Biodegradation of Some Sulfur Analogues of Sodium *p*-(*n*-Dodecyl) Benzenesulfonates

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ABSTRACT

The biodegradability of a novel class of thia derivatives of sodium *p*-(*n*-dodecyl) benzenesulfonate and of the corresponding sulfoxides and sulfones has been compared using the Shake Flask, River-Die-away, Aeration Chamber Die-away, and Warburg respirometric techniques. These studies have shown that the incorporation of the sulfur atom into the alkyl side chain of the linear alkylate sulfonate detergent molecule has failed to shorten the time required to biodegrade the linear alkylate sulfonate structure. The 7–9-thia derivatives, the 7–9-sulfoxides, and the 7–9-sulfones are rated as bioresistant compounds when using the Shake Flask and River Die-away tests, but, when testing these resistant isomers in synthetic sewage using a batch type Aeration Chamber Die-away procedure, all 11 thia compounds are found to be biodegradable. Some of the isomers do not appear to acclimatize sufficiently rapidly to the bacteria under the conditions of the Shake Flask or River Die-away tests. Under the more practical treatment conditions when using the Aeration Chamber Die-away procedure inoculated with activated sludge, a high order of biodegradability is obtained. The probable metabolic pathway and causes for variation in the biodegradation results obtained with these isomers are discussed.

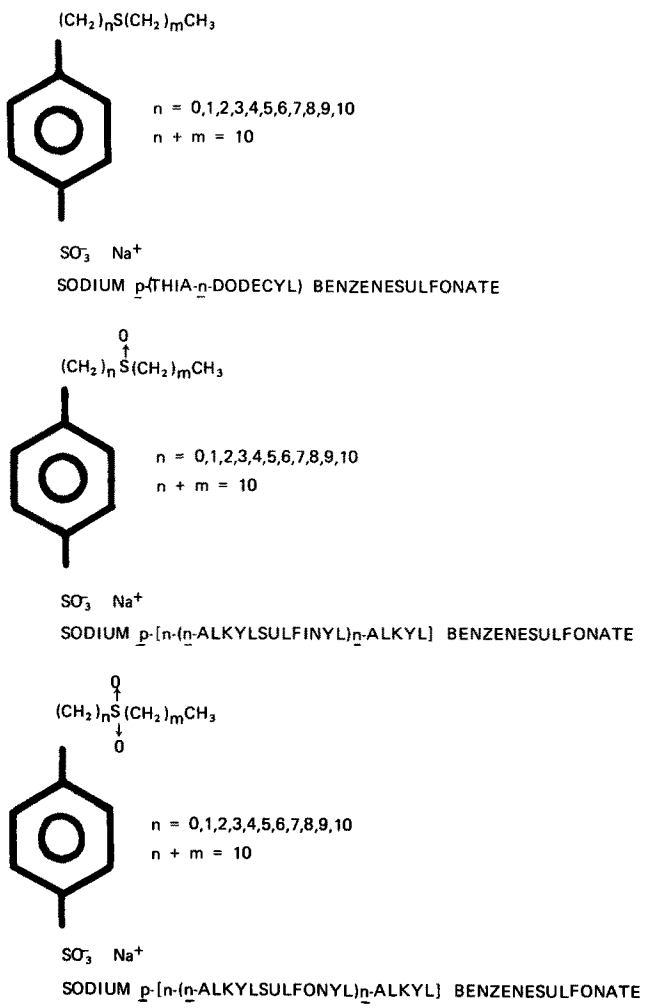

FIG. 1. Sulfur substituted sodium *p*-(*n*-dodecyl) benzenesulfonates.

INTRODUCTION

A Department of Defense Standardization Program (1) on cleansing and polishing compounds revealed a list of 22 federal and military specifications which included detergents in the various formulations. In an attempt to improve the ability of these products to meet special and general military requirements, a novel class of thia derivatives of sodium *p*-(*n*-dodecyl) benzenesulfonates and the corresponding sulfoxides and sulfones were synthesized (2-5) for study and possible use in the various formulations.

In all, a total of 39 compounds were synthesized by successively replacing the different methylene groups in the alkyl side chain by sulfur bearing groups, as detailed in this investigation.

This article summarizes studies carried out to determine the effect of addition of sulfur into the alkyl side chains of alkyl benzenesulfonates upon the biodegradability of the compounds as determined through Shake Flask, River Die-away, Aeration Chamber Die-away, and Warburg respirometric techniques. This information was essential to the overall evaluation of possible utility of these compounds.

MATERIALS AND METHODS

A series of thia derivatives of sodium *p*-(*n*-dodecyl) benzenesulfonate and the corresponding sulfoxides and sulfones, as shown in Figure 1, were synthesized for study. In addition, as a means of increasing the solubility of the compounds, an isomer of the sulfone analogues was prepared in which the benzene ring was located on carbon-2 of the aliphatic chain. Synthesis of these compounds has been described previously by Long, et al. (2-5).

