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Biodegradation of Fatty Alcohol Ethoxylates under the Conditions of the Die-Away Test

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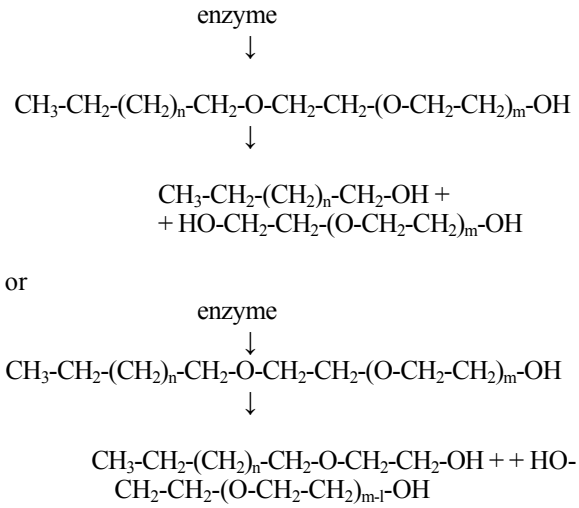
Abstract

Oxyethylated alcohol - surfactant C12E10 - was tested under the conditions of the die-away test with activated sludge as inoculum. The residual surfactant and its biotransformation by-products: short-chained ethoxylates (SCE) and long-and short-chained poly(ethylene glycols) (PEG) were separated and determined using the indirect tensammetric technique. A high percentage of primary biodegradation (98.5%) and high concentration of SCE and PEG was found at the end of the experiment. There is a clear sequence of biotransformation: central fission —> fragmentation of the long-chained PEG into short-chained PEG. Generally, the same pathway was observed as in the case of oxyethylated alcohol biodegradation under the conditions of the continuous flow activated sludge test.

Introduction

A relatively small number of papers is devoted to the biodegradation of non-ionic surfactants (NS), despite the fact that surfactants are the biggest source of anthropogenic organic carbon in surface water [1]. The ratio of the manufactured NS level to that of anionic surfactants has gradually grown over the last three decades [2, 3] and recently NS have become the major surfactant [4]. NS concentration in river water has been dramatically growing over the last decade [5] following the growth in NS consumption. Apart from NS, poly(ethylene glycols) (PEG), the NS biodegradation by-product were found in water of the Warta and Vistula rivers in concentrations of the same range as NS concentration [6, 7]. Therefore, not only primary biodegradation, but also by-products of partial NS biodegradation should be monitored in order to ensure minimum environmental risk. Oxyethylated alcohols (AE) are the major group of NS [4].

It has recently been shown [8, 9] that the fatty alcohol ethoxylates biodegradation pathway requires supplement ation. Under the conditions of the continuous flow acti vate sludge test (CAS), AE are split in accordance with the reactions:



Free fatty alcohol (FFA) or short-chained ethoxylates (SCE) containing 1-3 oxyethylene subunits (EO), as well as PEG are formed.

Though the occurrence of the central fission pathway has been confirmed in several papers [10-12], the by-products of central fission are not as readily biodegraded as judged previously [8]. Further FFA biodegradation, formed from AE by central fission, was considered as fast and complete [11]. This opinion, affirmed by Patter-

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son et al. [13], is based on the fact that FFA was not detected in AE biodegradation experiments. Apart from the central fission pathway the alternative pathways for ethoxylates have been published: co-oxidation of alkyl moiety and co-oxidation of oxyethylene moiety. The first leads to the shortening of the alkyl moiety, while the other to shortening of the oxyethylene moiety, co-oxidation of the oxyethylene chain was found to be the dominating biodegradation pathway in the case of oxyethylated alkylphenols [14]. In accordance with Schoberl et al. [15], the fast co-oxidation of branched alkyl chains of oxyethylated oxo-alcohols leads to the formation of dicarboxylated PEG. Carboxylated PEG may also be formed by the terminal oxidation of neutral PEG [11, 12]. In accordance with the most recent research [8], further PEG biodegradation under the CAS conditions can be described by the reaction:

$$HO\text{-}CH_2\text{-}CH2\text{-}(O\text{-}CH_2\text{-}CH_2)_m\text{-}OH$$

$$\downarrow$$

$$n\ H\ \text{-}(O\text{-}CH_2\text{-}CH_2)_p\text{-}OH$$

where p = 1- 6.

The main problem is whether AE may be biodegraded in accordance with different pathways due to the multicomponent nature of activated sludge (a consortia of different micro-organisms) and the divergent conditions of various tests, or whether there may be one dominating pathway selected independently of whether the test is dynamic or static and independently of the origin of activated sludge. The different pathways published in literature may appear due to the misinterpretation of results or the poor state-of-art analytical methods used in past investigations.

