The Biodegradation of Some Sulfated Alkanolamides[1]

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ABSTRACT

Biodegradability tests were carried out in a controlled nutrient medium under aerobic and microaerophilic conditions, in river water and in soil. Degradation occurred under all conditions but more rapidly when the oxygen concentration was high. Degradation was rapid in sterilized river water inoculated with anaerobic sewage sludge under microaerophilic conditions and in soil. LAS was not degraded in any of the microaerophilic tests.

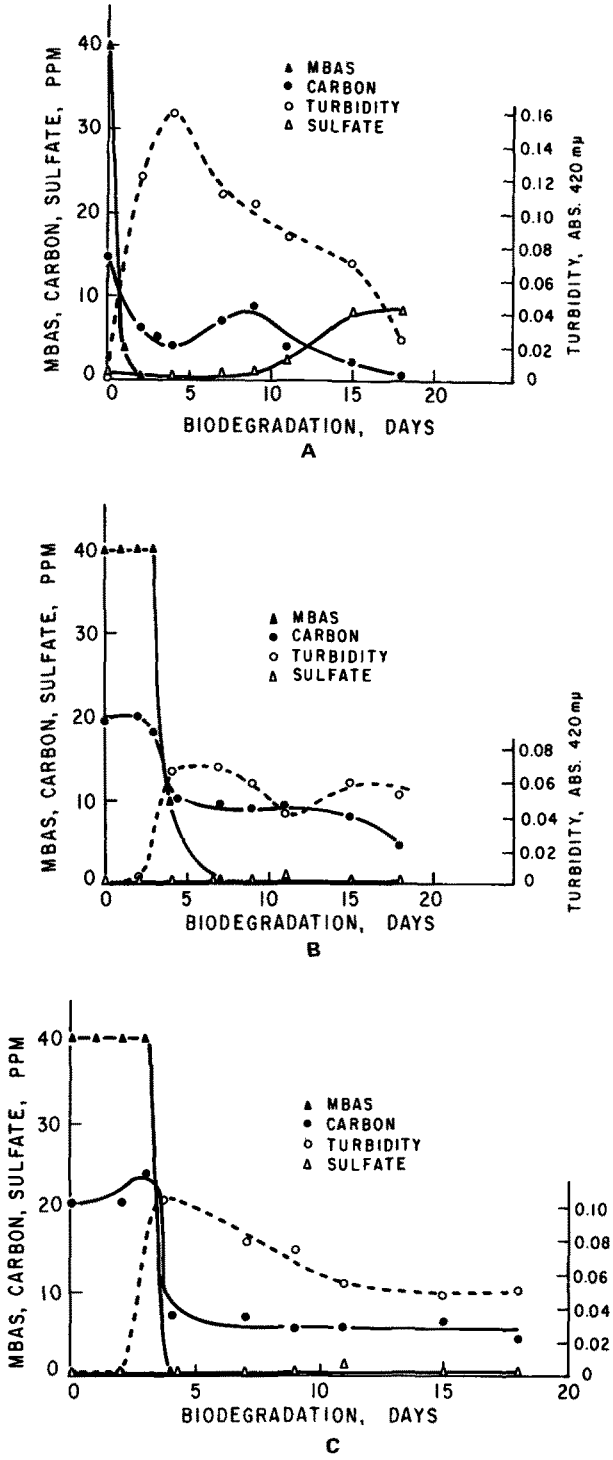

FIG. 1. Biodegradation of Sulfated Alkanolamides under aerobic conditions in a controlled nutrient medium at 25 C. A: Sulfated 2-hydroxyethyl palmitamide. B: Sulfated 2-hydroxypropyl stearamide. C: Sulfated 2-(2-hydroxyethoxy)ethyl stearamide.

INTRODUCTION

Sulfated alkanolamides have been shown to have desirable detergent and lime soap dispersing properties (1,2). Sulfonated amide surfactants have been found to be biodegradable under anaerobic conditions (3) and they along with the alcohol sulfates appear to be the only surfactants so far reported that are degraded in strictly anaerobic systems. Sulfated alkanolamides are degraded in river water under microaerophilic conditions (4). This paper deals with the biodegradability of sulfated alkanolamides under a variety of conditions as part of a continuing study of the biodegradability of fat-based detergents (4,5).

