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Influence of ozonation processes on the microbial degradation of surfactants

M. Lechuga, M. Fernández-Serrano a*, E. Jurado, A. Fernández-Arteaga, A. Burgos, F. Ríos

Department of Chemical Engineering, Faculty of Sciences, University of Granada, Campus Fuentenueva s/n, 18071 Granada, Spain.

Abstract

The influence of ozonation of aqueous solutions of non-ionic surfactants was investigated in this study. The research was conducted using solutions of amine oxide based surfactants. These surfactants constitute a particular class of non-ionic surfactants that exhibit cationic behaviour in acid solution, they show good foaming properties and are skin compatible. The ozonation process was carried out at 25ºC in a stirred tank reactor for 30 min, where gaseous ozone was bubbled. The degree of mineralization achieved after the ozonation process was evaluated by measuring the total organic carbon before and after ozonation. The influence of ozonation on biodegradability of the solutions was studied using the biodegradation test by *Pseudomonas putida*. Biodegradation efficiency of the ozonated amine oxides based surfactants was also evaluated. It was found that the effectiveness of ozonation depended on the chemical structure of surfactant molecule. Ozonation only slightly improved biodegradation of these surfactants.

Keywords: Ozonation; non-ionic surfactants, biodegradation; pseudomonas putida; amine oxide based surfactants

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1. Introduction

Surfactants are one of the most important components used in the formulation of detergents [1], both industrial and domestically uses, comprising from 15% to 40% of the total detergent formulation [2].

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* Corresponding author. Tel.:+34-958243311; fax: +34-958248992.
E-mail address: mferse@ugr.es

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However, many undesirable phenomena connected with the presence of surfactants in the water and wastewaters are observed.

Surfactants have negatively influences on fauna and flora living in surface waters, causing their eutrophication. Some detergents inhibit the growth of algae already at the concentration of 1 mg/dm^3, and their toxic dose for fish ranges from 2 to 8 mg/dm^3. For humans, detergents have slightly toxicity but could induce skin allergies. This implies the need for low toxicity and biodegradable surfactants. Biodegradability of chemical is one of the most important criterions in ecotoxicological risk assessment [3]. This is of great importance in the biological treatment of all types of sewage.

The methods for removal of surfactants can be divided into three groups: biological, mechanical, and chemical/physicochemical. Oxidation is the main method of the chemical decomposition 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. Direct reaction of the molecular ozone with the compounds, which are often highly solute selective and slow [6], and indirect reactions of the hydroxyl radicals resulting from the decomposition of ozone catalyzed by hydroxyl ion through a series of reactions including initiation and propagation [7–9]. The indirect hydroxyl radical reactions are non-selective, fast, and proceeded more rapidly with increasing pH [6]. Ozonation processes do not lead to complete mineralization but partial oxidation of surfactants to organic acids, aldehydes, and ketones [7]. Relationship between ozonation processes of surfactant solution and their biodegradability and aquatic toxicity has been discussed for a long time [1, 10–14]. It is known [15] that compounds containing aromatic rings, like surfactants, are less susceptible to microbial decomposition than analogous compounds without benzene rings. So the ozonation, as aromatic rings destruction agent, may have beneficial effects by increasing the biodegradability of surfactants present in aqueous media. In our research, we studied the effect of ozonation in the biodegradability of non-ionic surfactants by *Pseudomonas putida*.

2. Materials and methods

2.1. Surfactants and reagents

Two amine oxide based surfactants were used in this study: AO-R$_{14}$ (trade name: Oxidet® DM-4) and AO-R$_{12}$ (trade name: Oxidet® DM-20), supplied by Kao Corporation S.A. (Tokyo, Japan). These surfactants constitute a particular class of non-ionic surfactants that exhibit cationic behaviour in acid solution. They show good foaming properties and are skin compatible. Figure 1 shows the structure of these surfactants. Table 1 shows the alkyl chain length (R), the % of active matter, and the critical micelle concentration (CMC) of these surfactants.