The aim of this paper was the confirmation or falsification, under the conditions of the die-away test with the application of activated sludge as the inoculum, of a modified pathway of AE biotransformation in which the short-chained ethoxylates (including free fatty alcohol) as well as the long- and short-chained PEG are formed. In contrast to the CAS test, the tested surfactant in the die-away test is a sole source of organic carbon and the metabolic pathway should express the sequence of metabolic steps. The conditions corresponding to the slightly modified OECD 301E test [18] were selected for the test performance.

Materials and Methods

Apparatus

A Radelkis OH-105 polarograph and an ECO Chemie General Purpose Electroanalytical System μAUTOLAB were alternatively used for tensammetric measurements.

Surfactant

Surfactant C12E10 (Sigma) was selected as the model fatty alcohol ethoxylate. This surfactant is a polydispersal mixture. It consists of a C^{12} straight alkyl chain and oxyethylene chain with an average 10 oxyethylene subunits. The surfactant was used without additional purification.

Activated Sludge

Activated sludge used in the experiments originated from the sewage treatment plant (STP) in Szamotuly (near Poznan, Poland) which treats typical municipal sewage.

Dragendorff Reagent

Modified Dragendorff reagent [16] was prepared by mixing solutions A and B before use. Solution A: 1.7g of basic bismuth(III) nitrate, 65 g of potassium iodide and 220 ml of glacial acetic acid in 1000 ml. Solution B: an aqueous solution containing 290g of barium chloride dihydrate in 1000 ml.

The solution for dissolving the precipitate (solution C) was prepared from 12.4g of tartaric acid and 18 ml of ammonia solution (25%) made up with water to 1000 ml. A silica gel cartridge (Bakerbond spe silica gel 7086-03) was used for the purification of this solution.

Other Reagents and Materials

Purified sodium sulphate and sodium chloride were used for the preparation of the aqueous base electrolytes. All solutions used in analysis were prepared in water triply distilled from a quartz apparatus. Only freshly distilled water was used.

Freshly distilled ethyl acetate and chloroform were used. Other reagents used were of Analar grade as were methanol, glacial acetic acid and sodium hydrogencarbonate.

A glass filter G5 (Schott-Gerate) was used.

Procedures

The Die-Away Test with Activated Sludge as the Inoculum

Twelve conical flasks of 300 ml volume were filled with 200 ml of tested solution containing 10 mg l^{-1} of surfactant C12E10, 0.2 mg l^{-1} of activated sludge and 8.5 mg l^{-1} of KH_2PO_4, 21.5 mg $11^{-1}$of K_2HPO_4, 33.5 mg l^{-1} of Na_2HPO_4. $2H_2O$, 20 mg l^{-1} of NH_4CI, 22.5 mg l^{-1} of $MgSO_4 \cdot 7H_2O$, 27.5 mg l^{-1} of $CaCl_2$,0.25 mg l^{-1} of $FeCl_3 \cdot 6H_20$,40 μg l^{-1} of $MnSO_4 \cdot H_2O$, 57.5 μg l^{-1} of H_3BO_3, 42.5 μg l^{-1} of $ZnSO_4$-$7H_2O$, and 35 μg l^{-1} of $(NH_4)_6Mo_7O_{24}$. The necks of the flasks were stoppered with glass wool and wrapped with aluminium foil in order to protect against algae growth. The samples were shaken for 1 hour per day.

On days 1,2,3,5,7,9,12,16 and 20 of the experiment, single samples were taken from the set of samples and preserved with 1% formaline. Two samples were preserved just after preparation of the tested solution. These two samples served for the determination of "blanks". Addi-

tionally, a solution without surfactant C12E10 was preserved for the same purpose.

Separation and Determination of Residual Surfactant and its Biotransformation by-Products

Separation of residual surfactant, short-chained ethoxylates and poly(ethylene glycols) from a sample and the determination of concentration of residual surfactant by the BiAS-ITT have been described in a previous paper [8]. Briefly, the sample was separated into several fractions by sequential liquid-liquid extractions and precipitation. Ethyl acetate and chloroform were used for sequential extraction. The ethyl acetate fraction contained the residual surfactant, short-chained ethoxylates and free fatty-alcohol, while the chloroform fraction contained PEG. Precipitation with modified Dragendorff reagent was used for further separation of the examined mixtures. Residual surfactant was precipitated from ethyl acetate extract. Similarly, long-chained PEG (having more than 4 oxyethylene subunits) were precipitated. Concentration of analytes in particular fractions was determined by the indirect tensammetric method (ITM) after dissolving of the precipitates. Precision of determination of the concentration of residual surfactant was 5% and 4% in the case of the long-chained PEG.

