can be degraded by microbes in the environment, although some surfactants may be persistent under anaerobic conditions [5]. Different types of surfactants have different degradation behaviour in the environment.

Biodegradation tests can be used to evaluate the primary and ultimate biodegradability of anionic and non-ionic surfactants. The comparison of different types of surfactants by various biodegradability tests will identify the least damaging to the aquatic media and will determine the influence of the surfactant structure on the biodegradation process. On this basis, simple methods can be chosen to evaluate the biodegradability because results depend on the biodegradation test used. Tests can also determine the choice for including them in detergent formulas, also taking into account their effectiveness in the wash.

A wide variety of surfactants are used in detergents formulas. A mix of several surfactants is selected to find the formulation more appropriate for each kind of soiling. This chapter examines the biodegradation of some anionic and non-ionic surfactants which have noteworthy properties for use in the detergent formulas, and which represent one of the major families of surfactants used today.

Non-ionic surfactants: *Fatty alcohol ethoxylates* (FAEs) represent the economically most important group of non-ionic surfactants. Commercial FAEs generally consist of a mixture of several homologues differing in alkyl chain length and degree of ethoxylation. FAEs are widely used in domestic and commercial detergents, household cleaners, and personal care products. Thus, the major route of disposal of FAEs is down the drain, through sewage systems, and into municipal sewage-treatment plants (WWTP) [6]. *Nonylphenol polyethoxylate* (NPEO), as a

result of its field of application, its resistance to biodegradation at low temperatures, and the generation during the degradation process of some persistent metabolites which are much more toxic than the original compound [7], the use of NPEOs has been banned in domestic formulations in some countries of the European Union (Germany, Spain, and the United Kingdom, [8] as well as Switzerland and Canada [9]. The *alkylpolyglucosides* (APGs) belong to non-ionic surfactants of growing use. Because of their good foaming properties, as well as synergy with other surfactants, they have found application in dishwashing and laundry detergents, and in other cleaning products [10]. Also, their good skin tolerance makes them suitable for mild personal-care products [11]. They are prepared on the basis of renewable raw materials, namely (starch/sugar) and fatty alcohols (vegetable oils). As these chemicals belong to a new type of surfactant, few studies have addressed their environmental properties [12]. *Amine-oxide-based surfactants* constitute a particular type of non-ionic surfactants, they are classified as nitrogen non-ionic surfactants, exhibit cationic behaviour in acid solution, and can be ionized depending on the pH of the test medium. They show good foaming properties and are skin compatible [13]. These compounds, the consumption of which only in Western Europe is estimated at 14 ktonnes/year [14] are widely used in detergents, toiletries, and antistatic preparations, usually together with other surfactants. They are compatible with anionic surfactants and can be used to give synergistic advantage to formulations [15] and [16].

Anionic surfactants: Linear-chain alkylbenzenesulfonate types are the most popularly used synthetic anionic surfactants. They have been extensively used for over 30 years with an estimated global use of 2.8 million tonnes in 1998 [17]. There has been an emphasis over the past few years on the development of surfactants and builders with improved biodegradability and also non-polluting characteristics [18]. This growing concern has led to the development and use of other surfactants, such as *ether carboxylic derivative surfactants*. These anionic surfactants improve the foaming quality of the detergent, reducing the irritation level, and therefore they are used as co-surfactants in detergents which have to be in contact with the skin [19] [20]. These surfactants are marketed in concentrated acid form. For these surfactants, aerobic biodegradation has been studied employing standardized methods which use micro-organism to degrade the surfactant. The results enable us to analyse the behaviour of the surfactant in the environment or in the sewage-treatment plants, and then evaluate their biodegradability to evaluate the suitability of including them in detergent formulas.

2. Materials and methods

2.1. Non-ionic surfactants

The non-ionic surfactants used were: Fatty-alcohol ethoxylates (FAEs) and amine-oxide-based surfactants supplied by Kao Corporation S.A. (Tokyo, Japan), nonylphenol polyethoxylate (degree of ethoxylation 9.5) supplied by Tokyo Chemical Industry (Tokyo, Japan) and the alkylpolyglucosides from Henkel-Cognis (Dusseldorf, Germany) supplied by Sigma.

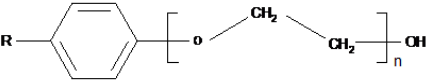
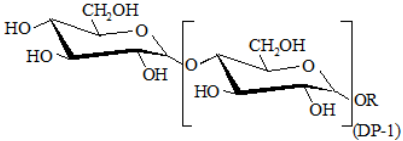
The FAEs used in this study were: FAE-R₁₀E₃, FAE-R₁₀E₆, FAE-R₁₂₋₁₄E₄, FAE-R₁₂₋₁₄E₁₁, FAE-R₁₆₋₁₈E₆, and FAE-R₁₆₋₁₈E₁₁. The alkylpolyglucosides were Glucopone 650 EC (APG-R₈₋₁₄DP_{1.35}),

Glucopone 600 CS UP (APG-R₁₂₋₁₄DP_{1.59}), and Glucopone 215 CS UP (APG-R₈₋₁₀DP_{1.42}). The amine-oxide-based surfactants used in this study were AO-R₁₄, AO-R₁₂, and AO-Cocoamido.

