TABLE X

Detergency—Average Rd Reflectance Values

U.S. Testing	1-Phenyl	2-Phenyl	6-Phenyl	7-Phenyl	Std ABS
50 ppm	30.2	32.8	34.1	34.5	30.3
150 ppm	31.7	32.9	35.1	36.4	36.6
200 ppm	28.6	32.8	35.8	36.4	36.6
Test Fabrics					
50 ppm	43.2	44.1	45.8	47.1	42.8
150 ppm	44.1	44.3	45.8	47.6	44.3
200 ppm	38.4	44.4	47.0	47.8	44.1

sebum foam test that follows because it had no initial foam.

The 2- and 3-phenyl compounds had a lower initial foam height which collapsed quickly under the initial sebum soil additions. The 4-phenyl had an intermediate foam pattern whereas the 5- and 6-phenyl compounds were equal to standard at 500 mg soil load and the whole foam pattern closely resembled that the standard. For a detailed analysis of the sebum soil and simulated dishwashing tests, refer to the work of W. Spangler (4) of our laboratories.

B. Detergency Studies

Detergency was measured as the increase in reflectance after washing U.S. Testing and Test Fabric soiled cloths in a Terg-O-Tometer at 0.15% conen, 6 swatches/liter, 10 min at 120F and 100 rpm. There was significant increase (1.2 units is significant at 95% confidence level) in detergency for the 6- and 7-phenyl tetradecylbenzenes as compared to the 1- and 2-phenyl tetradecylbenzenes. This is illustrated in Table X.

We have established that the internal 5- and 6-phenyl isomers are better foamers in mixed chain alkylates than the 1-,2-,3- and 4-isomers. We have shown that the performance of tridecylbenzene sulfonate (ABS) can be matched in detergency and foam performance by optimizing linear alkylate structure. This structure should be in the mol wt area of 252–266 with 90% of the carbon chains being C_{12}, C_{13} and C_{14} and with less than 10% C_{11} or C_{15} and should have approx 40% 5- and 6-phenyl content and 20% 2-phenyl content.

We have also shown that in the pure tetradecylbenzene series of isomers, those with the phenyl centrally located on the chain are ca. 50–80% better foam performers than the 2-isomer. Similarly, the detergency using standard soiled cloth is also better for the internal isomer.

ACKNOWLEDGMENT

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An Evaluation of the River Die-away Technique for Studying Detergent Biodegradability

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Abstract

The accuracy and reproducibility of the river die-away test has not been well defined. This information is required to establish the validity of observed differences in degradation behavior.

The present paper presents data on the accuracy and repeatability of the methylene blue analytical procedure using both the standard method and the automated "AutoAnalyzer" version of the methylene blue method.

Data are also presented showing reproducibility of the biodegradation curves obtained by replicate analysis of a single detergent under 1) identical die-away conditions, and 2) different conditions obtained by die-away studies in various river waters.

The effect of detergent mol wt and microbial adaptation to the test substance on the degradation pattern is also discussed.

Introduction

DETERGENT BIODEGRADATION STUDIES have been reported utilizing a variety of biological test systems. Generally, however, these systems may be classified as either static or dynamic. Recent efforts in the development of dynamic systems have been directed toward the development of apparatus and procedures that are simple to maintain and operate in an average laboratory. These dynamic units are operated on the continuous feed principle, designed to simulate conditions in an activated sludge type sewage plant.

The river die-away technique is an example of the static type biological system, and has been extensively used in detergent biodegradation studies. This test have several advantages over other biodegradation test systems. These include the experimental test simplicity and the low solids content of the system which facilitates isolation, or other analytical techniques

TABLE I

Analytical Deviation of the Standard Methylene Blue Method

LAS in:	Replicates	Average value (ppm)	Standard deviation (ppm)
Distilled water	6	8.62	0.36
Distilled water	6	0.42	0.005
River water	15	9.35	0.35

which would be hampered by the high organic nutrient level of other systems.

The accuracy and reproducibility of the degradation patterns obtained by this technique, however, have not been reported in the literature. Without this knowledge it is difficult to assess the significance of differences in degradation curves obtained by the river die-away technique. The interpretation of results from the test are complicated by large biological deviations which we define as variations between test jars not due to analytical measurement or detergent adsorption. Such biological deviations may be indirectly produced by the development of minute physical or chemical differences between jars, or more directly, to microbial genetic changes. While the adsorption of detergent can be fairly large, we have not found this factor to be significantly different between test jars of the same geometry and type of material.

