Biological Behavior of Some Soap-Based Detergents

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ABSTRACT

The biodegradability of tallow soap, three soap-based detergent formulations and their component lime soap dispersing agents—sodium methyl α-sulfotallowate, sulfated N-(2-hydroxypropyl) tallowamide, and sodium N-methyl N-(2-sulfoethyl) tallowamide—was determined under aerobic and microaerophilic conditions. Both sewage and river water microorganisms were used as the sources of inoculum. The course of biodegradation was followed by loss of carbon and methylene blue active substance, and by increase in turbidity and surface tension. Carbon analysis for soap in solutions containing Ca^{++} and Mg^{++}, which would precipitate soap, was performed by an improved technique using the disodium salt of ethylenediamine tetraacetic acid. Invariably a decrease in carbon content was accompanied by an increase in turbidity and surface tension. Also, loss in methylene blue active substance was concurrent with an increase in turbidity and surface tension of the degrading solutions of the detergent. Soap cannot be determined as methylene blue active substance because of the low pH of the test. Soap and the built soap formulations degraded under aerobic and microaerophilic conditions. Preliminary toxicity data upon mammals and fish indicated that the soap-based detergents are as safe as conventional commercial detergents.

INTRODUCTION

Tallow soap is an excellent detergent in soft or moderately hard water but is limited in usefulness because of its sensitivity to calcium and magnesium ions present in hard water. In recent work by this laboratory (1,2), it has been demonstrated that phosphate free soap-based detergents can be formulated with tallow soap to which lime soap dispersing agents (LSDA) have been added. The detergency performance of these detergents was enhanced by the addition of inorganic builders, such as glassy sodium silicates. Such formulations were found to have detergency properties comparable to those of phosphate built commercial detergents when tested over a wide range of water hardness and temperature. The LSDA used most extensively in these formulation studies were sodium methyl α-sulfotallowate (TMS), sulfated N-(2-hydroxypropyl) tallowamide (TAM), and sodium N-methyl N-(2-sulfoethyl) tallowamide (IGT). Since the main thrust of this type of detergent research was environmental safety, the ease of biodegradation of these three LSDA was confirmed under aerobic and microaerophilic conditions (3-5). Although soap has been thought of as being highly biodegradable, no proof for this hypothesis had been furnished until Loehr (6) showed that the sodium and corresponding calcium soaps of fatty acids through C_{18} degrade aerobically as long as the insoluble particles are divided finely. The biodegradation of a soap-based formulation cannot necessarily be predicted on the basis of the ease of degradation of its components. Therefore, this article is concerned with the measurement of the ease of biodegradation of formulated soap-based mixtures, as well as with the determination of the fate of soap under a variety of conditions. In addition, the formulations also were screened for toxicity to mammals and to fish.

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EXPERIMENTAL PROCEDURES

Materials

The preparation and properties of TAM, $CH_3(CH_2)_{16}CONHCH_2CH(CH_3)OSO_3Na$, and IGT, $CH_3(CH_2)_{16}CON(CH_3)CH_2CH_2SO_3Na$, have been described by Weil, et al., (7) and Bistline, et al., (8) respectively. TMS, $CH_3(CH_2)_{15}CH(SO_3Na)COOCH_3$, was purchased as Bioterge TMS (Stepan Chemical Co., Northbrook, Ill.), and tallow soap was purchased as Naminco 42 (National Milling Corp., Philadelphia, Pa.). For ease of reference the soap-based formulations are coded by numbers with the incorporated LSDA shown in parentheses as follows: no. 1 (TMS), no. 2 (TAM), and no. 3 (IGT). Each formulation contained 62% soap and 21% LSDA, 16% sodium silicate (SiO_2/Na_2O = 1.6), 1% carboxymethylcellulose (CMC), and a small amount of brightener. The linear alkylbenzenesulfonate (LAS) ingredient used as a reference material throughout this study was separated from inorganic salts by extraction with absolute ethanol from a commercial sample of LAS (mol wt 350).