2.2. Anionic surfactants

The anionic surfactants tested were: linear alkyl benzene sulphonate and ether carboxylic derivative surfactants EC-R₈E₈, EC-R₁₂₋₁₄E₃, EC-R₁₂₋₁₄E₁₀, EC-R₈E₅, EC-R₆₋₈E₃₋₈, EC-R₄₋₈E₁₋₈, supplied by Kao Corporation S.A. (Tokyo, Japan).

Table 1 shows the structure of the surfactants and the abbreviations used in this study.

Family	Surfactant	Structure	Abbreviation
	Fatty-alcohol ethoxylate	$R(-O-CH_2-CH_2)_E-OH$	FAE-R _V E _Z
	Nonylphenol polyethoxylate		NPEO
Non-ionic	Alkylpolyglucoside		APG
	Amine oxide	$RCONH(CH_2)_3-N(CH_3)_2-O$ and $R-N(CH_3)_2-O$	AO-R _V
		(AO-Cocoamido (AO-R ₁₄ & AO-R ₁₂))	
Anionic	Linear alkyl benzene sulphonate	$CH_3(CH_2)_5CH(CH_2)_4CH_2SO_3^-Na^+$	LAS
	Ether carboxylic derivative	$R-O(CH_2-CH_2O)_E-CH_2-COO-X$	EC-R _V E _Z

R: alkyl chain length. E: degree of ethoxylation. X= H⁺ or Na. DP: average number of glucose units per alkyl radical

Table 1. Chemical structure and abbreviation of the surfactants used in the tests

2.3. Biodegradation tests

Screening test: The test was conducted according to the OECD 301 E test for ready biodegradability [21]. 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 at 25°C for 21 days. The procedure consists of placing 1.2 liters of surfactant solution in a 2-liter 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 was 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) provided the necessary aeration. The surfactant solution was prepared by dissolving the desired quantity of surfactant in the nutrient solution.

The primary biodegradation was monitored by means of the residual-surfactant concentration over time using colorimetric methods in which the absorbance is directly proportional to the surfactant concentration. For the absorbance measurements, a double-beam spectrophotometer, VARIAN Cary 100 Bio, was used. The fatty-alcohol ethoxylates and the nonylphenol polyethoxylate were determined by the iodine-iodide colorimetric method [22]: 0.25 mL of iodine-iodide reagent was added on 10 mL of the test sample, after stirring and maintaining for 5 minutes at room temperature, the absorbance was measured against air at 500 nm in the spectrophotometer. The alkylpolyglucosides were quantified by a modification of the anthrone method proposed by Buschmann and Wodarczak [23]: 5 ml 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 10 min. The absorbance of this mixture at 622 nm was determined by spectrophotometer. The linear alkyl benzene sulphonate was quantified by a simplified spectrophotometric method for determining anionic surfactants, based on the formation of the ionic-pair anionic surfactant-methylene blue [24]: 5 mL of sample in 10-mL glass vials were made alkaline to pH 10.0 by adding of 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 5-min wait, the absorbance at 650nm was measured against air or against a blank with chloroform. The biodegradation was calculated according the following equation:

$$\text{Biodegradation \%} = \frac{[S]_i - [S]_t}{[S]_i} \cdot 100 \quad (1)$$

Where $[S]_i$ is the initial surfactant concentration and $[S]_t$ is the surfactant concentration at each time.

The biodegradation process for the amine oxides and ether carboxylic derivates was monitored by measuring the residual-surfactant concentration over time (21 days) by dissolved organic carbon (DOC) measurements. In the TOC-analyser used, the organic compounds were first oxidized to carbon dioxide, and then the CO₂ released was measured quantitatively by an IR-detector. The oxidation method was high-temperature catalytic oxidation. The Shimadzu VCSH/CSH TOC analyser equipped with an auto-sampler was used. Samples were filtered through a 0.45-µm polyvinylidene fluoride filter (Millipore S.A.) prior to TOC analysis. The biodegradation was calculated according the following equation:

$$\text{Biodegradation \%} = \frac{(DOC_i - DOC_t)}{DOC_i} \cdot 100 \quad (2)$$

Where DOC_i is the initial DOC concentration and DOC_t is the DOC concentration measured at each time.

Confirmatory Test: The test was performed according to the OECD 301 E test for ready biodegradability [21]. This test is used for surfactants which have failed in the screening test to confirm or reject the results. It consists of inoculating a small amount of microorganisms, from a secondary effluent-treatment plant which works preferably with domestic wastewater. The biodegradation process was performed in a small activated sludge plant at laboratory scale, where synthetic wastewater was used with a surfactant concentration of 10 mg/L at flow rate of 1 L/h. The test was run at room temperature (18-25°C).