The determination of short-chained PEG (1-4 EO) has also been described elsewhere [9]. Briefly, this fraction was extracted with chloroform from the filtrate after precipitation of long-chained PEG and determined by the ITM. Precision of determination was 7.5%.

The determination of FFA has been described in a previous paper [17]. Briefly, the residual surfactant, short-chained ethoxylates and FFA fraction were separated from the sample by extraction with ethyl acetate and the residual surfactant separated by precipitation with modified Dragendorff reagent. Free fatty alcohol and short-chained ethoxylates were extracted from the filtrate with chloroform and determined by the ITM. Precision of determination of the concentration was 10%.

The ultimate biodegradation was calculated assuming that PEG and FFA are the only metabolites of biodegradation. The rest of the carbon was assumed to be transformed into carbon dioxide or biomass.

Results and Discussion

Changes of concentration of residual surfactant C12E10, short-chained ethoxylates and total PEG during the test are shown in Fig. 1 (curves a, b and c, respectively). The residual surfactant C12E10 concentration (curve a) dropped from the value close to the initial concentration at the beginning of the test to a very low value (approximately 150 μg l^{-1}) at the end of the experiment. This indicates a very high percentage of primary biodegradation (98.5%). A high percentage of the primary biodegradation occurred in two steps: approximately 10% at the beginning of the test and approximately 90% between days 6 and 12 of the experiment. This meant that a 6 day acclimation period was required.

Simultaneously, with the rapid decrease of surfactant

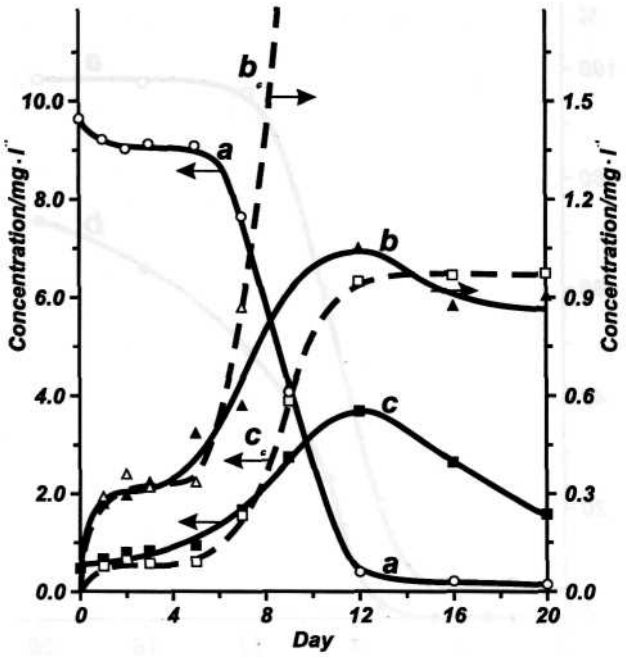

Fig. 1. Residual concentration of surfactant C12E10 (a), and short-chained ethoxylates (b) and total poly(ethylene glycol) (c)concentration during the test. Curves be and cc correspond to the concentrations of the short-chained ethoxylates (be) and total poly(ethylene glycols)(cc) calculated on the basis of central fission and curve a.

C12E10 concentration, a growth in the concentration of short-chained ethoxylates (curve b) and PEG (curve c) was observed. This is evidence that these substances are products of the biodegradation of surfactant C12E10, and that the further biodegradation of these by-products is slower than that of surfactant C12E10. After day 12 of the experiment, when the whole surfactant C12E10 is split, die-away of already formed short-chained ethoxylates and PEG was observed.

In order to compare the experimental results with those calculated on the basis of central fission, theoretical concentrations of short-chained ethoxylates and PEG were calculated. Calculations were performed assuming that dodecylmonoethoxylate (C12E1) and PEG having 9 oxyethylene subunits (E9) are formed from primary biodegraded surfactant C12E10. The results of calculation have been added to Fig. 1 as curves be and cc, respectively. Agreement of the calculated and experimental results is satisfactory during the initial 5-7 days of the experiment. This meant that during this initial period only the split of surfactant C12E10 is observed and the highly probable position of the split is between the first and second oxyethylene subunits. After the initial period, the experimental values of by-products were significantly lower than the calculated ones, showing progress in the subsequent steps of biodegradation.