Biological Methods

Aerobic: Biodegradation was determined at 25 C by the Esso controlled nutrient procedure (9) in which essential

TABLE I

Composition of Nutrient Stock Solution

Stock solution	Composition	g	Deionized water to make
No. 1	$Mg(NO_3)_2 \cdot 6H_2O$	5.375	1 liter
	$Ca(NO_3)_2 \cdot 4H_2O$	2.925	
	$Fe(NO_3)_3 \cdot 9H_2O$	0.375	
	$CoCl_2 \cdot 6H_2O$	1.025	
No. 2	$(NH_4)_2HPO_4$	4.76	1 liter
	K_2HPO_4	36.6	
	KH_2PO_4	31.1	
No. 3	KH_2PO_4	40	1 liter
No. 4	Difco nutrient broth	10	200 ml[a]
	Dextrose	10	
	$NaNO_3 \cdot H_2O$	12.5	

[a]Measure 5 ml aliquot portions into screw cap test tubes and sterilize in autoclave at 15 psi for 15 min to keep from spoiling.

TABLE II

Carbon Analysis of Soap, Soap-Based Detergents, and LAS[a] Solutions after 14 Days of Aerobic Biodegradation

Formula	Time	Soap, ppm		LSDA,[b] ppm		Total DOC[c] found, ppm	Soap % reduction
		Theory	Calculated	Theory	Calculated		
1	Start	20.96	15	5.46	5.0	20.0	
	14 days		0.5		1.8	2.5	97
2	Start	19.88	14.8	6.48	5.7	20.5	
	14 days		0.5		1.3	1.5	97
3	Start	20.96	14.6	5.84	3.7	18.3	
	14 days		3.0		0.8	3.5	80
Tallow soap	Start	27.98	20.5			20.5	
	14 days		0.5			0.5	98
LAS	Start	24.8	22.4			22.4	
	14 days		9.8			9.8	56

[a]LAS = linear alkylbenzenesulfonate.
[b]LSDA = lime soap dispersing agent.
[c]DOC = dissolved organic carbon.

TABLE III

Biodegradability by Oxygen Depletion (11)

Test sample	Total amount of oxygen in mg/liter consumed as measured on day			
	2	5	9	15
Sodium tallowate	3.8	4.1	4.8	5.7
LAS[a]	0.1	1.6	3.1	3.9
Formulation no. 1	2.9	3.5	3.9	4.3
Formulation no. 2	2.7	3.2	3.6	3.9
Formulation no. 3	2.7	3.1	3.7	4.2

[a]LAS = linear alkylbenzenesulfonate.

TABLE IV

Biodegradability of Lime Soap Dispersing Agents by the Presumptive Soap and Detergents Association Method (11)

Lime soap dispersing agent[a]	Percent degradation after 7 days
TMS	100
TAM	100
IGT	100
LAS	92

[a]TMS = sodium methyl α-sulfotallowate, TAM = sulfated N-(2-hydroxypropyl) tallowamide, IGT = sodium N-methyl N-(2-sulfoethyl) tallowamide, and LAS = linear alkylbenzenesulfonate.

TABLE V

Biodegradation of Soap and Soap-Based Detergents Solutions under Microaerophilic Conditions at 35 C

Formula	Time days	ST[a]	MBAS[b] ppm	
			Found	Theory
Na tallowate	0	62.0		
	3	68.0		
No. 1	0	55.0	2.3	2.5
	20	68.0	0	
No. 2	0	43.6	2.6	2.9
	3	68.5	0.2[c]	
No. 3	0	39.9	2.3	2.5
	6	68.0	0.3[c]	

[a]ST = surface tension.
[b]MBAS = methylene blue active substance.
[c]Zero MBAS 10 days.

minerals (Table I) were supplied, and the test detergent at 40 ppm was the sole source of carbon and energy. Stock solution no. 1 (1 ml) and 5 ml of stock solution no. 2 were added/liter of medium. Activated sludge from a local sewage plant that treats mainly domestic sewage was maintained in a fill and draw aerator following the Soap and Detergents Association Procedure (10). However, 5 ml each of nutrient stock solutions no. 1, 3, and 4 (Table I), which are devoid of sulfates, were used daily as synthetic sewage to maintain the culture. Before use, the culture was acclimated to the above synthetic sewage for 1 week and maintained thereafter for 6 months. Sludge (10 mg/liter, dry wt basis) from the fill and draw aerator was used as the inoculum. The course of biodegradation was followed by the measurement of the loss of carbon and methylene blue active substance (MBAS), as well as by the determination of the increase in turbidity and surface tension. The results are summarized graphically in Figures 1-3. The results of the carbon analysis are shown in Table II.