Chemical oxygen demand (COD), and dissolved organic carbon (DOC) were measured daily to determine the biodegradation efficiency.

$$COD\ reduction\ \% = \frac{(COD_i - COD_t)}{COD_i} \cdot 100 \quad (3)$$

Where COD_i is the initial COD and COD_t is the COD measured at each time.

$$Mineralization\ \% = \frac{(DOC_i - DOC_t)}{DOC_i} \cdot 100 \quad (4)$$

Where DOC_i is the initial DOC concentration and DOC_t is the DOC concentration measured at each time.

Respirometry Test: The test was made using the system Oxitop Control® (WTW, Weilheim, Germany), which determines the manometric changes that occur when oxygen is consumed to transform the surfactant into CO_2 by the microorganisms inoculated (from a mixed population and aerated) in a mixture formed by the nutrient solution and the surfactant. The Oxitop system offers an individual number of reactors consisting of glass bottles (510 nominal volume) with a carbon dioxide trap (sodium hydroxide) in the headspace. The volume of the test mixture is usually 164 mL. The bottles were furnished with a magnetic stirrer and sealed with a cap containing an electronic pressure indicator. An incubator box was used to maintain the respirometer units at constant temperature (25°C) during a test run. The decrease in headspace pressure in the closed test vessel was continuously recorded and the biochemical oxygen demand (BOD) was calculated according the following equation:

$$DBO = \frac{M(O_2)}{R \cdot T} \cdot \left(\frac{V_{Total} - V_{Liquid}}{V_{Total}} + \alpha \cdot \frac{T_{25}}{T_0} \right) \cdot \Delta p(O_2) \quad (5)$$

Where $M(O_2)$ is the molecular weight of oxygen (32g/mol), R is the gas constant (83.144 mbar/(molK)), T_0 is the temperature at 0 °C (273.15 K), T_{25} is the incubation temperature, 25°C (298.15 K), V_{Total} is the total volume in the test vessel, V_{Liquid} is the volume of the test mixture, α is the Bunsen absorption coefficient (0.03103) and $\Delta p(O_2)$ is the difference of the partial pressure of

oxygen (mbar). Biodegradation of the test compound was calculated from the measured DBO as a percentage of its theoretical oxygen demand (ThOD).

***Pseudomonas putida* biodegradation test:** A monoculture strain *P. putida* CECT 324, provided by the Spanish Type Culture Collection (Valencia, Spain), was used in the biodegradation test. Erlenmeyer flasks were filled with the surfactant solution, enriched with an inorganic medium and with a trace mineral solution [25] [26]. The pH was adjusted to 7.0 and the flasks were inoculated with bacterial stock of *P. putida*. Flasks were incubated at 30°C on a rotary platform shaker for 72 h. At the beginning and after 72 h, a sample of each flask was filtered and used to determine the dissolved organic carbon (DOC). Biodegradation efficiency (E_f) was evaluated as a percentage by:

$$E_f \% = \frac{[DOC_i - (DOC_f - DOC_m)]}{DOC_i} \cdot 100 \quad (6)$$

Where DOC_i is the initial DOC concentration, DOC_f is the DOC concentration measured at the end of the incubation (72 h) and DOC_m is the minimum concentration that cannot be metabolized by the bacteria [26].

3. Results and discussions

3.1. Screening test

The screening test was applied to the amine-oxide-based surfactants AO-R₁₄, AO-R₁₂, AO-Cocoamido, to the ether carboxylic derivative surfactants EC-R₈E₅, EC-R₆₋₈E₃₋₈, EC-R₈E₈, EC-R₁₂₋₁₄E₃, EC-R₁₂₋₁₄E₁₀, to the fatty-alcohol ethoxylates FAE-R₁₀E₃, FAE-R₁₀E₆, FAE-R₁₂₋₁₄E₄, FAE-R₁₂₋₁₄E₁₁, FAE-R₁₆₋₁₈E₆, FAE-R₁₆₋₁₈E₁₁, to the alkylpolyglucosides APG-R₈₋₁₄DP_{1.35}, APG-R₁₂₋₁₄DP_{1.59}, APG-R₈₋₁₀DP_{1.42}, to the nonylphenol polyethoxylate and linear alkyl benzene sulphonate.

Results for ether carboxylic derivative, fatty-alcohol ethoxylates, alkylpolyglucosides, nonylphenol polyethoxylate and linear alkyl benzene sulphonate, show that the biodegradability is influenced by the initial concentration of surfactant; that is, the degree of biodegradation achieved is higher when the initial concentration of surfactant is lower. Lower concentrations, 15mg/L and 25 mg/L, result in a percentage of biodegradation close to or above 90%. Current legislation requires a minimum level of biodegradation of over 80% for surfactants to be considered biodegradable, when the OECD test is applied. For the amine-oxide-based surfactants the effect of the concentration is the opposite, the biodegradation is higher when the initial concentration is higher.

Figure 1 shows the influence of the concentration for one example of each family of surfactants tested.