The control test detergent used in these studies was dodecene-1 derived reference linear alkylate sulfonate (LAS) no. 2 (November 1964) obtained from the Soap and Detergent Association (SDA). In the course of these studies, pure sodium *p*-(*n*-dodecyl) benzenesulfonate and 2-phenyl C_{12} LAS were synthesized at these laboratories and also were used in some of the tests as a control standard for biodegradation.

Biodegration test: The first biodegradation method selected to evaluate the series of test surfactants was the River Die-away test technique (6,7). The static River Die-away test gives the rate of disappearance, as well as the degree of ultimate disappearance, normally obtained with an activated sludge unit or trickling filter or in a stream or river receiving the effluent from a sewage disposal plant. The advantages and disadvantages of this test procedure have been reported previously in the literature (8-10).

In these studies, 8 oz. screw cap bottles were prepared containing 80 ml Sudbury River (Massachusetts) water and 20 ml detergent solution, so that the final volume contained 5-20 mg/liter of the test detergents. A magnetic stirring bar was placed in each jar, the jar lids were fitted loosely, and the solutions incubated statically at 37 C or at room temperature for 30 days.

At sampling intervals, ordinarily daily, the test solutions were mixed thoroughly for 1 min by means of a magnetic stirrer and an appropriate aliquot removed for analysis by the standard methylene blue colorimetric method (11), which is used for the purpose of measuring biodegradability. This method depends upon the formation of a blue colored salt when methylene blue reacts with anionic detergents, which include not only LAS but also alkyl sulfates. Thus, the materials determined by this method often are designated methylene blue-active substances (MBAS). The chloroform-soluble salt will vary in color density in proportion to its concentration in the solvent. A spectrophotometer is used to make the necessary color readings at a wavelength of 652 nm.

Although the biodegradation data on the River Die-away can be used as a measure or index of the disappearance of the detergent over the test period, the Shake Flask (12) method has been found to be more reliable and was, therefore, adopted by SDA as the presumptive part of its standard test procedure for determining the biodegradability of alkyl benzenesulfonate (ABS) and LAS surfactants (13). The basal medium used in the Shake Flask method consists of mineral salts, yeast extract, and distilled water. The surfactant was added to each shake flask containing the basal medium at 20-30 mg/liter prior to autoclaving. After sterilization and cooling, the flasks were inoculated with 10 ml/liter of a 72 hr old adapted mixed culture originally obtained from an activated sludge recycle line of an activated sludge treatment plant. Normally, the culture was passed through the Shake Flask medium containing the surfactant under study for two 72 hr adaption periods before use in the actual degradation experiment. In the actual tests, 500 ml medium were placed in 1500 and 2000 ml Erlenmeyer flasks, inoculated, and incubated on a reciprocating shaker at 25 C.

Warburg tests: The culture used for the Warburg investigation was a stable mixed culture originally obtained from an activated sludge type waste treatment plant located in Enid, Okla. as specified in the SDA Presumptive Test. The culture was maintained in the Shake Flask medium containing 30 mg/liter SDA LAS no. 2.

The culture was acclimated and grown in a plexiglass aeration chamber (as specified in B2.1 of the Confirming Test, [13]) at room temperature using a synthetic sewage described by McKinney and Donovan (14) to which was added 75 mg/liter detergent being tested. The medium was aerated at the rate of 1 cu ft/hr. Fifty ml stock culture was used to inoculate the system. The system was allowed to incubate without any further addition or withdrawal of medium until the methylene blue analysis showed 95% biodegradation of the test detergent was complete, and then an 80 ml sample was withdrawn from the aeration chamber, centrifuged at 1000 rpm for 20 min, and washed 4 times in Krebs-Ringer solution (15). After the final resuspension, a 2 ml suspension was used to determine the dry wt of the bacterial suspension. Dry wt ranged between ca. 11-16 mg/ml. The final suspension was standardized by adjusting to 45% transmission at 420 nm in a spectrophotometer. Two ml microbial suspension was dispensed into the main compartment of each Warburg flask. The endogenous flasks received 1 ml distilled water and the other half 1 ml 150 mg/liter surfactant solution (to give a final concentration of 50 mg/liter). The flasks then were placed on the manometers and incubated at 30 C. The manometers were read every hr for the first 5 hr. The manometers then were readjusted to operate overnight without attention. On the following day, readings were continued at hourly intervals through the thirtieth hr of incubation. The final recorded readings were corrected for endogenous respiration and are plotted in Figure 3.