EXPERIMENTAL PROCEDURES

Materials

The preparation and properties of the sodium salts of sulfated 2-hydroxy-ethyl palmitamide, $CH_3(CH_2)_{14}CONHCH_2CH_2OSO_3Na$, sulfated 2-hydroxypropyl stearamide, $CH_3(CH_2)_{16}CONHCH_2CH(CH_3)OSO_3Na$, and sulfated 2-(2-hydroxyethoxy)-ethyl stearamide, $C_{17}H_{35}CONHC_2H_4OC_2H_4OSO_3Na$, used in these tests, were described by Weil et al. (1,2). Sodium hexadecyl sulfate (6) and the active ingredient extracted from a commercial sample of linear alkyl-benzene sulfonate with absolute ethanol were used as reference materials.

Procedures

Biodegradability under aerobic conditions was determined using the Esso controlled nutrient procedure (7). Essential minerals were supplied, but the medium was devoid of sulfates. Detergents at 40 ppm were the sole source of carbon and energy. The inoculum was prepared from activated sludge taken from a sewage plant that treats mostly domestic sewage. The biodegradation was carried out at 25 C in stirred solutions, and the course of

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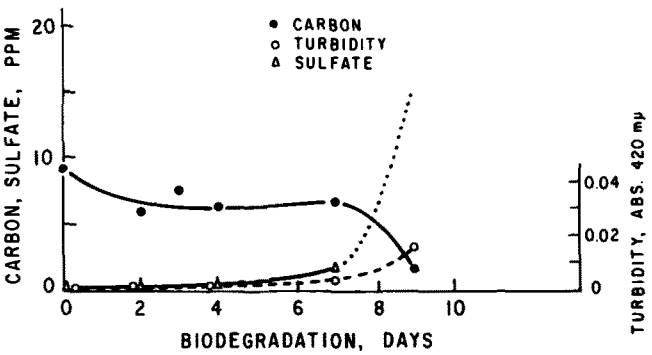

FIG. 2. Biodegradation of N-methyltaurine under aerobic conditions in a controlled nutrient medium at 25 C.

breakdown was followed by measuring the loss of methylene blue active substance (MBAS) and organic carbon, and the formation of sulfate ions (8).

Microaerophilic tests (4) were carried out at 35 C in 4 liter aspiration bottles containing 2 liters medium. Deionized water was boiled to remove dissolved oxygen and cooled in an atmosphere of nitrogen. Nutrient salts were added from the following stock solutions:

Stock Solution No. 1	Dissolved in 1 liter deionized water
$Mg(NO_3)_2 \cdot 6H_2O$	5.375g
$Ca(NO_3)_2 \cdot 4H_2O$	2.925g
$Fe(NO_3)_2 \cdot 9H_2O$	0.375g
$CoCl_2 \cdot 6H_2O$	1.025g
Stock Solution No. 2	**Dissolved in 1 liter deionized water**
$(NH_4)HPO_4$	4.76g
K_2HPO_4	36.6g
KH_2PO_4	31.1g

One milliliter of Solution No. 1 and 5 ml of Solution No. 2 in deionized water were used to make 1 liter of nutrient salts solution. Forty milligrams per liter of detergent and 10 mg/liter of sewage sludge as inoculum were added. The inoculum was sludge obtained from the anaerobic digester of a sewage treatment plant that treats mostly domestic sewage. It was transported to the laboratory in bottles which were completely filled in order to eliminate head space gases. The sludge was held at 35 C until use, but never longer than 24 hr before use.

The contents of the aspirator bottles were stirred with magnetic stirrers and nitrogen was passed into the vented bottles at the rate of 50-100 ml/min. It was found that passing the nitrogen over the surface of the solution excluded air, and sparging was not necessary which eliminated the problem of foaming.

Biodegradation in soil was carried out as previously described (9).

Analytical Methods

MBAS was determined with a Technicon Auto Analyzer (10) and carbon with a Beckman Carbonaceous Analyzer (5). Sulfate ion concentration was measured using a turbidimetric method (8) developed in this laboratory that included the determination of inherent turbidity.