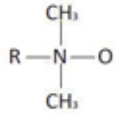

Fig. 1. Structure of the amine oxide based surfactants

Table 1. Description of the amine oxide bases surfactants used in the study

Surfactant	**Structure**	**Active matter, %**	**CMC, mg/L (25 ºC)**
AO-R_{14}	R:14	30	107.7
AO-R_{12}	R:12	30	474.3

The rest of the reagents used were PA quality and supplied by Panreac. All the solutions used in the experiments were prepared with deionised water (Milli Q® quality).

2.2. Ozonation process

The ozonation process was carried out in a stirred tank reactor where gaseous ozone was bubbled (Figure 2). 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 rate was first passed through an ozone analyzer (Anseros Ozomat GM-6000), and then bubbled for 30 minutes into the stirred tank reactor (200 ml), filled up with the surfactant solution to be ozonizated. The unreacted ozone in the outlet gas was collected in two potassium iodide traps (5% w/w) connected in series. A constant temperature (25º C) was maintained in the reactor by circulating water a thermostatic bath through the reactor jacket.

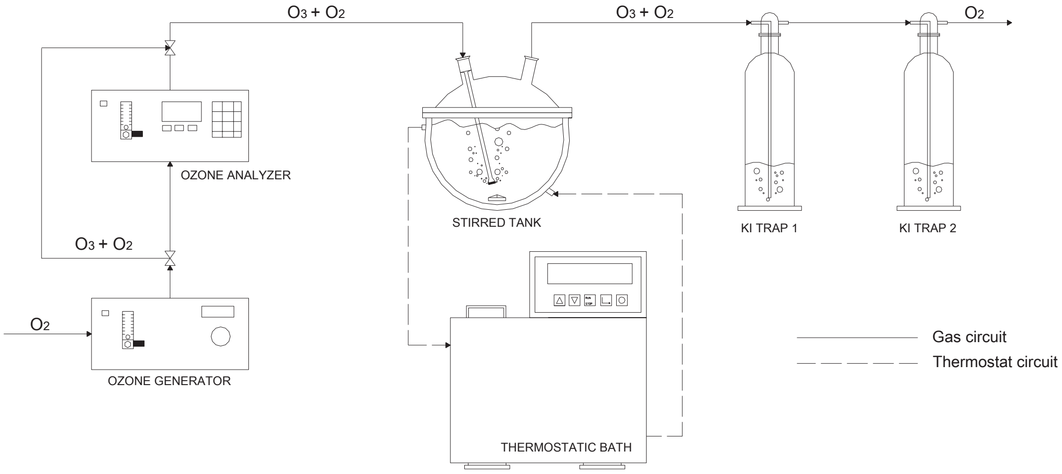

Fig. 2. Schematic diagram of the ozonation system

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 carry out the biodegradation test by *Pseudomonas putida*.

2.3. Pseudomonas putida biodegradation test

A monoculture strain *Pseudomonas putida* CECT 324, provided by the Spanish Type Culture Collection (Valencia, Spain), was used in the biodegradation test. Bacteria were procured in lyophilized form. These cultures were revived on solid agar Petri dish at pH 7.2, 1 g/L beef extract, 2 g/L yeast extract, 5 g/L peptone, 5 g/L NaCl and 15 g/L agar powder.

Bacterial stock was prepared with a colony of *Pseudomonas putida* from solid agar Petri dish, which was incubated for 24 hours in fluid growth medium, 1 g/L beef extract, 2 g/L yeast extract, 5 g/L peptone, 5 g/L NaCl, on a rotary platform shaker (100 rpm). 250 ml erlenmeyer flasks were filled to 100 ml with the surfactant solution to different concentrations and enriched up to NH_4Cl, 0.5 g/L, K_2HPO_4, 0.5 g/L, KH_2PO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$, 0.5 g/L and 10 ml/L of trace mineral solution, $CaCl_2 \cdot 2H_20$, 0.98 g/L, $MnSO_4 \cdot H_20$, 1 g/L, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_20$, 0.8g/L, $ZnSO_4 \cdot 7H_20$, 0.2 g/L and nitrilotriacetic acid, 0.2 g/L [16] [17]. The final pH was adjusted to 7.2 by adding either 0.1 N HCl or 0.1M NaOH. The flasks were inoculated with 200 µL of bacterial stock. Flasks were incubated at 30ºC on a rotary platform shaker (100 rpm) for 72 hours.