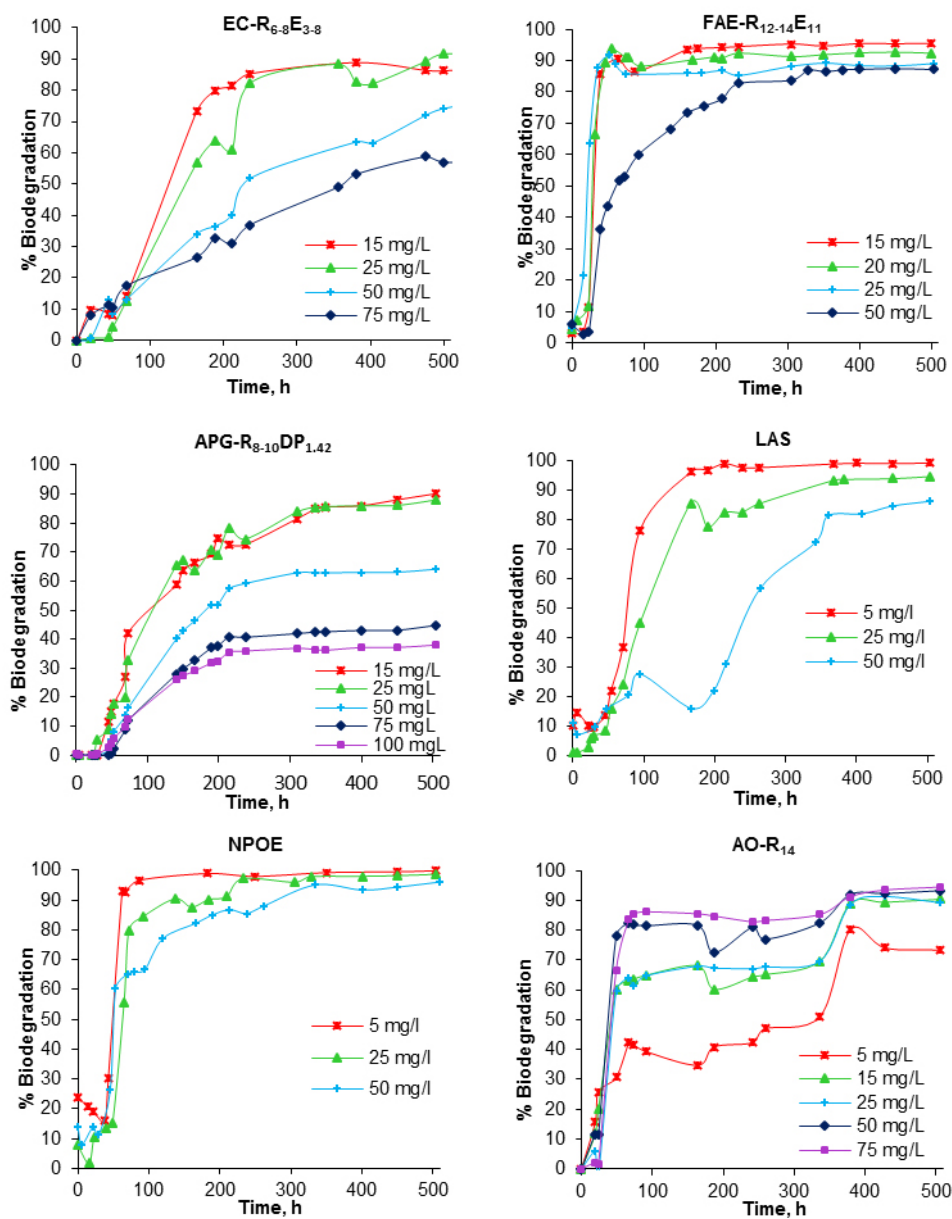


Figure 1. Screening-test results for the surfactants. Influence of the initial concentration.

The degrees of biodegradation achieved for linear alkyl benzene sulphonate and nonylphenol polyethoxylate are among the highest (Figure 1), but NPOE reportedly produces toxic

byproducts [27] which can be harmful to human health. This surfactant has been withdrawn in most European countries and North America [28].

An analysis of the screening-test results indicates that for all tested concentrations of fatty-alcohol ethoxylates, there was a preferential surfactant biodegradation of the surfactant with longer alkyl chain and higher degree of ethoxylation. Figure 2 shows the comparison of three fatty-alcohol ethoxylates with different alkyl length and degree of ethoxylation. The results for ether carboxylic derivate surfactants show that the biodegradability was higher for the surfactants with shorter alkyl chains. For the surfactants with the same chain length, biodegradability is higher for those with higher degrees of ethoxylation (Figure 2). For amine-oxide-based surfactants, the results indicate that AO-Cocoamido is less biodegradable than AO-R₁₂ and AO-R₁₄, the AO-R₁₄ (with the longest alkyl chain) being the most biodegradable amine oxide tested (Figure 2). These surfactants can be considered readily biodegradable, according to García et al. [13], because amine-oxide-based surfactants are rapidly and easily converted into carbon dioxide, water, and biomass under aerobic conditions.