RESULTS

Thia compounds: Table I shows the comparison of the breakdown of the thia derivatives using both the Shake Flask and the River Die-away methods. In these tests, the SDA dodecene-1 derived reference LAS no. 2 (November 1964) and the pure sodium *p*-(*n*-dodecyl) benzenesulfonate synthesized at these laboratories were used as control standards for biodegradability comparison. The results show that the 1-thia through 4-thia degrade slightly faster by both test methods than the SDA LAS no. 2 reference standard but do not degrade any more rapidly than the parent 1-or 2-phenyl nonsulfur LAS controls. The 5-thia appears to be degraded slightly slower than the 6-thia by the Shake Flask method but ca. equal in degradation with the River Die-away method. However, as the sulfur is moved further out the chain (7-thia through 9-thia) the degradation becomes slower. However, at the 10-thia and the 11-thia, a reversal in the trend takes place, and the degradation of these compounds is increased when monitored by the River Die-away method. Unfortunately, this observation was not observed in the Shake flask procedure.

The 4-, 6-di thia and the 3-, 5-di thia also were evaluated and found to be biodegraded rapidly.

Sulfoxides: The results of the Shake Flask and River-Die away tests for the sulfoxides (Table II) revealed a biodegradation pattern similar to that shown by the thia compounds. Between 1-8 days were required for the 1–5-sulfoxides to degrade 90% or more. Although the Shake Flask results showed more than 95% biodegradation of the 1-sulfoxide after only 1 day, this rapid biodegradation was not confirmed by the River Die-away tests.

Subsequent Shake Flask runs showed that the 1 day biodegradation of this compound could not be repeated. Several days were required to achieve more than 90% degradation, and once degradation was complete, adding an additional charge of 20-30 mg/liter 1-sulfoxide to the same flasks resulted in 95% biodegradation in 24 hr. The 6–9-sulfoxides were rated as only partially degraded while the 10- and 11-sulfoxides required 3-23 days to biodegrade. Since there was no dramatic increase in the biodegradation of the sulfoxides, it tentatively was concluded that the introduction of the sulfoxide group in the alkyl chain did not appear to enhance biodegradation by permitting an alternate point of attack where the sulfoxide group was located in the alkyl chain.

Sulfones: The 1–4-sulfones proved to be soft by both the Shake Flask and River Die-away methods (Table III). However, the 5–6-sulfones are soft by the River Die-away method but hard to the Shake Flask method. The 7–11-sulfones are hard by the Shake Flask method, while by the River Die-away method, only the 7–9-sulfones are hard and the 10- and 11-sulfones are soft. Other than being more soluble than the thia derivatives, the sulfones failed to show any advantage in terms of biodegradability.

Additional tests: Despite several attempts to acclimate the inoculum to biodegrade the 7-thia through 11-thia isomers by the Shake Flask method, these compounds were found to be consistently resistant to rapid biodegradation. An alternate technique used in an attempt to show biodegradation of these resistant thia isomers by the Shake Flask method involved adding ca. 20 mg/liter each resistant thia surfactant to a Shake Flask which previously had biodegraded rapidly either the non-thia substituted LAS control or one of the 1-thia through 4-thia compounds, as shown in Figure 2. After 5 days exposure in the Shake Flask environment which previously had demonstrated rapid biodegradation of non-thia LAS and the 1-4-thia derivatives, none of the 7-11-thia derivatives showed any sign of being biodegraded when analyzing for loss of MBAS.

Because we were not able to resolve the discrepancies in the biodegradation of the 10-thia and the 11-thia com-