Biodegradation of the formulations and of lime soap dispersants was also determined by the oxygen depletion procedure of Dias and Alexander (11), in which each test solution initially was adjusted to a total carbon content of 2 mg/liter. Dissolved oxygen was measured by the azide modification of the iodometric method (12). The results are given in Table III. In addition, the biodegradability of the individual LSDA was determined by the conventional presumptive test of the Soap and Detergents Association (10) which is not strictly applicable to soap, since, under the acidic pH conditions of the MBAS analysis, soap is converted to fatty acid. The results of the presumptive tests are shown in Table IV.

Microaerophilic: Water from the Schuylkill River at Philadelphia was allowed to stand for 6 hr, filtered through glass wool, and maintained under an atmosphere of N_2 overnight before use. Water hardness ranged from 180-200 ppm (as $CaCO_3$). Biodegradation of 10 ppm of test material was determined in the dark at 25 C and 35 C in 4 liter aspirator bottles containing 2 liters media. The contents were stirred with magnetic stirrers, and nitrogen was passed into the vented bottles at the rate of 50-100 ml/min (4,5). The results of these tests are given in Table V.

Toxicological evaluations: Formulations no. 1 and 2 were subjected to the following tests: acute toxicity (LD_{50}) in mice, rabbit skin, and eye irritation, sensitization, and fish toxicity (TLm).

Acute toxicity: Young adult mice (Swiss-Webster strain of both sexes) were used for the LD_{50} tests. The test material was administered by stomach tube in single doses/animal, food having been withheld for 18 hr prior to

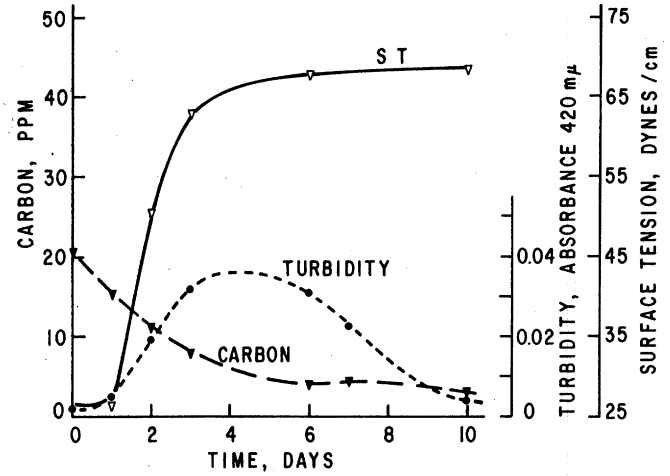

FIG. 1. Biodegradation of sodium tallowate under aerobic conditions in a controlled nutrient medium at 25 C inoculated with microorganisms from a continuously operated fill and draw aerator. ST = surface tension.

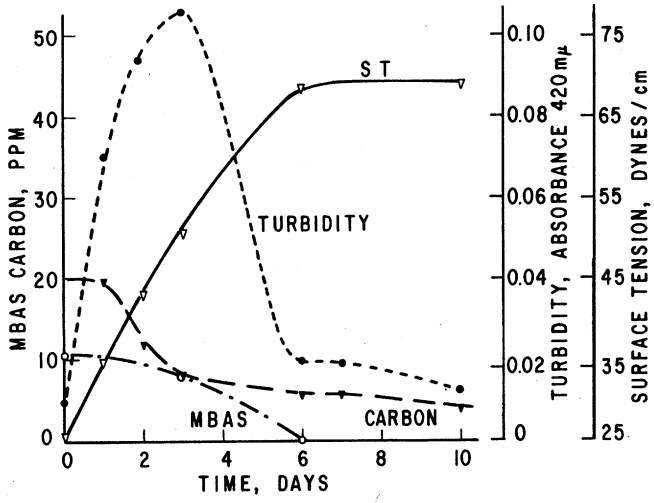

FIG. 2. Biodegradation of formulation no. 1 under aerobic conditions in a controlled nutrient medium at 25 C inoculated with microorganisms from a continuously operated fill and draw aerator. ST = surface tension and MBAS = methylene blue active substances.

dosage.