RESULTS AND DISCUSSION

Aerobic Biodegradation

Figure 1A shows the course of the degradation of sulfated 2-hydroxy-ethyl palmitamide. MBAS was reduced to zero in 2 days, and most of the organic carbon was gone in 4 days. The lack of sulfate ion formation indicates that hydrolysis did not occur at the sulfate group. The turbidity was probably caused by hydrolysis at the amide group giving insoluble carbon chains. The degradation of sodium hexadecyl sulfate, however, produced high turbidity with the concomitant formation of sulfate ions (5). No significant amount of sulfate ion was formed from the sulfated alkanolamide until the 11th day, and this, together with the presence of residual carbon, points to the formation of somewhat resistant molecular fragments. In the next 7 days the residue was decomposed as shown by the formation of 87% of the theoretical sulfate and almost complete loss of carbon.

Fragments resembling the sodium salt of N-methyltaurine

$$\begin{array}{l} HN\text{-}C_2H_4SO_3Na \\ | \\ CH_3 \end{array}$$

might result from the degradation of sulfated 2-hydroxy-

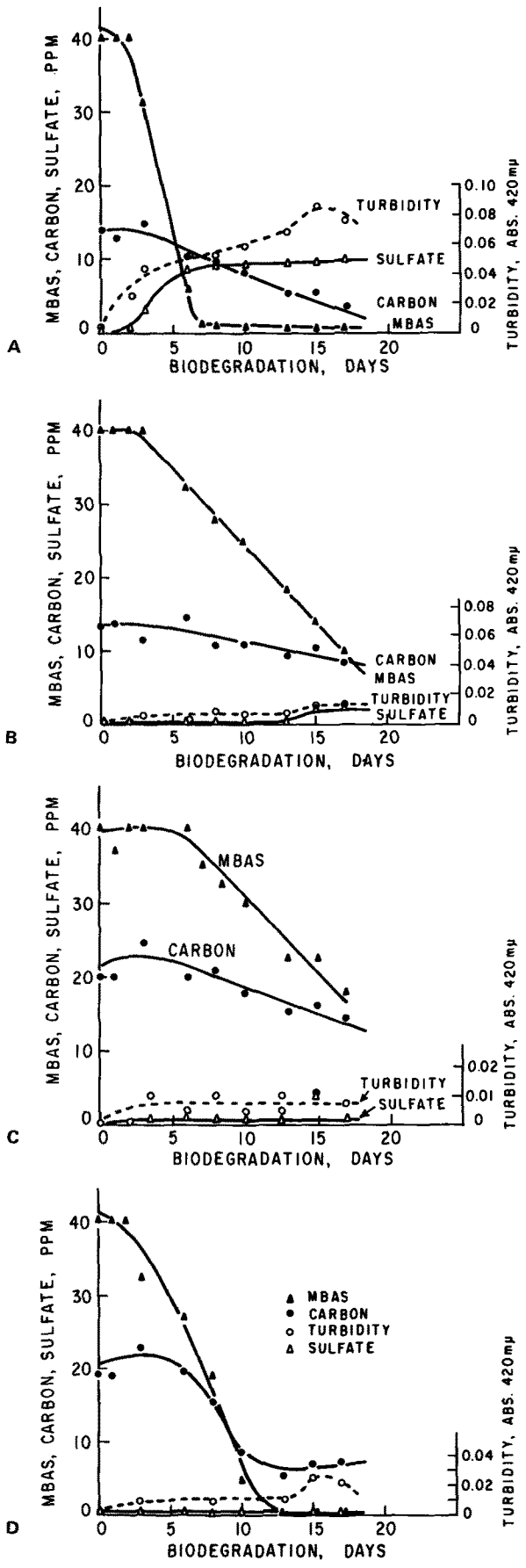

FIG. 3. Biodegradation of three sulfated alkanolamides and sodium hexadecyl sulfate under microaerophilic conditions in a controlled nutrient medium at 35 C. A: Na hexadecyl sulfate. B: Sulfated 2-hydroxyethyl palmitamide. C: Sulfated 2-hydroxypropyl stearamide. D: Sulfated 2-(2-hydroxyethoxy)ethyl stearamide.