TABLE I

Biodegradation of Thiaalkylbenzenesulfonates

Compound	Structure[a]	Shake Flask, days									River Die-away, days							
		1	2	3	4	5	6	7	8	9	3	6	9	12	15	18	21	24
LAS[b] no. 2	Dodocene-1 derived (SDA)[c]	40.0[d]	56.6	65.0	81.6	92.5	97.5	98.3	98.3	-	2.5	15.0	32.5	75.0	87.5	91.5	94.0	95.0
LAS (1-phenyl)	CCCCCCCCCCCC$\phi\Sigma$	56.4	87.5	96.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LAS (2-phenyl)	CCCCCCCCCCC(ϕ)Σ	18.1	91.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ABS[e]	ABS, Lot no. 5 (SDA)	-	31.6	36.6	40.0	43.3	50.0	50.0	-	-	12.5	20.0	25.0	29.0	30.0	35.0	36.5	40.0
1-Thia	CCCCCCCCCCCS$\phi\Sigma$	42.6	93.3	96.6	97.6	98.3	99.3	100.0	100.0	100.0	71.5	86.5	90.0	94.0	95.0	96.5	97.5	98.8
2-Thia	CCCCCCCCCCSC$\phi\Sigma$	71.6	87.6	91.6	94.3	96.6	97.6	98.3	98.3	99.3	52.5	70.0	94.0	95.0	97.5	97.5	99.0	100.0
3-Thia-2-phenyl	CCCCCCCCCSCC(ϕ)Σ	9.3	98.3	99.3	99.6	98.6	100.0	-	-	-	10.0	85.0	98.8	99.0	100.0	-	-	-
3-Thia	CCCCCCCCCSCC$\phi\Sigma$	86.6	93.3	95.0	96.6	97.6	98.3	99.0	99.3	100.0	85.0	92.5	94.0	95.0	96.5	97.5	99.0	100.0
4-Thia	CCCCCCCCSCCC$\phi\Sigma$	88.3	91.6	96.0	97.3	97.6	98.3	99.3	99.3	100.0	25.0	90.0	95.0	96.5	97.5	98.0	100.0	100.0
5-Thia	CCCCCCCSCCCC$\phi\Sigma$	11.0	17.6	26.6	31.6	47.6	92.6	97.6	98.0	98.3	35.0	42.5	65.0	90.0	94.0	96.5	97.5	99.0
6-Thia	CCCCCCSCCCCC$\phi\Sigma$	45.0	73.3	88.3	91.0	96.0	96.0	97.6	98.3	98.3	32.5	61.5	87.5	90.0	91.5	94.0	96.5	99.0
7-Thia	CCCCCSCCCCCC$\phi\Sigma$	5.0	16.6	19.3	23.3	25.0	26.6	26.6	28.3	30.0	10.0	12.5	17.5	20.0	21.5	22.5	25.0	27.5
8-Thia	CCCCSCCCCCCC$\phi\Sigma$	4.3	11.6	20.0	23.3	26.0	28.3	31.0	33.3	33.3	1.5	12.5	17.5	20.0	22.5	24.0	27.5	30.0
9-Thia	CCCSCCCCCCCC$\phi\Sigma$	4.3	9.3	11.6	15.0	19.3	21.0	26.0	26.6	28.3	4.0	7.5	10.0	20.0	22.5	25.0	30.0	36.5
10-Thia	CCSCCCCCCCCC$\phi\Sigma$	2.6	8.3	14.3	15.0	17.6	20.0	21.6	21.6	23.3	6.5	25.0	27.5	30.0	77.5	95.0	96.5	97.5
11-Thia	CSCCCCCCCCCC$\phi\Sigma$	1.6	3.3	6.0	13.3	15.0	17.6	19.3	20.0	24.3	14.0	27.5	30.0	35.0	94.0	95.0	96.5	97.5
4,6-Di-thia	CCCCCCSCSCCC$\phi\Sigma$	13.3	41.6	58.3	93.3	96.6	97.6	99.3	100.0	-	42.5	72.5	80.0	86.5	94.0	95.0	95.0	95.0
3,5-Di-thia	CCCCCCSCSCCC$\phi\Sigma$	79.5	98.0	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] ϕ = Benzene ring and Σ = sulfonate group.

[b] LAS = linear alkylate sulfonate.

[c] SDA = Soap and Detergent Association.

[d] Values expressed as percent methylene blue-active substance degraded.

[e] ABS = Alkylbenzenesulfonate.

TABLE II

Biodegradation of Sulfoxides

Compound	Structure[a]	Shake Flask, days									River Die-away, days							
		1	2	3	4	5	6	7	8	9	3	6	9	12	15	18	21	24
1-Sulfoxide	CCCCCCCCCCCS(=O)φΣ	95.0[b,c]	96.6	97.6	98.3	99.3	100.0	-	-	-	10.0	90.0	44.0	96.5	99.0	100.0	-	-
2-Sulfoxide	CCCCCCCCCCS(=O)CφΣ	21.6	35.0	66.6	96.6	97.6	98.3	99.3	100.0	-	42.5	72.5	80.0	86.5	94.0	95.0	95.0	97.5
3-Sulfoxide	CCCCCCCCCS(=O)CCφΣ	26.6	36.6	48.3	86.6	98.3	99.3	99.9	100.0	-	27.5	39.6	71.5	95.0	95.0	95.0	95.0	97.5
4-Sulfoxide	CCCCCCCCS(=O)CCCφΣ	15.0	32.6	73.3	89.3	96.0	99.0	99.3	100.0	15.0	22.5	50.0	75.0	89.0	91.5	95.0	96.5	-
5-Sulfoxide	CCCCCCCS(=O)CCCCφΣ	10.0	43.3	91.6	99.3	100.0	-	-	-	-	29.0	56.5	95.0	97.5	99.0	100.0	-	-
6-Sulfoxide	CCCCCCS(=O)CCCCCφΣ	1.0	6.6	9.3	11.6	16.0	21.0	31.6	32.6	33.3	0.0	0.0	1.4	1.4	2.4	2.4	2.4	3.9
7-Sulfoxide	CCCCCS(=O)CCCCCCφΣ	2.6	4.3	6.0	8.3	11.6	19.3	28.3	32.6	40.0	30.0	37.5	40.0	42.5	44.6	54.5	55.0	57.5
8-Sulfoxide	CCCCS(=O)CCCCCCCφΣ	1.6	6.0	15.0	33.3	35.0	36.6	38.3	39.3	41.0	0.0	0.0	0.0	0.9	0.9	2.4	2.4	3.4
9-Sulfoxide	CCCS(=O)CCCCCCCCφΣ	1.0	2.6	4.3	6.0	8.3	16.6	31.0	42.6	48.3	35.0	36.5	40.0	42.5	45.0	49.0	50.0	52.5
10-Sulfoxide	CCS(=O)CCCCCCCCCφΣ	33.	23.3	32.6	43.3	54.3	62.6	93.3	97.6	99.3	25.0	35.0	49.0	51.5	57.5	71.5	87.5	96.5
11-Sulfoxide	CS(=O)CCCCCCCCCCφΣ	20.0	66.6	93.3	95.0	99.3	100.0	-	-	-	29.0	47.5	57.5	62.5	75.0	90.0	96.5	100.0