Skin irritation: Patch tests were applied to the clipped skin of young adult New Zealand male or female rabbits which were immobilized during the 24 hr period of exposure to the test materials. Each material was applied to an area of ca. 1 sq. in. of skin surface in the form of a thick paste (0.5 g solids plus few drops of water). The paste was covered by applying a 2 sq. in. gauze pad which was 2 layers in thickness and secured by surgical tape around the edges. Then a rubberized cloth was wrapped completely around the body between the front and rear legs to hold the patches tightly against the skin. Both abraded and nonabraded skin areas were used. After a 24 hr period of exposure, the patches with the material were removed and the resulting reactions were evaluated on the basis of a scoring system described by Draize (13).

Eye irritation: The same rabbits involved in the skin test also were used to evaluate the effects of the detergents when either 0.1 ml 10% suspension in water or 0.1 ml dry powders were instilled directly into the conjunctival sac of the eye. The scoring system used to evaluate the degree of irritation was that described by Draize (13).

Sensitization: The possible allergic properties of the two detergents were investigated using the standard guinea pig intracutaneous injection technique. Two weeks after a series of 10 injections of 0.1 ml 0.1% solution/injection site, a retest injection was made and read for evidence of enhanced irritation and an edema formation.

Fish toxicity: The detergents were tested for toxicity to the fish *Gasterosteus aculeatus* or Stickleback, a fish which lives in both brackish and fresh water. Ca. 200 fish were used in testing each product (12).

In these tests, 5 individuals were placed in each 5 liter volume of test solution. Each tank continuously was oxygenated by bubbling an excess of pure oxygen through the solution.

Dead fish were removed as deaths occurred during the work day, and again at 8:30 the following morning. Deaths were recorded at 24 hr intervals for not less than 96 hr and for as long as 144 hr. Water temperature was steady at 72 F; pH was ca. 6.7. No buffers were added, and the fish were not fed for 24 hr prior to nor during the tests. Control fish were maintained in similar tanks and in large holding tanks at the same temperature. Deaths of controls never exceeded 2% during the course of the tests.

Analytical Methods

Carbon and turbidity: Since soap is inherently sensitive to the Ca, Mg, and other polyvalent ions present in the nutrient salt solutions, as well as in the river water, precipitates are formed that would be centrifuged out during the clarification step required to remove particulate matter before carbon analysis. To prevent such precipitation, a technique was developed using the disodium salt of ethylenediamine tetraacetic acid (Na_2EDTA) under highly alkaline conditions. A correction for the carbon content in Na_2EDTA was made by a blank determination using the biodegradation medium. The treated solution then was used for both carbon and turbidity determinations.

Solutions were prepared for analysis as follows: 50 ml of test solution were pipetted into a 150 ml beaker and made strongly alkaline by the addition of 1 drop of 18N NaOH. Fifty μliters 2% Na_2EDTA then were added, and the solution was heated to clearing (80-90 C). For turbidity determinations, a 25 ml portion of the hot solution was pipetted into a 50 ml volumetric flask, diluted to volume with deionized water and mixed thoroughly with a magnetic stirring bar. The solutions were kept warm at ca. 80 C on the steam bath until analyzed. Turbidity measurements of the hot solutions were made at 420 nm against a blank nutrient salts solution containing all the reagents except the test detergent.

For dissolved organic carbon analysis, the remaining aliquot of treated solution was heated to ca. 80 C, weighed, and centrifuged at 4500 rpm (1760 Gs) for 5 min to remove particulate matter, including cells of microorganisms. The supernatant liquid was kept warm, made strongly acidic (pH paper) with a drop of conc HCl, purged with N_2 for 5 min to remove CO_2, then made strongly alkaline (pH paper) with ca. a drop of 18N NaOH, capped, and kept warm until analyzed. Carbon analyses were performed with a Beckman carbonaceous analyzer (3). Sensitivity was increased by standardizing the instrument so that a 20 μliter sample of 50 ppm sodium oxalate gave a full scale peak height of 100 ppm on the recorder scale.

MBAS and surface tension: MBAS was determined with a Technicon auto analyzer (14) and surface tensions with a du Noüy tensiometer at room temperature.