Since the well known environmental factors reported by Fuhrman et al. (8) which do affect biodegradation patterns were held constant in our studies, we conclude that these factors did not affect our biodegradation results.

The deviations observed in this study are particularly serious when degradation results in regard to both detergent disappearance and the shape of the degradation curve are to be compared between physically different rivers.

Previous detergent degradation studies have demonstrated the importance of microbial adaptation to specific structures when evaluating degradation patterns (1,2,3). The data to be presented will show the importance of this parameter in the river die-away test.

Experimental Methods

The requirements for the operation of a detergent die-away study are very simple. Briefly, the test involves adding a specific quantity of the detergent test substance to a sample of river water contained in a glass jar and allowing the solution to incubate at room temp. Degradation of the detergent is then measured by some suitable analytical procedure.

The studies reported in this paper utilized waters from the Mississippi, Missouri, Ohio, Wabash, Kaskaskia and Arkansas rivers. In conformity with standard practice, not more than 24 hr elapsed between collection of the samples and initiation of the test.

The straight-chain alkyl benzene sulfonates used in our studies were prepared in our Petrochemical Research laboratories both by alkylation of benzene with an α-olefin or by alkylation with a straight-chain

TABLE II

Analysis of River Waters Used in Die-Away Studies

River	MBAS (ppm)	D.O.[a]	Bacterial count/ml	pH	C.O.D.[b]	Suspended solids	Chloride (ppm)
Ohio	.13	1.0	53,000	7.4	29.3	1.1	30
Mississippi	.06	1.0	178,000	6.9	18.4	0.7	30
Wabash	.05	0.7	200,000	7.6	19.3	0.8	30
Missouri	.05	1.0	118,000	7.6	34.1	1.2	30
Kaskaskia	0	0.8	50,000	7.1	28.1	2.5	33

[a] Dissolved oxygen.
[b] Chemical oxygen demand.

TABLE III

Biodegradation of LAS in Various River Waters

River	Days of incubation (values in ppm)				
	0	11	14	18	32
Ohio	21.5	16.0	8.5	4.9	0.3
Mississippi	21.7	15.3	11.3	6.6	1.3
Wabash	22.0	16.3	15.3	5.1	0.9
Missouri	21.1	15.5	11.3	3.5	0.3
Kaskaskia	21.1	16.7	13.5	4.9	0.6
Average value, ppm	21.5	16.0	12.0	5.0	0.68
Standard deviation, ppm	0.39	0.58	2.6	1.1	0.43

chloroparaffin. In either case, an alkylbenzene is produced with an unbranched carbon side chain, which yields the straight-chain alkylbenzene sulfonate upon reaction with oleum or SO_3. These new materials are becoming known as LAS detergents, abbreviated from the term Linear Alkylate Sulfonate.

In our studies, the river water was added to half-gallon Mason fruit jars and sufficient detergent added to provide an approx 10 ppm concn. After the addition of a magnetic stirring bar, the jar lids were loosely fitted and the solutions allowed to incubate at room temp (25C).

At sampling intervals, ordinarily daily, the solutions were thoroughly mixed by means of a magnetic stirrer and an appropriate aliquot removed for analysis by the standard methylene blue colorimetric method (4).

Samples for the AutoAnalyzer were removed and diluted volumetrically to approx 2 ppm to provide the desired response using the Technicon large volume sampler. As the degradation progressed to 2 ppm and below, samples were analyzed directly without dilution.

The effect of microbial adaptation was studied by detergent readdition after the original concn had decreased to 0.5 ppm or less.

Results and Discussion

Analytical Deviation—Standard Methylene Blue Method. The standard deviation of the standard methylene blue analytical method was determined by replicate analysis of distilled water solutions of detergent at about 10 ppm and 0.5 ppm, and also at about 10 ppm in a river water medium.

The results are shown in Table I. These findings are generally in good agreement with the values reported in "Standard Methods for the Examination of Water and Wastewater" (4).

Biological Deviation—Standard Methylene Blue Method. The reproducibility of the river die-away technique itself was studied in two different experiments. In the first study, five geographically different river waters were obtained and a standard detergent added to each river as substrate for the die-away experiments. The standard methylene blue method was used for the detergent analyses. The initial characteristics of the five river water samples are shown in Table II.