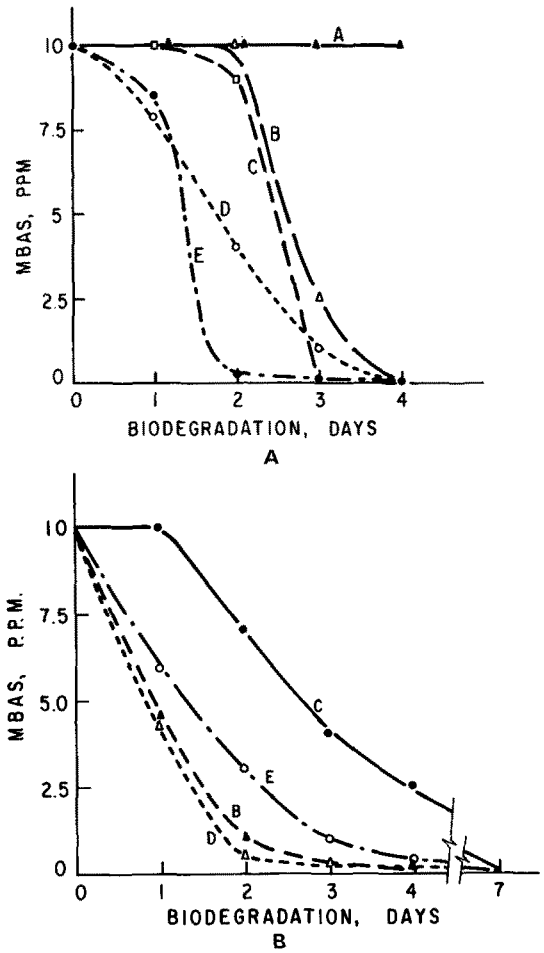

FIG. 4. Biodegradation of detergents in sterilized river water by sewage microorganisms under microaerophilic conditions at 25 C and 35 C. A-LAS, B-Sulfated 2-hydroxypropyl stearamide, C-Sulfated 2-(2-hydroxyethoxy)ethyl stearamide, D-Sulfated 2-hydroxymethyl palmitamide, E-Sodium hexadecyl sulfate. A and B: Inoculated river water.

ethyl palmitamide and might show similar properties when biodegraded. We have shown (5) that sulfate ion is formed readily from alkyl sulfonates as well as from alcohol sulfates. The course of the degradation of N-methyltaurine is shown in Figure 2. For 7 days there was very little change. The carbon then decreased rapidly and nearly the theoretical amount of sulfate ion was formed, indicating the destruction of the molecule.

The results obtained with sulfated 2-hydroxypropyl stearamide are shown in Figure 1B and with sulfated

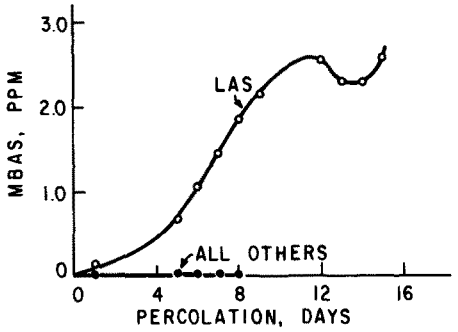

FIG. 5. Analysis of lysimeter effluents for MBAS in the soil degradation tests of sulfated 2-hydroxymethyl palmitamide, sulfated 2-hydroxypropyl stearamide, sodium hexadecyl sulfate and LAS.

2-(2-hydroxyethoxy)ethyl stearamide in Figure 1C. These curves resemble those obtained with sulfated 2-hydroxyethyl palmitamide, but the degradation was slower and no significant amount of sulfate was formed. The amount of carbon remaining indicated cleavage at the amide group. The complicated nature of residues from sulfated 2-hydroxypropyl stearamide and sulfated 2-(2-hydroxyethoxy) ethyl stearamide could account for their increased resistance to decomposition and lack of sulfate ion formation.

