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BIODEGRADATION OF ALKYL ETHOXYLATES AND ALKYLPHENOL ETHOXYLATES IN RIVER WATER IN A STATIC DIE-AWAY TEST AS STUDIED BY HPLC-ESI/MS

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ABSTRACT

Biodegradation of nonylphenol ethoxylates and dodecyl ethoxylates ($C_9H_{19}-C_6H_4-(OCH_2CH_2)_n-OH$ and $C_{12}H_{25}-(OCH_2CH_2)_n-OH$, respectively) in river water in a static die-away test was analyzed by using high performance liquid chromatography with mass spectrometric detection using the Applied Biosystems quadrupole-linear ion trap mass spectrometer Model 4000 QTrap. Surfactants containing 2-4 ethoxylene units may be regarded as persistent metabolites of longer surfactants. Homologues $C_9H_{19}-C_6H_4-(OCH_2CH_2)_5-OH$ and $C_{12}H_{25}-(OCH_2CH_2)_5-OH$ underwent substantial biodegradation till the 9th day of the test, their further biodegradation proceeded very slowly. The longer homologues ($n > 5$) were fully biodegraded during the test. Although the biodegradation of the surfactants studied consisted in shortening the ethoxylene chain (hydrophilic part), it was found that the hydrophobic part also affected their biodegradation.

Keywords: non-ionic surfactants, biodegradation, HPLC-ESI/MS

INTRODUCTION

Alkyl- and alkylphenol ethoxylates (AEs and APes) are the most widely used non-ionic surfactants. They have been used in domestic detergents, pesticide formulations and industrial products. It is well known that, nowadays, the-

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se compounds and their metabolites are present in the environment, as reviewed recently [1-5]. Thus, it is important to know how these compounds behave in the environment, mainly to know their biodegradation pathways. Therefore, analysis of these compounds, mainly with the use of mass spectrometric techniques, in the environmental samples was performed [6-11]. There are also a number of papers devoted to the biodegradation of non-ionic surfactants in biological sewage treatment plants (STP) or the so-called activated sludge (as a source of microorganisms) from STP is used for biodegradation of non-ionic surfactants [11-21]. There are also several interesting studies of the surfactants subjected to the action of specific bacteria [22-25].

In some of the biodegradation tests performed in laboratory conditions, surfactants are placed in environmental water (which contains different microorganisms) and biodegradation products are analyzed. Mann and Boddy have studied biodegradation of alkylphenol ethoxylates in lake water under conditions of complete darkness and a 12 h light, 12 h dark photoperiod [26]. Potter et al. have studied the biodegradation of Intan-100 in estuarine water [27]. Jonkers et al. have studied biodegradation of nonylphenol ethoxylates in river water [28]. Perez-Carrera et al. have studied biodegradation of dodecyl ethoxylate (and other surfactants) in sea water (from the Atlantic Ocean at the Spanish seashore) [29]. In this work the biodegradation of nonylphenol ethoxylate and dodecyl ethoxylate ($C_9H_{19}-C_6H_4-(OCH_2CH_2)_n-OH$ and $C_{12}H_{25}-(OCH_2CH_2)_n-OH$, respectively) in river water was analyzed by means of high performance liquid chromatographic analysis with electrospray ionization mass spectrometric detection (HPLC/ESI-MS, which seemed the best method for the purpose of the study [30]).

EXPERIMENTAL

The water used in the biodegradation study was sourced from the River Warta (October 2009, Poznań, Poland; the physical and chemical parameters of the water can be found on <http://www.poznan.pios.gov.pl/glowna/index.php>). Dodecyl ethoxylates (Tergitol TMN-10) were obtained from Sigma-Aldrich (Poznań, Poland). Nonylphenol ethoxylates (Rokafenol N-10) was obtained from PCC Rokita S.A. (Brzeg Dolny, Poland).

The tests were performed in 200 cm³ bottles: the bottle was filled with river water and 10 mg of surfactant was added (there were two bottles: one for dodecyl ethoxylates and one for nonylphenol ethoxylates). Such concentration is typically used in the biodegradation test [28]. The bottles were opened (aerobic conditions), stored at a room temperature and protected against dust. Each day a 0.5 cm³ sample was collected from each bottle and 2 cm³ of acetonitrile was added to the collected portion in order to stop the biodegradation process. The samples collected were subjected to HPLC/ESI-MS analysis.