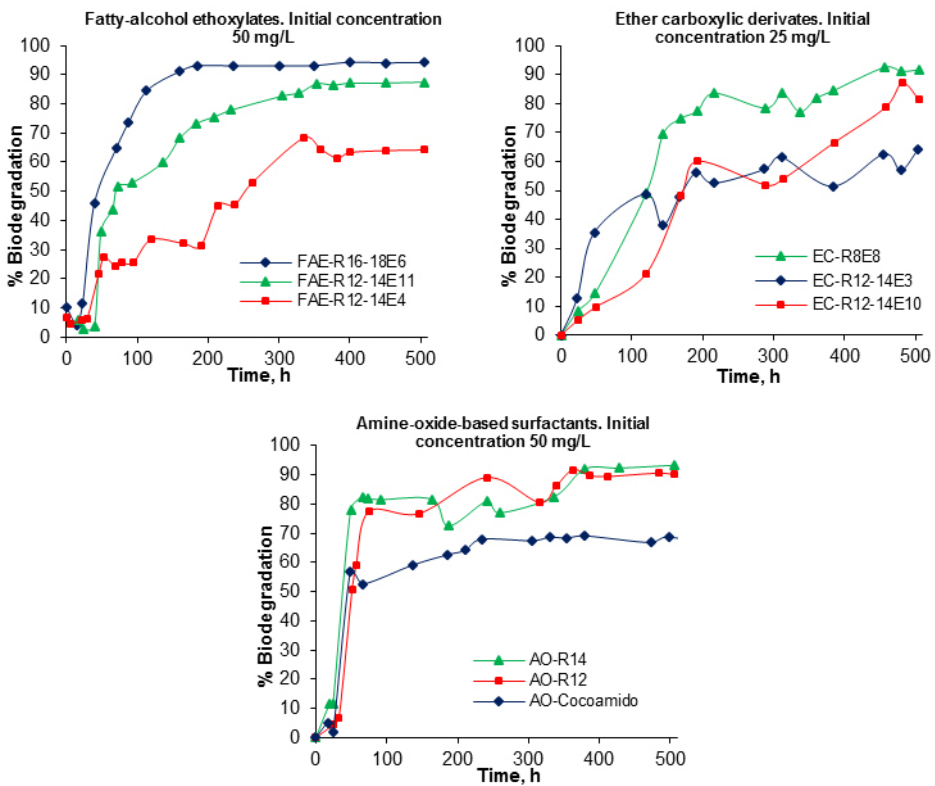


Figure 2. Screening-test results for the surfactants. Influence of the alkyl chain length and the degree of ethoxylation.

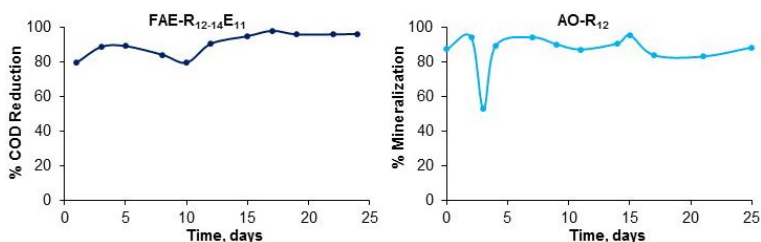


Figure 3. Confirmatory test results.

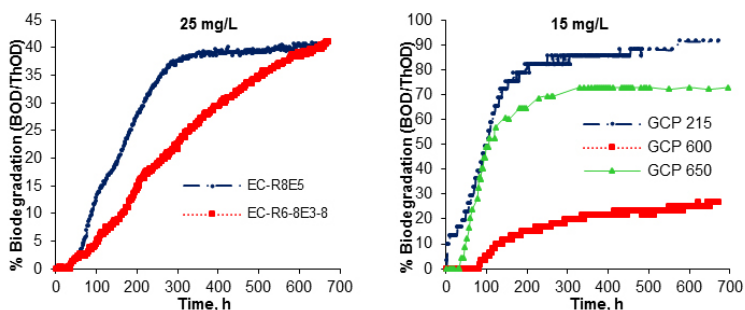


Figure 4. Respirometry test results. Influence of the alkyl chain length, degree of ethoxylation, and average number of glucose units.

3.2. Confirmatory test

After the screening test, a confirmatory test was performed over a period of 21 days for the amine-oxide-based surfactants and two fatty-alcohol ethoxylates. The results show that the surfactants tested can be considered easily biodegradable because, after a few days from the start, biodegradation exceeded 90% and remained steady for 21 days. Figure 3 shows one example for each family of surfactants tested. In case of fatty-alcohol ethoxylates, FAE-R₁₂₋₁₄E₁₁, the evolution is shown for the COD reduction (Eq. 3) between the synthetic wastewater in the feedtank and the treated water at the outlet. For the amine oxide, AO-R₁₂, Figure 3 shows the evolution of the mineralization achieved at the outlet, calculated on the basis of the DOC, (Eq. 4).

3.3. Respirometry test

The respirometry test was applied for the ether carboxylic derivative surfactants, for the alkylpolyglucosides and for the fatty-alcohol ethoxylates.