The primary and total biodegradation of surfactant C12E10 during the test have been compared in Fig. 2. The percentage of total biodegradation is significantly lower than the primary biodegradation due to the presence of high concentrations of short-chained ethoxylates and PEG.

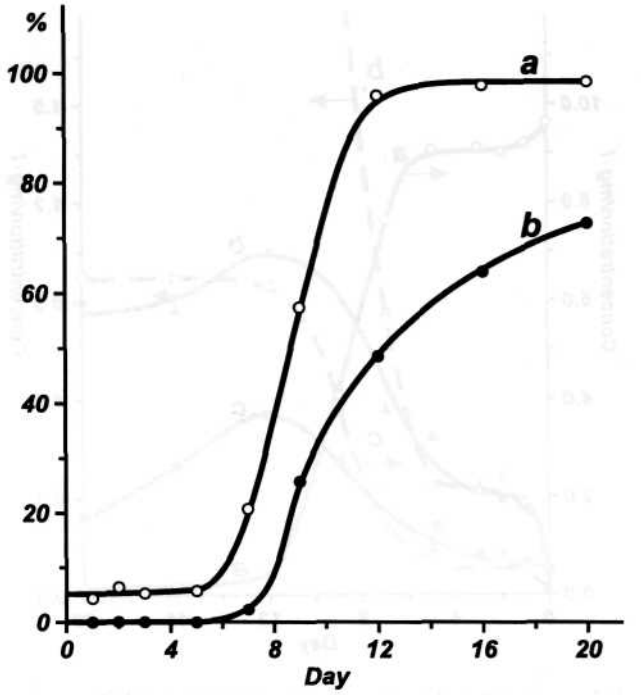

Fig. 2. Primaiy(a) and total(b) biodegradation of surfactant C12E10 during the test.

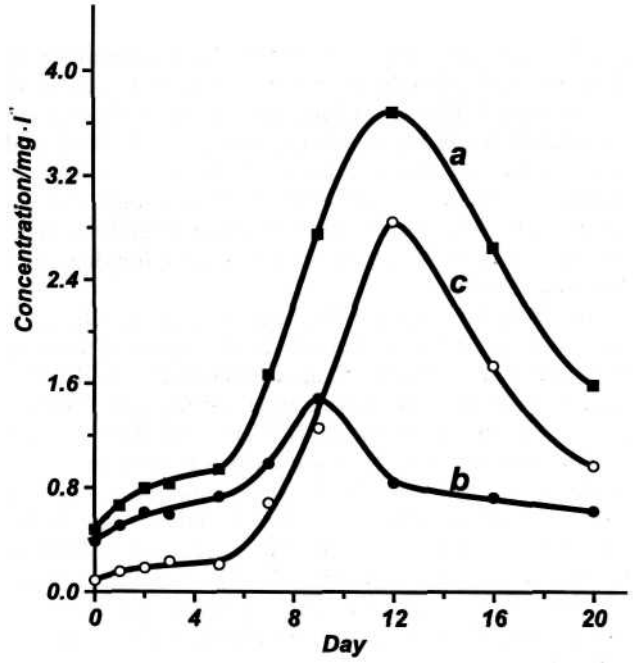

Fig. 3. Fractional changes of poly(ethylene glycols) during the test: (a) total poly(ethylene glycol) concentration, (b) long-chained PEG concentration, (c) short- chained PEG concentration.

The changes in a fractional PEG concentration during the test are shown in Fig. 3. There is no doubt that the short-chained PEG (i.e. PEG having less than 5-6 oxyethylene subunits) were the major fraction. The maximum of short-chained PEG concentration is delayed by 3 days in comparison to the long-chained PEG concentration maximum.

Generally, the experiment shows that oxyethylated alcohols are biodegraded in accordance with the central fission pathway with the formation of short-chained ethoxylates and PEG; further PEG biodegradation leads to the fragmentation of the long-chained PEG into short-chained PEG. The biodegradation of the by-products is delayed with respect to central fission of the surfactant. This pathway is the same as that observed in the case of biodegradation of oxyethylated alcohols under the conditions of the continuous flow activated sludge test [8]. It is worth stressing that, in the continuous flow activated sludge test, the surfactant is supplied together with an excess of organic nutrients (beef meat extract and peptone) and is treated by activated sludge for only several hours; whereas, in the die-away test, the surfactant is the sole source of organic carbon and is continuously treated over 4 weeks. Nevertheless, one general pathway is observed for the biodegradation of oxyethylated alcohols, independently of the test conditions. This is the main conclusion of this paper.

Acknowledgements

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