In order to prove that the observed changes in the surfactant concentration are really the result of the biodegradation process, the next two bottles were filled with mixtures of 9:1 river water/acetonitrile (the microorganisms are not

expected to be able to survive such conditions) and the next two bottles were filled with tap water (tap water contains very low amounts of microorganisms in comparison with river water). A portion of the surfactant was added to each bottle. No changes in the homolog concentrations (described further for bottles filled with river water) were observed in the bottles filled with river water/acetoneitrile and with tap water.

The HPLC/ESI-MS analyses were performed using the Applied Biosystems quadrupole-linear ion trap mass spectrometer Model 4000 QTrap. The ESI source parameters were declustering potential 80 V, ion spray 3500 V, source temperature 400°C. The sample solutions were injected into a Hypersil Gold C18 RP column (100 mm x 3.9 mm i.d.) (Thermo Scientific) by means of an autosampler. The gradient started from 60% CH₃OH – 40% H₂O with 5 mmol CH₃COONH₄ reaching 90% CH₃OH after 8 min, and 100% CH₃OH after 1 more minute maintained for 6 min.

RESULTS AND DISCUSSION

Depending on the test conditions, the biodegradation of alkyl- and alkylphenol ethoxylates yields shortening of the ethoxylene chain, polyethylene glycols (a result of the central fission pathway) and/or carboxylated derivatives (as the results of oxidation of the ethoxylene chain) [11-29]. In this work, the biodegradation process leads to the shortening of the ethoxylene chains, while the effects of the other biodegradation pathways are not observed.

In order to demonstrate the relative changes in each homolog concentration during the biodegradation test, Figures 1 and 2 show the breakdown plots of the peak areas of the sodiated molecule (ions [C₉H₁₉-C₆H₄-(OCH₂CH₂)_n-OH + Na]⁺ and [C₁₂H₂₅-(OCH₂CH₂)_n-OH + Na]⁺) against time, as expressed in the number of days of the biodegradation test. For clarity, the top plots concern the short homologues of which the concentration increased during the test, and the bottom plots concern the longer homologues of which the concentration decreased during the test. Also for the sake of clarity, only the results of every other day (odd day numbers) were taken into account in making the plots.

As shown in Figures 1 and 2, the concentration of the homologues containing 2, 3 and 4 ethoxylene units increased during the test, for both surfactants. There was a rapid increase in the concentration of C₉H₁₉-C₆H₄-(OCH₂CH₂)₂-OH (in the first three days) and then its concentration was practically constant. The concentration of C₉H₁₉-C₆H₄-(OCH₂CH₂)₃-OH also increased rapidly during the first three days and then its concentration increased slowly. In contrast to the concentration changes of C₉H₁₉-C₆H₄-(OCH₂CH₂)₂-OH and C₉H₁₉-C₆H₄-(OCH₂CH₂)₃-OH, the concentration of C₁₂H₂₅-(OCH₂CH₂)₂-OH and C₁₂H₂₅-(OCH₂CH₂)₃-OH increased systematically throughout the test. The concentration of C₁₂H₂₅-(OCH₂CH₂)₄-OH, C₁₂H₂₅-(OCH₂CH₂)₅-OH and C₁₂H₂₅-(OCH₂CH₂)₆-OH also increased rapidly at the beginning of the test, while no such rapid increase was observed for C₉H₁₉-C₆H₄-(OCH₂CH₂)₄-OH, C₉H₁₉-C₆H₄-(OCH₂CH₂)₅-OH

and $C_9H_{19}-C_6H_4-(OCH_2CH_2)_6-OH$. Although the biodegradation of the surfactants studied concerns the ethoxylene chain (hydrophilic part), the above described differences between the behavior of $C_9H_{19}-C_6H_4-(OCH_2CH_2)_n-OH$ and $C_{12}H_{25}-(OCH_2CH_2)_n-OH$ ($n = 2-6$) allow the conclusion to be drawn that the hydrophobic part also affects the biodegradation of the surfactants.