In this test, unlike the screening, the biodegradation of ether carboxylic derivatives was not higher for the surfactant with a shorter alkyl chain (Figure 4). However, the surfactant with the highest degree of ethoxylation was the most biodegradable, as in the screening test.

In the case of the alkylpolyglucosides, the comparison of the biodegradability between the three surfactants tested depended on the initial concentration. Thus, for low concentrations, 15 mg/L, 25 mg/L, and 50 mg/L, the most biodegradable was the APG-R₈₋₁₀DP_{1.42}, with the shorter alkyl chain and a middle number of glucose units. However, instead, for higher concentrations, 75 mg/L and 100 mg/L, the most biodegradable alkylpolyglucoside was the APG-R₈₋₁₄DP_{1.35}, which had the lowest number of glucose units and with a medium-length alkyl chain (data not shown). Notably, for all the concentrations tested, the biodegradability proved lowest for the surfactant with the longest alkyl chain and greatest number of glucose units (APG-R₁₂₋₁₄DP_{1.59}).

Analysing the influence of the initial concentration of surfactant, (Figure 5), for the ether carboxylic derivate surfactants, as in the screening method, the results show that the biodegradability was higher when the initial concentration was lower. For the fatty-alcohol ethoxylates tested, FAE-R₁₆₋₁₈E₆, the biodegradation was lower at the highest initial concentration, but the lowest initial concentration did not give the highest percentage of biodegradation, as was expected.

The influence of the initial concentration on the biodegradation process was the same for the three alkylpolyglucosides tested. The biodegradability was higher when the initial concentration of alkylpolyglucoside was lower (Figure 5).

3.4. *Pseudomonas putida* biodegradation test

The *P. putida* biodegradation test was applied for the amine-oxide-based surfactants at different initial surfactant concentrations. This test did not provide a comparable biodegradation value with the other biodegradation tests, but the results can be used to compare the surfactants in order to make decisions concerning their use in the surfactant formulations. The results show that the surfactant AO-Cocoamido was the most biodegradable amine-oxide-based surfactant tested; this surfactant is different from the others because incorporates an amino group in the alkyl chain, which probably increases the hydrophilic character of the surfactant [13]. For the amine oxides with the same structure but different alkyl-chain lengths, AO-R₁₂ and AO-R₁₄, the biodegradability was similar, although the AO-R₁₂, with a shorter alkyl chain, was slightly more biodegradable. Figure 6 shows the results for 30 mg/L of initial concentration.

The biodegradation process is influenced by the initial concentration, with the biodegradation efficiency (Eq. 6) being higher when the initial concentration is lower. This trend was found for the three amine-oxide-based surfactants. Figure 7 shows the biodegradation efficiency at different values of initial concentration for the AO-R₁₂.

3.5. Biodegradation parameters

Biodegradation profiles resulting from the screening test as well as from the respirometry test allowed us to determine the kinetics of the biodegradation process, this being to evaluate the persistence of surfactants and to assess the risks of exposure to humans, animals, and plants.

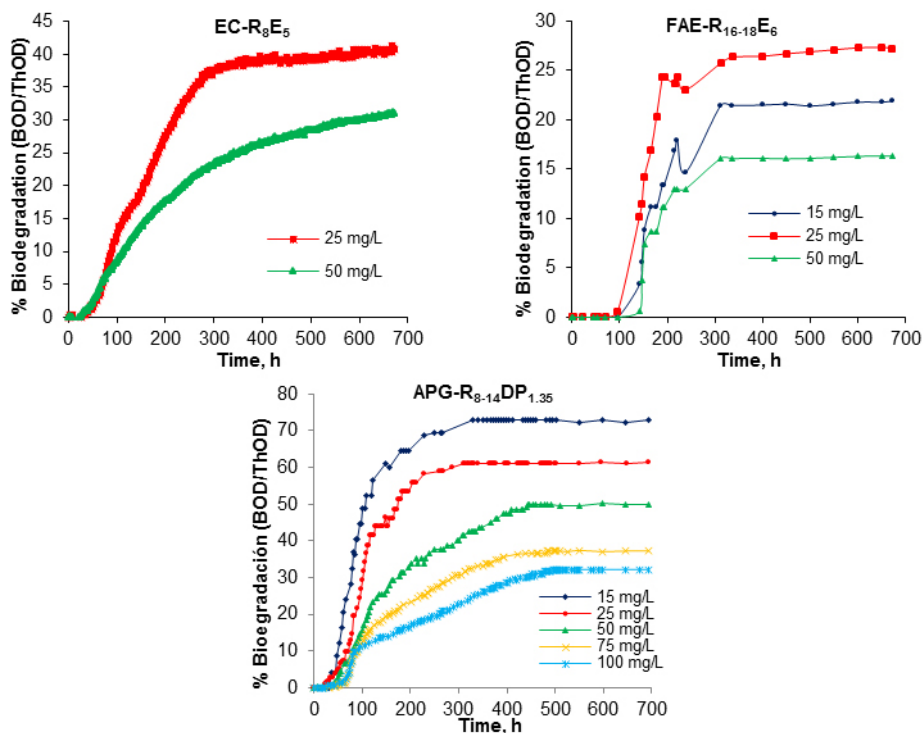


Figure 5. Respirometry test results. Influence of the initial concentration.