At the beginning and after 72 hours, a sample of each flask was filtered using 0.45 µm Millipore® filters. The filtered sample was used to determine the dissolved organic carbon (DOC) using the TOC analyzer Shimadzu TOC-V CHS. Biodegradation efficiency (E_f) of the amine oxides based surfactants before and after ozonation, was evaluated as a percentage by:

$$Ef(\%)=[[DOC_i - (DOC_f - DOC_m)]/DOC_i]\cdot 100 \qquad (1)$$

Where DOC_i is the initial DOC concentration, DOC_f is the DOC concentration measured at the end of the incubation (72h) and DOC_m is the minimum concentration that cannot be metabolized by the bacteria [17].

3. Results and Discussion

3.1. Ozonation processes

The ozonation of aqueous solutions of non-ionic surfactants was analyzed using two selected oxide amine based surfactants: Oxidet® DM-4 and Oxidet® DM-20. The tested aqueous solutions had a concentration of 50 and 100 mg/L for the surfactant AO-R_{14} and 200 mg/L for the surfactant AO-R_{12}. The initial concentrations of the surfactants that underwent ozonation, were chosen in such way that they were below the values of critical micelle concentration of the surfactants. The tests conditions and the results of mineralization for the surfactants studied are showed in tables 2 and 3.

Table 2. Results of ozonation of surfactant AO-R_{14}

Time of ozonation (min)	Ozone production (%)	Surfactant concentration (mg/L)	Mineralization* (%)	T (ºC)
30 min	50	50	5.67 (4.52-6.82)	25
30 min	100	100	37.64 (35.48-39.80)	25

*Mineralization values (95% CI)

Table 3. Results of ozonation of surfactant AO-R_{12}

Time of ozonation (min)	Ozone production (%)	Surfactant concentration (mg/L)	Mineralization (%)	T (ºC)
30 min	75	200	13.92 (12.09-15.75)	25
30 min	100	200	6.41 (5.75-7.07)	25

*Mineralization values (95% CI)

Ozonation of the surfactants solution causes a decrease of TOC for the surfactants AO-R_{14} and AO-R_{12}, but the mineralization degree due to ozonation is not high. Despite the moderately mineralization the molecular structure changes induced by ozone results in the formation of more biodegradable products. For the surfactant AO-R_{12} the increase of the ozone production does not increase the degree of mineralization. The decomposition by ozone of the surfactants depends on the chemical structure of the surfactants. Comparing the % reduction loss of surfactant concentrations, we observed that the mineralization increases for the surfactant with the alkyl chain higher (Tables 2 and 3).

According to this results and previously published data on surfactant oxidation, it can be stated that surfactant mineralization caused by ozone is slow and incomplete. However the formation of oxygenated compounds, mainly short chain carboxylic acids, could reduce the hydrophobic character of the original surfactant [18].

3.2. Influence of Ozonation on the biodegradability of surfactants

Assays using *Pseudomonas putida* might be particularly advantageous, as they are moderately time consuming and involve a definite living system instead of the complex and variable consortium of microorganisms as are activated sludge. The solutions used in the *Pseudomonas putida* assays were the aqueous solutions obtained in the ozone tests. The initial concentrations in the assays were 5 mg/L for AO-R_{14} and 10 mg/L for AO-R_{12}. Figure 3 shows the biodegradation efficiency from *Pseudomonas putida* tests and figure 4 shows the comparison of biodegradation efficiency from *Pseudomonas putida* tests before and after ozonation.