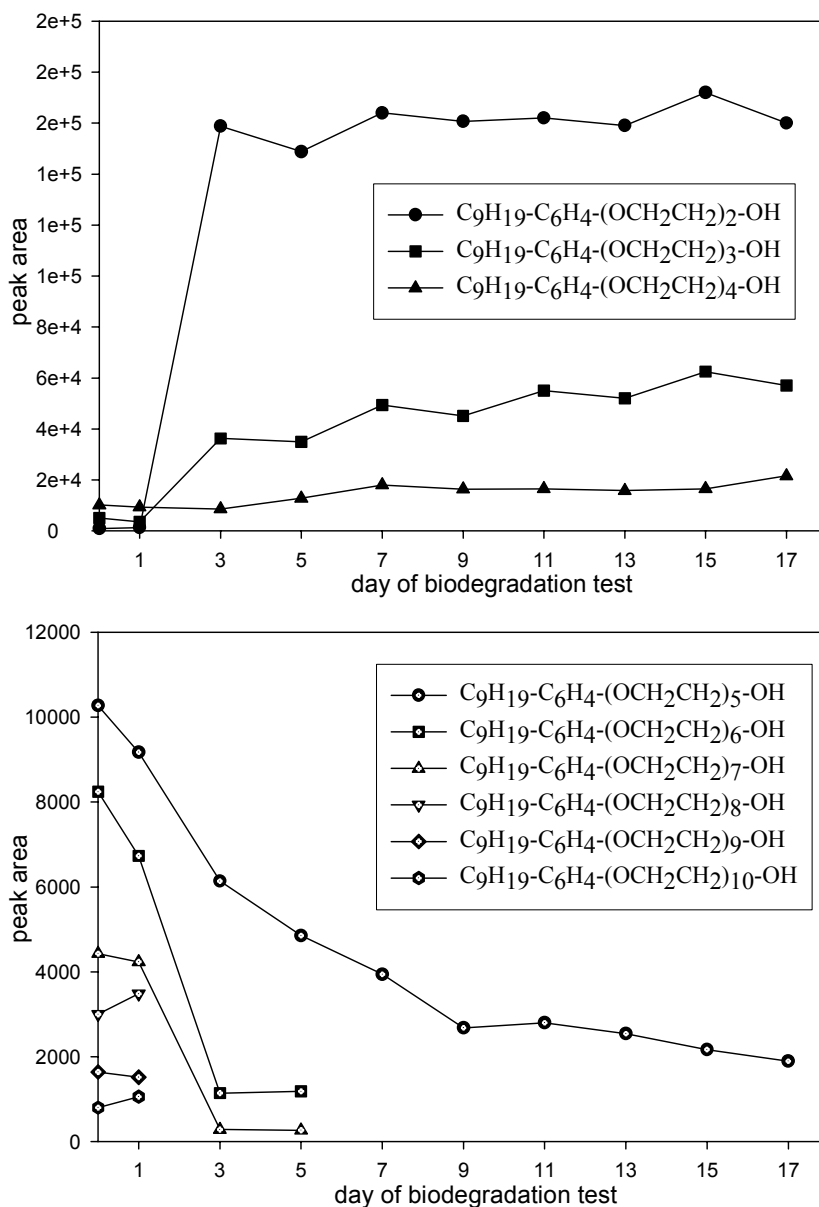


Fig. 1. A breakdown plot of peak areas of $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_n-OH + Na]^+$ ions against days of biodegradation test in river water.