[a] φ = Benzene ring and Σ = sulfonate group.

[b] Values expressed as percent methylene blue-active substance degraded.

[c] In a repeat run, several days were required to achieve 95% degradation.

TABLE III

Biodegradation of Sulfones

Compound	Structure[a]	Shake Flask, days									River Die-away, days							
		1	2	3	4	5	6	7	8	9	3	6	9	12	15	18	21	24
1-Sulfone	CCCCCCCCCCCS(=O)(=O)Oφ Σ	9.3[b]	90.0	96.6	97.6	98.3	100.0	-	-	-	86.5	90.0	92.5	96.5	98.5	99.0	99.0	99.0
2-Sulfone	CCCCCCCCCCS(=O)(=O)Cφ Σ	6.6	33.3	91.6	93.3	96.6	97.6	98.3	98.3	99.3	45.0	55.0	77.5	82.5	97.5	99.0	100.0	-
3-Sulfone	CCCCCCCCCS(=O)(=O)CCφ Σ	5.0	58.3	95.0	96.6	96.6	97.6	97.6	98.3	98.3	77.5	87.5	96.5	97.5	100.0	-	-	-
4-Sulfone	CCCCCCCCS(=O)(=O)CCCφ Σ	6.6	57.6	96.6	98.3	99.3	100.0	-	-	-	97.5	98.0	99.0	99.0	100.0	-	-	-
5-Sulfone	CCCCCCCS(=O)(=O)CCCCφ Σ	14.3	25.0	26.6	28.3	29.3	30.0	31.0	31.6	38.3	24.0	29.0	44.0	51.5	92.5	96.5	99.0	100.0
6-Sulfone	CCCCCCS(=O)(=O)CCCCCφ Σ	1.6	2.6	3.3	8.3	11.0	13.3	15.0	16.6	17.6	2.5	7.5	15.0	22.5	50.0	84.0	91.5	100.0
7-Sulfone	CCCCCS(=O)(=O)CCCCCCφ Σ	0.0	1.6	3.3	5.5	7.6	8.3	12.6	13.3	16.6	4.0	7.5	15.0	21.5	22.5	26.5	32.5	35.0
8-Sulfone	CCCCS(=O)(=O)CCCCCCCφ Σ	0.0	1.6	3.3	6.0	8.3	10.0	12.6	15.0	17.6	2.5	6.5	9.0	11.5	17.5	21.5	22.5	24.0
9-Sulfone	CCCS(=O)(=O)CCCCCCCCφ Σ	0.0	1.6	2.6	3.3	5.0	8.3	11.0	11.6	12.6	4.0	6.5	11.5	12.5	16.5	22.5	26.5	29.0
10-Sulfone	CCS(=O)(=O)CCCCCCCCCφ Σ	5.0	7.6	8.3	9.3	11.6	13.3	16.0	16.6	18.3	25.0	35.0	87.5	90.0	92.5	95.0	95.0	97.5
11-Sulfone	CS(=O)(=O)CCCCCCCCCCφ Σ	1.6	3.3	6.0	13.3	15.0	17.6	19.3	20.0	24.3	14.0	27.5	30.0	35.0	94.0	95.0	96.5	97.5

[a] φ = Benzene ring and Σ = sulfonate group.

[b] Values expressed as percent methylene blue-active substance degraded.