RESULTS AND DISCUSSION

Effect of EDTA upon Soap Solutions

The sequestering effect of Na_2EDTA upon soap solutions containing polyvalent ions is shown in Table VI. At pH 12.0, the soap solution alone in deionized water had an absorbance of 0.05. However, when the nutrient salts were

TABLE VI

Effect of 2% Na_2EDTA[a] Solution upon the Turbidity of 40 ppm Sodium Tallowate Solution in the Presence of Polyvalent Ions

Solutions	pH	Absorbance at 420 nm
Soap solution alone	7.9	0.074
	12.0	0.050
Nutrient salts added	12.0	0.161
Nutrient salts + 50 mμ 2% Na_2EDTA	11.8-12.0	0.043-0.047

[a]EDTA = ethylenediamine tetraacetic acid.

TABLE VII

Analysis of Solutions Showing Recovery of EDTA[a] and Tallow Soap[b]

Solution	Carbon, ppm		Average % recovery
	Calculated	Found	
H_2O + EDTA	6.5	6.5	100
1 ppm soap + EDTA	0.69	0.5	72.5
40 ppm soap	27.98	16.0	57.3
40 ppm soap + EDTA	27.98	20.5	73.5

[a]EDTA = ethylenediamine tetraacetic acid.
[b]Results based upon 4 determinations.

added, the absorbance increased about 3 times to 0.161, whereas the addition of Na_2EDTA reduced the absorbance to a value slightly less than that for soap alone, indicating complete sequestering of cations present.

Aerobic Biodegradation

Esso controlled nutrient procedure: Figures 1 and 2 show respectively the course of biodegradation for tallow soap and formulation no. 1 containing TMS. Maximum turbidity (bacterial growth) and surface tension values closely followed each other (3-6 days). The carbon content values dropped rapidly until maximum surface tension values were reached, then gradually tapered off to 0.5 ppm for soap and 2.6 ppm for no. 1 (Fig. 2) after 14 days. Carbon values will be discussed in greater detail later. The correlation between loss of MBAS in no. 1 and increase in surface tension is clearly evident. Graphs for formulations nos. 2 and 3 containing TAM and IGT, respectively, were so similar to that for no. 1 that they were omitted.

Figure 3 shows the course of biodegradation for LAS. Reduction of MBAS corresponded to increase in surface tension and turbidity as observed in Figures 1 and 2. The MBAS dropped rapidly in 3 days to 4 ppm and thereafter tapered off to 3 ppm in 14 days. This unusually short acclimation time may be due to the greater initial activity of the inoculum from laboratory acclimated sewage after

TABLE VIII

Analysis of LSDA Solutions used for Calculating Values Shown in Table II

LSDA[a]	Carbon, ppm		14 Days
	Theory	Start	
TMS	22.8	21.0 (92%)	7.6 (36%)
TAM	22.7	20.0 (88%)	4.5 (22%)
IGT	21.2	13.3 (63%)	3.0 (22%)

[a]LSDA = lime soap dispersing agent, TMS = sodium methyl α-sulfotallowate, TAM = sulfated N-(2-hydroxypropyl) tallowamide, and IGT = sodium N-methyl N-(2-sulfoethyl) tallowamide.

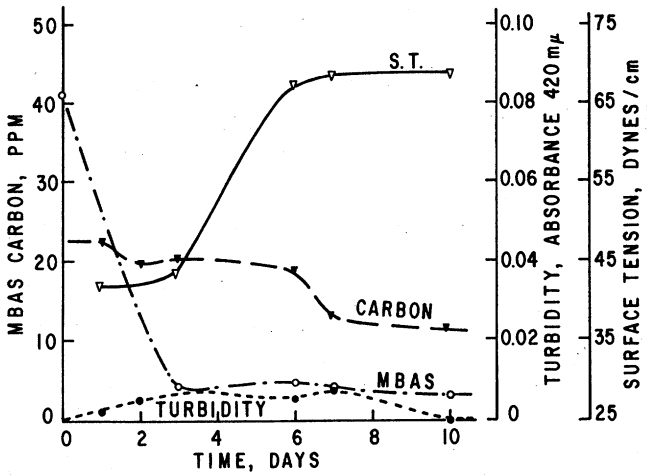

FIG. 3. Biodegradation of linear alkylbenzenesulfonate under aerobic conditions in a controlled nutrient medium at 25 C inoculated with microorganisms from a continuously operated fill and draw aerator. ST = surface tension.

several months' operation of the fill and draw aerator. LAS, in our experience, degrades more gradually in 7-10 days (3,4) when the inoculum from fresh activated sludge is used after 1 week's acclimation to synthetic sewage. A significant feature of this study is the relatively small change in carbon analyses, even though the surface activity disappeared in 6 days. Thus, it would appear that the disappearance of MBAS is not a reliable index of ultimate biodegradability.