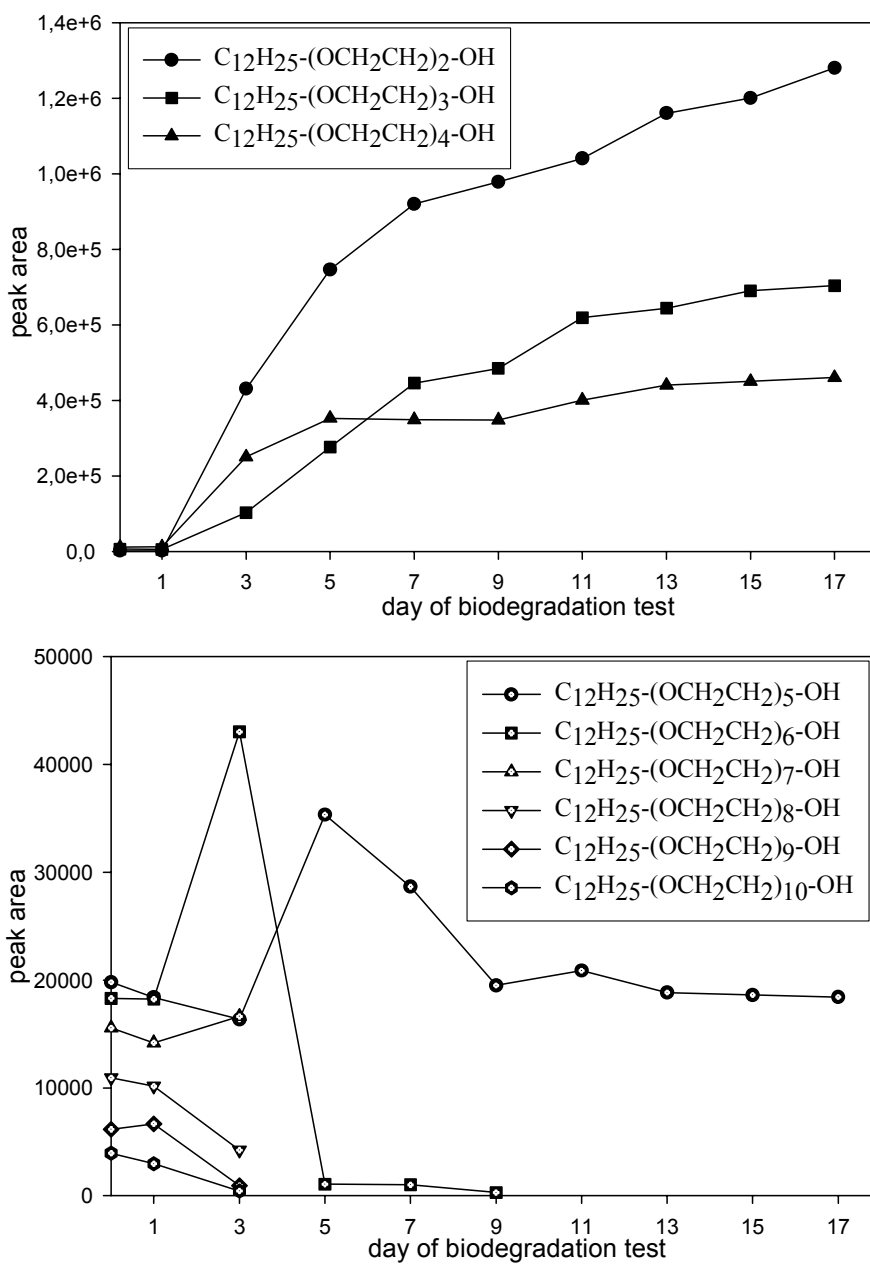


Fig. 2. A breakdown plot of peak areas of $[C_{12}H_{25}-(OCH_2CH_2)_n-OH + Na]^+$ ions against days of biodegradation test in river water.

As clearly follows from Figures 1 and 2, the surfactants containing 2-4 ethoxy-ylene units did not undergo the biodegradation process consisting in shortening the ethoxy-ylene chain. These surfactants may be regarded as persistent metabolites

(under the conditions used) of the longer surfactants. Homologues $C_9H_{19}-C_6H_4-(OCH_2CH_2)_5-OH$ and $C_{12}H_{25}-(OCH_2CH_2)_5-OH$ underwent the biodegradation process until day 9 of the test, their further biodegradation occurred very slowly. The longer homologues ($n > 5$) were fully biodegraded during the test.

It should be noted that the shortest homologues $C_9H_{19}-C_6H_4-OCH_2CH_2-OH$ and $C_{12}H_{25}-OCH_2CH_2-OH$ ($n = 1$) were not observed. It is possible that they were formed in a low amount, however, they were undetectable because of their low ESI response [31].

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