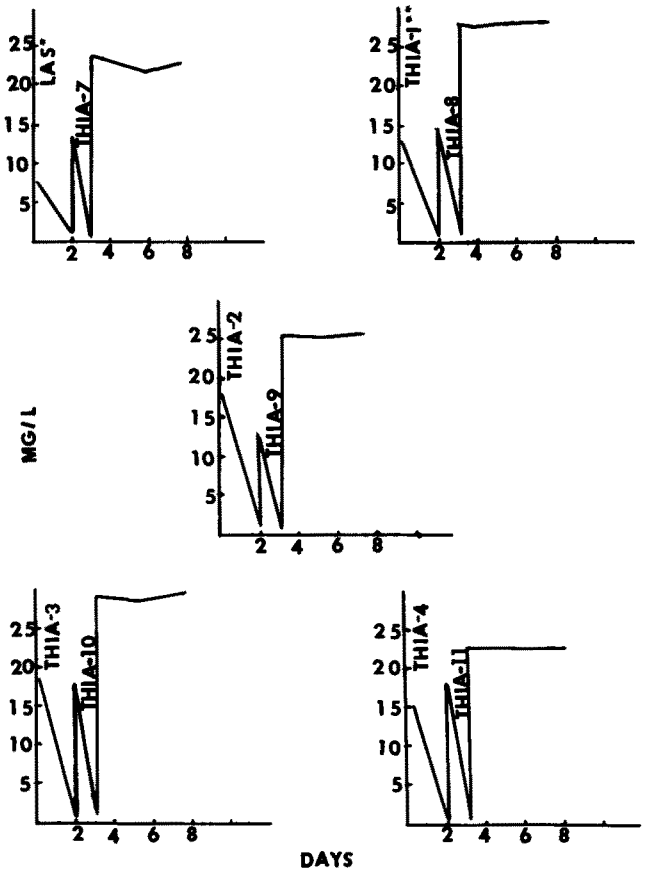

FIG. 2. Results of Shake Flask experiments containing cultures previously adapted to the biodegradable linear alkylate sulfonate (LAS) or the 1–4-thia compounds and spiked on day 3 with the bioresistant 7–11-thia compounds. *Pure 1-phenyldodecane-*p*-sulfonate (control) synthesized at Natick Laboratories. **1-Thia, etc., designates the S position on the alkyl side chain.

pounds as revealed by the Shake Flask and the River Die-away methods, it was decided to conduct a die-away test with synthetic sewage and Warburg studies in an attempt to develop additional data on the biodegradation of these compounds. The small scale aeration chamber previously referenced (13) was used utilizing synthetic sewage medium (14), not only as a final attempt to develop adapted cells capable of degrading all of the thia isomers, but also to provide sufficient cells for Warburg manometry. A summary of the biodegradability results obtained for the 11 thia isomers by this procedure is presented in Table IV. The subsequent Warburg data are presented (Fig. 3). The 7–9-thia isomers which previously had been found resistant to biodegradation by both the Shake Flask and River Die-away procedures using the loss of MBAS as the index of biodegradation were found to undergo biodegradation in the aeration chamber

Oxidation of these resistant thia isomers was confirmed in the Warburg tests. Figure 3 shows that all 11 thia derivatives showed higher initial oxygen uptake during the first 6 hr, as compared with the non-thia control (SDA-LAS). After the initial oxidation, the curves for all 11 derivatives approximated the same slope, indicating that the sulfur appeared to influence only the initial oxidation rate of the compounds.

At the time these experiments were conducted, there was not a sufficient quantity of the locally synthesized non-thia LAS available, and, therefore, the SDA reference LAS no. 2 had to be used as the control detergent.

Mann and Reid (16) reported that laboratory-scale biodegradation tests sometimes may yield misleading indications of low biodegradability on LAS products of higher mol wt, since such products do not appear to acclimatize

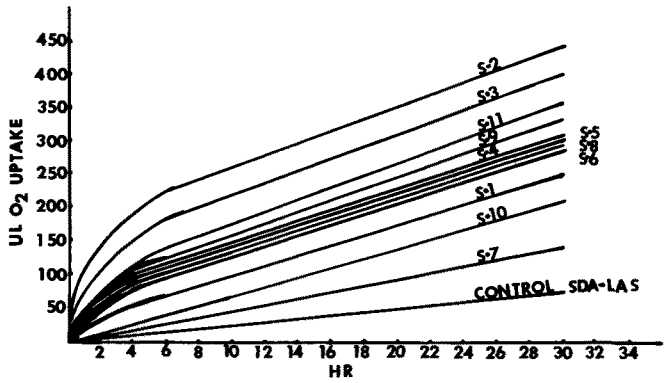

FIG. 3. Biodegradation of sodium *p*-(thia-*n*-dodecyl) benzenesulfonate in Warburg respirometry using microorganisms harvested from a semicontinuous activated sludge aeration chamber. S-1, etc., = position of substitution of S on the alkyl side chain. SDA = Soap and Detergent Association and LAS = linear alkylate sulfonate.