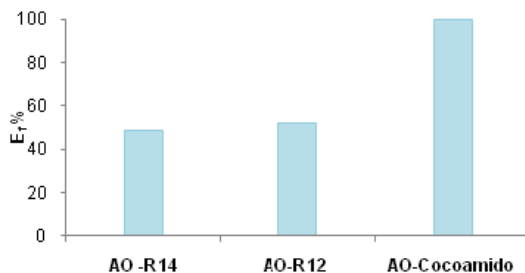


Figure 6. *P. putida* biodegradation test at 30 mg/L to amine-oxide-based surfactants

This is also useful for the design of industrial plants and equipment needed to eliminate these products.

Using the profiles of the biodegradation process, we can define and evaluate some characteristic parameters for the comparison and quantification of the biodegradation assays [29]. In this study two were selected:

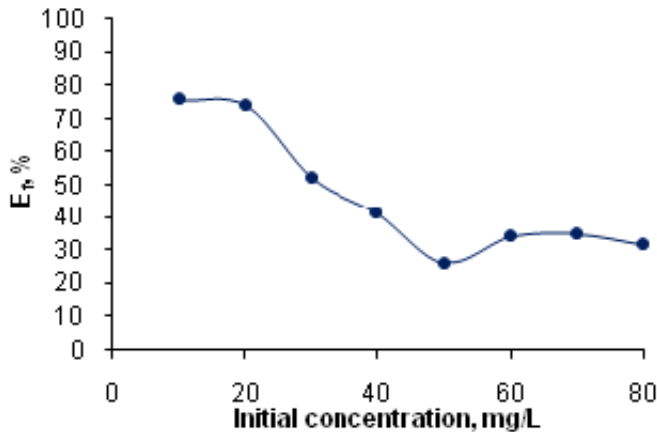


Figure 7. *P. putida* biodegradation test for the amine-oxide-based surfactant AO-R₁₂. Effect of the concentration.

- *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. This time corresponds to the period during which a mild change occurs in the residual concentration. For the screening test, it was calculated by drawing two tangents to the adaptation and biodegradation stages. The latency term was the cut-off point of both straight lines. For the respirometry test, it was calculated as the time necessary to achieve 10% biodegradation.
- *Mean biodegradation rate* (V_M) has been defined as the mean velocity of biodegradation reached until achieving 50% biodegradation of the surfactant and it has been calculated as 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.

Figure 8 shows the latency times obtained for the surfactants tested at the initial concentration of 25 mg/L, for the screening test and the respirometry test.

The latency times obtained show that the behaviour of the microorganism varies considerably depending on the surfactant tested, as well as the test used. According to the result, the non-adapted microorganisms need more time to acclimatize when the surfactant tested is the APG-R₁₂₋₁₄DP_{1.59} in case of the screening test, and the EC-R_{6.8}E_{3.8} when the respirometry test is employed.

The mean biodegradation rate was also evaluated in cases where possible, Figure 9 shows the mean biodegradation rate for the surfactants tested at the initial concentration of 25 mg/L, in the screening test and the respirometry test.

The mean biodegradation rate V_M enabled us to compare the biodegradation processes of the surfactants. According to the results (Figure 9), the mean biodegradation rate was higher for the fatty-alcohol ethoxylates while the carboxylic derivative surfactants showed the slowest mean biodegradation rate in case of the screening test. In case of the respirometry test, the carboxylic derivative surfactants registered the best values and the alkylpoliglucosides the worst.

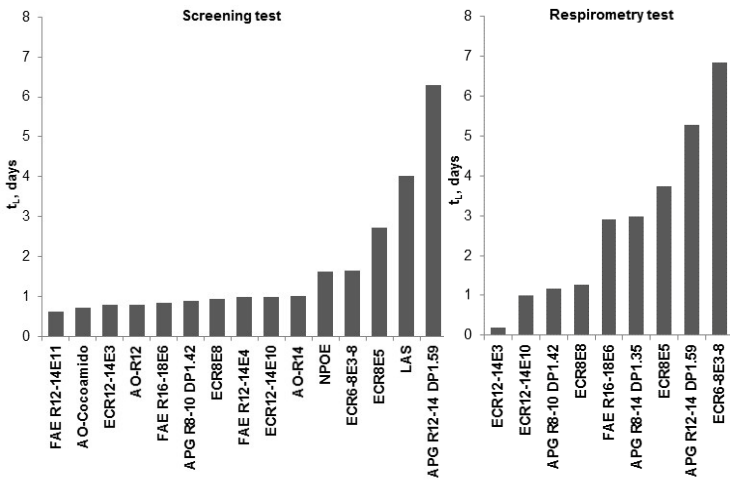


Figure 8. Latency time at the initial concentration of 25 mg/L.