On the other hand, the LSDA of the soap formulations (3,4), as well as soap itself, degraded easily with both fresh inoculum (3,4) and inoculum from the continuously operated fill and draw aerator, indicating the ease of acclimatization and assimilation by the microorganisms for these tallow-based surfactants. Table II gives the carbon content of the formulations, soap alone and LAS at the beginning and end of a 14 day degradation period.

Tables VII and VIII, respectively, provide data for the control system and data for individual LSDA used in calculating the breakdown of components in Table II. The carbon values for the LSDA alone (Table II, column 6) were obtained from data supplied in Table VIII and subtracted from the total dissolved organic carbon content (column 7) to obtain the calculated carbon values assumed to be due to soap only (column 4). Carbon reduction of soap in formulas 1 and 2 equaled that for soap alone, whereas the carbon reduction in formulation 3 was somewhat less than that for the other two formulations. Thus, the nature of the LSDA conceivably might have some effect upon the biodegradation of soap. Significant, however, is the fact that soap alone is removed to a minimum of 97-98% either by itself or in formulations 1 and 2. All three formulated products were removed efficiently to an extent of 80% or above. LAS, on the other hand, under the same bacteriological conditions as the tallow-based detergents had a carbon reduction of only 56%. Even when fresh inoculum was used, the average reduction of carbon in previous experiments was only 82% in 14 days (3,4).

Oxygen depletion: Table III lists the oxygen consumption for each formulation, as well as for LAS and tallow soap. The amount and rate of oxygen depletion indicate the ease of biodegradability. In this study, since the carbon content of each test solution was adjusted to 2 mg/liter, the theoretical oxygen consumption is 7.5-7.7 mg/liter provided certain interferences can be ruled out. Unfortunately the situation is complicated by oxygen uptake by the microorganism cells and by nitrification. Although a blank corresponding to the oxygen consumption by the sludge microorganisms has been subtracted from the observed

data, it must be borne in mind that these biological data are not very precise.

The rate of oxygen consumption shown in Table III is more important than absolute consumption values. Thus, it will be observed, that all materials, except LAS, showed a substantial oxygen uptake after two days corresponding to a biodgradation of 36-50%. It required 9 days before LAS attained a similar level of biodegradation. After the fifth day the rate of biodegradation generally slowed down, and the significance of the observations thereafter is somewhat doubtful.

Presumptive test: In the presumptive test, microorganisms were acclimated to each test sample at a concentration of 30 ppm for 72 hr. Following two adaptive transfers, biodegradation was determined by the amount of MBAS reduction during the test period of 7-8 days. Table IV lists the percent biodegradation after 7 days. However, the table does not tell the entire story, because each of the LSDA, TMS, TAM, and IGT, degraded completely during the acclimatizing period. After the 7 day test period, LAS had degraded to the extent of 92%. Since a minimum 90% degradation is considered adequate by the test, it can be concluded that the LSDA used in the formulations had more than adequate biodegradability.

Microaerophilic Biodegradation

Since biodegradation is more difficult and slower under microaerophilic conditions, experiments were performed at 10 ppm concentrations of test materials to detect differences in biodegradation rates. Very little difference in degradation was observed between tests run at 25 and 35 C, except for no. 1 (TMS) which does not degrade easily at 25 C. Table V shows the analysis for surface tension and MBAS at 3 stages of biodegradation at 35 C: (A) 0 time, (B) complete loss of surface activity, and (C) loss of MBAS. The effectiveness of the LSDA in controlling the river water hardness is shown by the lower initial surface tension values of the formulations (10 ppm) compared to that of soap alone (10 ppm). Loss of surface activity was complete in 3-6 days for all except no. 1 (20 days). For all practical purposes, nos. 2 and 3 degraded to 0 MBAS in 3-6 days.