TABLE IV

Biodegradation of Thiaalkylbenzenesulfonates by Aeration Chamber Die-away Process

Detergent	Days required to obtain >95% MBAS[a] removal
1-Thia	2
2-Thia	2
3-Thia	3
4-Thia	3
5-Thia	3
6-Thia	3
7-Thia	8
8-Thia	13
9-Thia	8
10-Thia	18
11-Thia	11

[a]Methylene blue-active substance.

sufficiently under the conditions of the laboratory test. Under practical sewage treatment plant conditions or with tests that simulate these conditions, a higher order of biodegradation is obtained. It would appear that acclimation of the microorganisms to the 7–9-thia isomers in the aeration chamber die-away tests is a result of more favorable conditions and the higher concentration of organisms present which probably accounts for the rapid acclimatization and degradation of the thia isomers previously found resistant in the Shake Flask and River Die-away methods.

With regard to an anaerobic biodegradation of these thia compounds, an experiment was devised where sodium *p*-(4, 6-dithia-*n*-dodecyl) benzenesulfonate was compared with the pure non-thia control, sodium *p*-(*n*-dodecyl) benzenesulfonate using Postgate's medium for sulfate reducers (17). Test results revealed that both the di-thia and the non-thia control equally required 8 days to bring about 100% biodegradation, as determined by the standard methylene blue colorimetric method. Others have shown that linear alkylsulfates undergo biodegradation in anaerobic systems where cesspools serve as domestic sewage systems, and the work of Ōba, et al., (18) suggests that this may be simply hydrolysis.

DISCUSSION

The metabolic pathway used in the biodegradation of LAS compounds generally is agreed to begin with the attack of the end of the alkyl chain to form a carboxyl group followed by rapid β-oxidation of the chain, then more slowly by oxidation of the ring with conversion of the sulfonate group to inorganic sulfate. Other reactions may be involved, for example, fatty ester formation or diterminal oxidation wherein α-oxidation is repeated at the other end of the alkyl chain in the biodegradation of

n-paraffins. Central attack on a hydrocarbon chain also can occur. Abbot and Casida (19) fed *n*-hexadecane to glucose grown resting cells of *Nocardia salmonicolor* which resulted in the oxidation of hexadecane to a mixture of internal monohexadecenes. It was postulated that a double bond insertion may represent an early step in a new pathway of aliphatic hydrocarbon degradation.

An alternate pathway postulated at the beginning of this study was the possible attack and subsequent rupture at an intermediate point in the alkyl chain where a 2-carbon-sulfur bond was introduced in preference to a continuous carbon-carbon bond. This hypothesis was tested by the synthesis of compounds where sulfur derivatives of sodium *p*-(*n*-dodecyl) benzenesulfonate were prepared by successively replacing methylene groups with sulfur.

The general biodegradation pattern observed with these three series of sulfur containing compounds (using the results obtained from the Shake Flask tests and the River Die-away tests) is that, as the sulfur is moved further out on the side chain, the degradation becomes slower, except that, when the sulfur substitution reaches C_{10} and C_{11}, the compounds become readily biodegradable again.

Although the results of the biodegradation tests show that some of the thia substituted compounds degraded more rapidly than the commercially produced SDA LAS no. 2, there was no evidence to show that thia substituted compounds were superior to the parent 1-phenyl LAS in biodegradability. The sulfoxides and sulfones also were found to be less biodegradable than the thia compounds. Based upon the tests conducted, the mechanism of biodegradation of the sodium *p*-(thia-*n*-dodecyl) benzenesulfonates, does not appear to be the initial oxidation to the sulfoxide with subsequent rupture of the carbon-sulfur bond. Instead, the presence of the sulfoxide and sulfone groups in some instances may have an adverse effect upon the biodegradation of the side chain, indicating that bacterial oxidation of the terminal methyl group probably is preferred to that of a sulfur atom. Attempts to isolate and identify metabolic intermediates from these sulfur containing compounds by gas chromatographic and mass spectrometric analysis to postulate what biodegradation route was involved were unsuccessful.

Swisher (20) divided organic compounds, including detergents, into three categories as potential food sources for bacteria: (A) readily utilizable, (B) utilizable after acclimation, and (C) not utilizable. There is no sharp division between these categories, and the classification of the given compound may vary from one situation to another. Many organic compounds not capable of immediate assimilation can serve as food sources after the development of the necessary adaptive enzymes has taken place by the microorganisms involved. Throughout our testing of the LAS sulfur compounds, acclimation of these compounds was necessary before biodegradation would take place. However, as noted in Tables I-III, acclimation of the inoculum to degrade some of the compounds satisfactorily (7–9-thia, 7–9-sulfoxides) was never accomplished via the Shake Flask and River Die-away tests.