Microaerophilic Biodegradation

The anaerobic sludge for the early experiments was obtained from a tank where it was pumped just prior to dewatering. Results were not always consistent, and attempts were made to improve the degradation. First, 10 ppm of yeast extract (Difco) was added to the growth medium to supply possibly needed growth factors. Second, the bacteria in the inoculum were activated prior to use. Five hundred milliliters sludge was mixed with an equal amount of anaerobic thioglycollate medium (Difco) and incubated at 35 C. On two successive days 50 ml portions were removed and replaced with fresh thioglycollate medium. The vigor of the culture was shown by copious gas evolution. Third, sludge was obtained directly from the bottom of the digester. Exposure to air and cooling of the sludge while it was being pumped to the surge tank might enervate the bacteria.

The loss of MBAS in representative tests is shown in Table I. Although the results were not uniformly consistent the inoculum taken directly from the digester appeared superior. In the latter set each detergent was at least 55% degraded. Neither activation of the inoculum nor addition of yeast extract increased the overall effectiveness. Sludge taken directly from the digester was used in the remainder of the tests.

TABLE I

Biodegradation of Sulfated Alkanolamides Under Microaerophilic Condition in a Controlled Nutrient Medium at 35 C

Detergent (initial concentration 40 ppm)	Fresh inoculum: From surge tank MBAS ppm	Days	From digester MBAS ppm	Days	From surge tank enriched[a] MBAS ppm	Days	Activated inoculum MBAS ppm	Days
Sodium hexadecyl sulfate	30	16	0	7	0	6	30	17
Sulfated 2-hydroxyethyl palmitamide	0	16	10	17	25	10	0	17
Sulfated 2-hydroxypropyl stearamide	33	16	18	17	25	10	33	17
Sulfated 2-(2-hydroxyethoxy)-ethyl stearamide	0	12	0	11	27	10	0	11

[a]Yeast extract added to growth medium.

Microaerophilic degradation of sodium hexadecyl sulfate, graphed in Figure 3A, followed a similar pattern to that under aerobic conditions (5) except that changes were much more gradual. Reduction of MBAS to 1.2 ppm required 7 days and a small amount persisted throughout the test, whereas under aerobic conditions it was zero in 2 days. Likewise, formation of sulfate, loss of carbon and increase in turbidity were relatively slow. Results with sulfated 2-hydroxyethyl palmitamide (Fig. 3B) and sulfated 2-hydroxypropyl stearamide (Fig. 3C) show a gradual loss of MBAS and carbon but no significant formation of sulfate ion. The low turbidity values indicated that if hydrolysis occurred it was at approximately the same rate as the degradation. Figure 3D shows that degradation of sulfated 2-(2-hydroxyethoxy)ethyl stearamide was faster than the other sulfated alkanolamides. No sulfate ion was formed. The small amount of turbidity was probably due to bacterial cell growth. Hydrolysis was not more rapid than degradation.

Experiments were conducted to determine the effect of sewage seed on detergent degradation in river water at very low or zero oxygen concentration. Water from the Schuylkill River in Fairmount Park, Philadelphia, was allowed to stand overnight, filtered through glass wool and autoclaved for 30 min at 122 C. After the water cooled in an atmosphere of nitrogen, detergents were added to 2 liter portions to make 10 ppm solutions. These were inoculated with 10 mg/liter of anaerobic sewage sludge and stirred with magnetic stirrers at 35 C in an atmosphere of nitrogen. The loss of MBAS is shown in Figure 4A. MBAS decreased to zero within 4 days except for LAS which did not degrade. The results of a repetition of the experiment at 25 C are shown in Figure 4B. MBAS values again decreased to almost zero in 4 days except for sulfated 2-(2-hydroxyethoxy)ethyl stearamide which became zero between days 4 and 7.

The results indicate that microorganisms from the anaerobic digester of a sewage plant rapidly reduce the MBAS of sulfated alkanolamides in sterilized river water and that the nutrient medium we have used may not be optimum for their activity under these conditions.

Biodegradation in Soil

Figure 5 shows the results of effluent analysis for MBAS of lysimeters used in the treatment of 20 ppm detergent solutions. Effluents from the fat-based detergents showed no methylene blue activity but LAS reached 2.5 ppm in 15 days.

ACKNOWLEDGMENTS

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