Previous work (4) indicated that sterile river water inoculated with anaerobic sewage sludge rapidly reduced the MBAS of sulfated alkanolamides. We, therefore, adapted the use of sterile river water to the degradation of the formulations using anaerobic sludge as the inoculum. Under these conditions, both no. 2 (TAM) and no. 3 (IGT) degraded to 0 MBAS easily at 25 and 35 C. Apparently, the anaerobic sludge microorganisms adapted readily to TAM and IGT and easily assimilated them at either temperature. On the other hand, the anaerobic microorganisms per se do not appear to assimilate TMS even at 35 C. Earlier work (15) showed that, with anaerobic sludge and high concentrations of sodium methyl α-sulfostearate, no degradation occurred, but the compound was not toxic to the microorganisms. However, in river water, facultative microorganisms under microaerophilic conditions appear to adapt more readily to TMS than microorganisms from the anaerobic sludge, because biodegradation in river water at 35 C usually was complete. In fact, biodegradation to 0 MBAS sometimes occurred even at 25 C.

Toxicological Evaluation for Formulations 1 and 2

Acute toxicity: The mouse LD_{50} dosage for the formulations (5-8 g/kg body wt) was ca. double that for a commercial phosphate built LAS containing detergent (2.5-3.5 g/kg body wt), indicating that nos. 1 and 2 are less toxic than this control detergent. The reverse appears to be true with the fish TLm test where the dosage ranged from 6.5-9 ppm for the formulations against 14 ppm for the control. However, it appears likely that sodium tripolyphosphate is less toxic to fish than either soap or surfactants. Both formulations were either degraded, adsorbed, or otherwise removed from the test solution by the fish. This was shown by removal of the dead fish and then, after 96 hr, the live fish and then introduction of new test fish into the used test solution. When solutions above the TLm concentrations were so reused (or retested), there were no deaths among the second batch of fish. The mechanism for this detoxification was not examined.

Skin irritation: The soap-based formulations, when tested on rabbits, produced a slight to well defined erythema but no edema, similar to control. The primary irritation index for both materials was less than 2, which is described as mildly irritating.

Eye irritation: Solutions (10%) produced a slight irritation, while 0.1 ml dry powders produced a severe irritation that was reversible. The control was initially more severe but also reversible.

Sensitization: During a 2 week period, intracutaneous injection of 10 0.1 ml 0.1% solutions produced negative results.

None of the various toxicological tests showed effects greater than those anticipated and were of the same order of magnitude as those observed in parallel studies with a leading commercial detergent built with phosphates.

ACKNOWLEDGMENTS

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REFERENCES

1. Bistline, R.G., Jr., W.R. Noble, J.K. Weil, and W.M. Linfield, JAOCS 49:63 (1972).
2. Noble, W.R., R.G. Bistline, Jr., and W.M. Linfield, Soap Cosm. Chem. Spec. 48:38 (1972).
3. Cordon, T.C., E.W. Maurer, and A.J. Stirton, JAOCS 47:203 (1970).
4. Cordon, T.C., E.W. Maurer, and A.J. Stirton, Ibid. 49:174 (1972).
5. Maurer, E.W., T.C. Cordon, and A.J. Stirton, Ibid. 48:163 (1971).
6. Loehr, R.C., and J.C. Roth, J. Water Pollut. Contr. Fed. 40:R385 (1968).
7. Weil, J.K., N. Parris, and A.J. Stirton, JAOCS 47:91 (1970).
8. Bistline, R.G., Jr., E.S. Rothman, S. Serota, A.J. Stirton, and A.N. Wrigley, Ibid. 48:657 (1971).
9. Konecky, M.S., R.J. Kelly, J.M. Symons, and P.L. McCarty, Paper presented at the 36th Annual Meeting, Water Pollution Control Federation, Seattle, Wash., October 1963.
10. Soap and Detergents Association, JAOCS 42:986 (1965).
11. Dias, F.F., and M. Alexander, Appl. Microbiol. 22:1114 (1971).
12. American Public Health Association, "Standard Methods for the Examination of Water and Wastewater," Thirteenth Edition, American Public Health Association, Washington, D.C., 1971, p. 477,562.
13. Draize, J.H., in "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics," Association of Food and Drug Officials of the U.S., Topeka, Kans., 1959, pp. 46-59.
14. Technicon Instruments Corp., "Instruction Manual," Technicon Instruments Corp., Ardsley, N.Y., 1963.
15. Maurer, E.W., T.C. Cordon, J.K. Weil, M.V. Nuñez-Ponzoa, W.C. Ault, and A.J. Stirton, JAOCS 42:189 (1965).