As an indirect test to determine if these seemingly bioresistant sulfur compounds could be degraded by organisms growing on other isomers, the bioresistant 7–11-thia isomers were added separately to shake flasks containing cultures previously acclimated to biodegrade rapidly the parent LAS 1-phenyl and the 1–4-thia compounds, as shown in Figure 2. In no instance, were any of these previously acclimated cultures able to utilize the 7–11-thia compounds. The data would seem to indicate that the enzyme systems may be matched specifically to the chemical structure of each of these thia containing compounds. The need for the continual availability of a highly mixed bacterial population in the cultures used to assess the biodegradability of organic compounds, as found in the laboratory semicontinuous activated sludge or die-away type aeration chambers but not in the Shake Flask, may be the limiting factor which results in erroneously classifying a compound as biologically resistant to breakdown. Since bacterial species differ in their reservoir of constitutive enzymes and in their assimilatory development, the inocula routinely used in the Shake Flask test may be inadequate to provide the needed sequence of enzymes capable of handling the 7–9-thia derivatives.

In a previous publication by the authors (21), 4 of the 19 pure cultures isolated from the "standard" Shake Flask inoculum were found incapable of biodegrading the SDA standard LAS detergent in pure culture even after extended acclimation periods different from that specified by the presumptive SDA Shake Flask method (13). The routine procedure of carrying the mixed culture on artificial media in the laboratory for extended periods may result in lowering the number of species in the mixed culture and, thereby, reduce the ability of the mixed inoculum to assemble the necessary enzyme systems required to assimilate a variety of exotic compounds, such as seen with the 7–9-thia compounds. This may account, in part, for the degradation of the 10–11-thia compounds by the River Die-away procedure but failure of the compounds to degrade by the Shake Flask method. The river water undoubtedly contained a wide variety of organisms capable of acclimating to the 10–11-thia compounds or to some breakdown products as required to bring about 95-97% degradation. The 25% or more degradation of all the resistant thia compounds tested also probably indicated that some degradation does take place, but the organisms with the necessary enzyme systems to continue the assimilation of the detergent are not immediately available.

Based upon the biodegradability data resulting from the Shake Flask and River Die-away tests, the 7–9-thia compounds presumptively were classified as biologically resistant. According to the SDA Shake Flask method (13), if percent reduction falls below 80% in the Presumptive test, the material is considered to be not adequately biodegradable, and no further testing is justified. Therefore, in a normal course of screening detergents for biodegradability, the 7–9-thia compounds would have been rejected as not adequately biodegradable. However, when testing the resistant thia compounds using an aeration chamber die-away process, all 11 thia compounds were found to be biodegradable. It is interesting to note (Table IV) that the 7–11-thia compounds were more slowly biodegradable than the 1–6-thia. However, cells harvested from the aeration chamber and used in the Warburg tests also confirmed the biodegradability of all 11 thia compounds as indicated by oxygen up-take. This would indicate that, once the acclimated cells are available, the sulfur appears to influence only the initial oxidation rate of the thia compounds, since the curves (Figure 3) for all 11 derivates approximated the same slope. One other possible explanation for the bioresistance of the 7–9-thia compounds based upon Horvath and Koft's (22) observations with ABS compounds is the need for the presence and accumulation of some intermediate compound which will permit cometabolism of the otherwise bioresistant molecule. River water or synthetic sewage as used in the SDA Confirming Test (13) may provide the intermediate ingredient necessary for cometabolism of the resistant compounds, while, in the SDA Presumptive Shake Flask test (12) procedure, this compound is not available.

These studies have shown that the incorporation of the sulfur atom into the alkyl side chain of the LAS molecule has failed to shorten the time required to biodegrade the LAS structure and that the mechanism of biodegradation of the sodium *p*-(thia-*n*-dodecyl) benzenesulfonates is not initial oxidation to a sulfoxide with subsequent rupture of the carbon-sulfur bond (as postulated by Long, et al. [3]). Biodegradation of these sulfur containing detergents proba-

bly proceeds via the usual β-oxidation mechanism used for fatty acid oxidation. Since some of the sulfur compounds were found to be slowly biodegradable using standard methods, the differences in the biodegradation are probably due to difference in acclimation properties. The need for a variety of organisms in the test environment to provide the necessary sequence of enzyme systems or the presence of an intermediate compound which will permit cometabolism are probably prerequisites for the biodegradation of the bioresistant sulfur containing detergents.

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