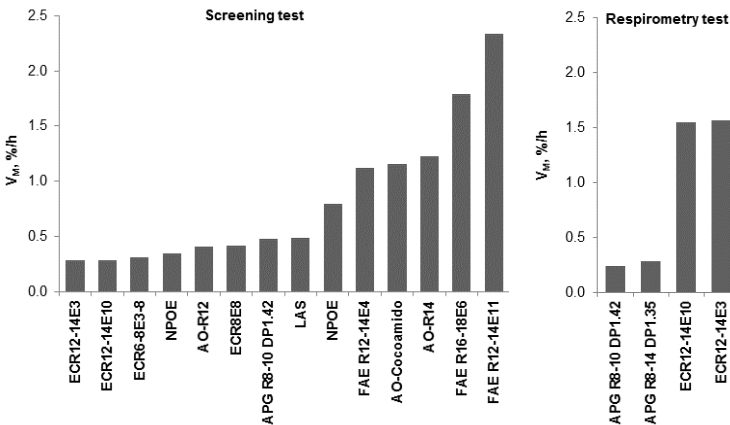


Figure 9. Mean biodegradation rate at the initial concentration of 25 mg/L.

3.6. Comparison of the biodegradation test used

The results of biodegradation and the biodegradation parameters for each family of surfactants tested may vary significantly depending on the biodegradation test used. It is therefore useful to determine the method which is the best to perform the aerobic biodegradation test of surfactants. For this, it is important to ascertain which method most accurately represents the actual conditions in the environment where the surfactants are dumped and the advantages and disadvantages when the method is applied.

Table 2 shows the advantages and disadvantages of the different biodegradation tests used in this study.

Biodegradation Test	Advantages	Disadvantages
Screening	Easy to prepare and to carry out Easy analysis of the results	Problems in the analysis of low surfactant concentrations Each inoculum differs Duration: 21 days, representing many measures.
Confirmatory	Similar conditions to a	Bulking sludge. This causes solid loss by flotation Each sludge for each test is different Duration: more than one month because of the adaptation phase of the sludge This requires much time and experimentation
Respirometry	Easy to prepare and to carry out Automatic tracking	
<i>P. putida</i>	Fast, 72h Bacteria <i>P. putida</i> are commonly present in activated sludge. Reproducibility Involve a definite living system	Complications to measure the DOC for low concentrations, but high concentrations cannot be tested because they can cause inhibition. The bacterial stock provides DOC that interferes mainly at low concentrations. Possibility of contamination of the strain. Need to work in sterile conditions

Table 2. Advantages and disadvantages of the aerobic biodegradation tests.

Based on these considerations, the screening test and the respirometry test are the most reproducible and the easiest to perform, and they supply more information.

4. Conclusions

According to the analysis, the biodegradation results depend on the biodegradation test used, the microorganisms used in the test, and the family of the surfactants tested. An important aspect is the adaptation of the microorganisms to the type of surfactant used as a sole carbon source.

Taking into account the screening test results, we can demonstrate the influence of the surfactant structure on the biodegradability. Regarding the length alkyl chain, the effect depends on the family of surfactant: for the fatty-alcohol ethoxylates and amine-oxide-based surfactants the biodegradability is higher when the alkyl chain is longer, while, for the carboxylic derivative surfactants and alkylpolyglucosides, the opposite occurs.

With respect to the influence of the initial surfactant concentration, the importance that this parameter has on the biodegradability has been evidenced. For all the surfactants tested, the greater the initial concentration is, the lower the biodegradability is, except for the amine oxides for which the effect is otherwise.

For the surfactants analyzed in this study, the fatty-alcohol ethoxylates and especially the FAE-R₁₂₋₁₄E₁₁ can be considered the most biodegradable but the carboxylic derivative surfactants the least biodegradable, according to the mean biodegradation rate.

Nomenclature

AO: amine-oxide-based surfactant

APG: alkylpolyglucoside

BOD: biological oxygen demand

COD: chemical oxygen demand

COD_i: initial chemical oxygen demand

COD_t: chemical oxygen demand at time t

DOC: dissolved organic carbon

DOC_e: dissolved organic carbon at the end of the incubation

DOC_i: initial dissolved organic carbon

DOC_m: minimum dissolved organic carbon that cannot be metabolized

DOC_t: dissolved organic carbon at time t

DP: average number of glucose units per alkyl radical

E: degree of ethoxylation

EC: ether carboxylic derivate
 E_f : biodegradation efficiency
FAE: fatty-alcohol ethoxylate
LAS: linear alkyl benzene sulphonate
NPEO: nonylphenol polyethoxylate
R: alkyl chain length
[S]₀= initial surfactant concentration
[S]_t= surfactant concentration at time t
ThOD: theoretical oxygen demand
 t_L : latency time
TOC: total organic carbon
 V_M : mean biodegradation rate

Author details

Encarnación Jurado, Mercedes Fernández-Serrano, Francisco Ríos and Manuela Lechuga

*Address all correspondence to: mferse@ugr.es

Department of Chemical Engineering, University of Granada, Granada, Spain

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