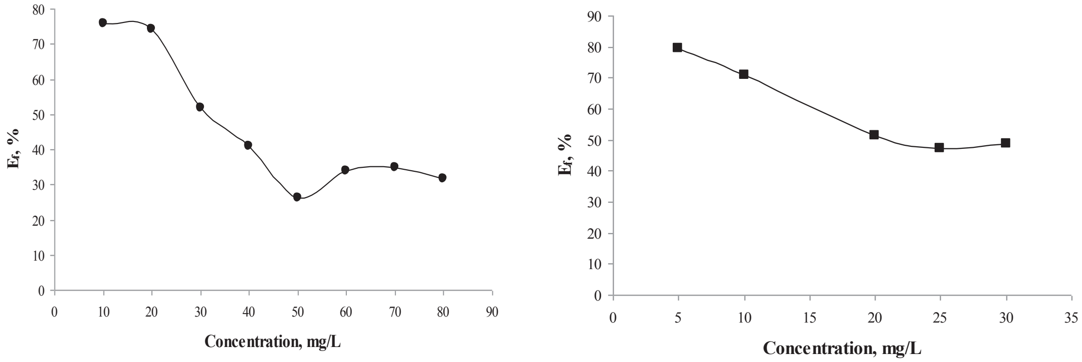

Fig. 3. Biodegradation efficiency from *Pseudomonas putida* tests before ozonation. (a) Surfactant AO-R_{14}; (b) Surfactant AO-R_{12}

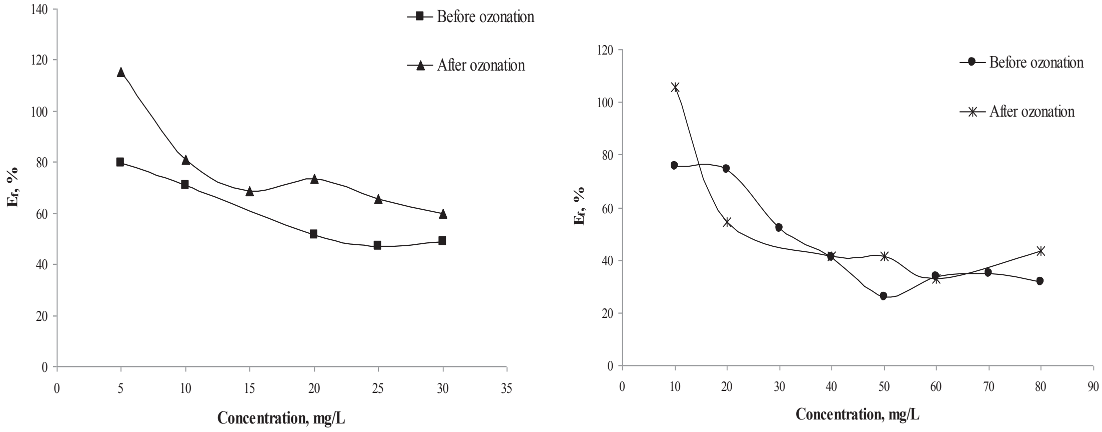

Fig. 4. Comparison of biodegradation efficiency from *Pseudomonas putida* tests before and after ozonation (a) Surfactant AO-R_{14}; (b) Surfactant AO-R_{12}

An increase in biodegradation efficiency was obtained with *Pseudomonas putida* test after ozonation, indicating that more biodegradable substances had been generated during the oxidation. In general, the *Pseudomonas putida* test shows an increase in efficiency with the progress of the ozonation reaction, so ozonation improved biodegradation of the surfactants.

4. Conclusions

Ozonation of the surfactants solution causes a decrease of TOC for the surfactants AO-R_{14} and AO-R_{12}, but the mineralization degree due to ozonation is moderately high. For the surfactant AO-R_{12} the increase of the percentage of ozone in gas mixture does not increase the degree of mineralization. The effectiveness of ozonation depended on the chemical structure of surfactant molecule. Ozonation only slightly improved biodegradation of amide oxide based surfactants. An increase in biodegradation efficiency was obtained with *Pseudomonas putida* test, indicating that more biodegradable substances had been generated during the oxidation. Ozonation improved biodegradation of the surfactants

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