The biodegradation patterns of an LAS surfactant in the five river waters are shown in Table III. The

TABLE IV

Analysis of Five Lots of Mississippi River Water Taken from the Same Sampling Point at 15-Minute Intervals

Water sample No.	pH	D.O. (ppm)	C.O.D. (ppm)	MBAS (ppm)	Bacterial count/ml
1	7.9	2.7	30	.08	60,000
2	8.0	3.0	30	.12	56,800
3	7.8	2.8	30	.07	52,600
4	7.7	2.6	30	.10	27,800
5	7.6	2.6	30	.13	25,400

TABLE V

Biodegradation of LAS in Various Lots of Mississippi River Water

344 MW LAS	Days of incubation (values in ppm)						
	0	3	5	10	17	24	38
River water No.							
1	10.0	2.8	0.90	0.75	0.49	0.28	0.20
2	10.0	6.2	.91	.77	.42	.26	.23
3	10.0	7.8	.80	.63	.39	.20	.19
4	10.0	6.6	.91	.69	.61	.20	.20
5	10.0	8.2	.98	.67	.44	.21	.21
Average value, ppm		6.3	.90	.70	.47	.23	.21
Standard deviation, ppm		2.1	.07	.06	.09	.04	.02
367 MW LAS							
River water No.							
1	10.0	....	....	9.0	2.7	0.65	0.15
2	10.0	....	....	8.8	3.3	1.7	.37
3	10.0	....	....	8.4	1.7	0.97	.34
4	10.0	....	....	9.3	2.3	1.1	.29
5	10.0	....	....	9.8	1.3	0.64	.32
Average value, ppm		....	....	9.1	2.3	1.0	.29
Standard deviation, ppm				.53	.79	.43	.09

degradation patterns do not appear to be specifically correlative with any of the physical properties of the river waters shown in Table II.

The data in Table III shows a standard deviation of the starting concentration which is in good agreement with the analytical deviation shown in Table I. The eleventh day marks the end of the induction or lag period for this detergent and this point is reflected reasonably well in all five rivers with a standard deviation of 0.58. However, the high (2.6) standard deviation found at 14 days during the active period of degradation prevents an accurate drawing of the shape and slope of the biodegradation curve. Greater sampling frequency would not be beneficial since the deviation at 18 days (5 ppm average level) is still quite high. At 32 days the standard deviation of 0.43 at the 0.7 ppm level is far higher than the approx analytical deviation of 0.01 ppm at this general level. In terms of percentage detergent remaining at 32 days in the various rivers, the range is from 1.4% in the Ohio River to 6.0% in the Mississippi River.

The data in Table III indicate that a consistent profile of the biodegradation of the particular surfactant could not be drawn from its behavior in any single river in a river die-away test.

The reproducibility of the river die-away technique was also studied by replicate analyses from five different lots of Mississippi River water. Each individual lot of water was drawn from the same point in the river at 15-min intervals. Two linear alkylate sulfonate samples, one with an average equivalent wt of 344 and the other 367, were added as substrates in the ensuing river die-away study.

A partial analysis of the five lots of river water is shown in Table IV.

The biodegradation patterns of the two linear alkylate sulfonate samples in the different lots of Mississippi water are shown in Table V. The standard methylene blue method was used for the detergent analysis.

TABLE VI

Analytical Deviation of the Methylene Blue AutoAnalyzer Method (LAS in distilled water)

(Added ppm)	Replicates	Avg found (ppm)	Standard deviation
0.5	15	0.53	0.06
1.0	15	1.0	0.04
2.0	18	2.0	0.06
5.0	5	4.7	0.41
10.0	5	9.9	0.13

TABLE VII

Comparison of LAS Biodegradation—Repilcate Tests in Identical and in Different River Water Samples

Water sample	Days of incubation								
	4			5			6		
	Test replicate No.			Test replicate No.			Test replicate No.		
	1	2	3	1	2	3	1	2	3
1	8.1	7.7	8.1	7.9	6.2	7.4	5.8	0.92	0.49
2	7.8	7.6	7.7	5.2	7.6	7.3	.42	2.4	4.4
3	7.7	8.0	7.8	7.4	7.1	7.0	5.8	1.2	5.7
4	7.8	7.8	8.0	7.3	7.4	5.9	.53	2.0	.53
5	8.1	8.1	8.0	7.7	7.4	7.1	5.4	5.0	.59

The standard deviation of the values obtained from the biodegradation study in the five lots of Mississippi River water are much better for the 344 MW sulfonate than the data obtained when geographically different river waters were used, as shown in Table III, except during the third day portion of the degradation curve. However, the pattern of the 367 MW sulfonate in the five waters is ragged throughout the 10 to 38-day period shown, with deviations far in excess of analytical deviations at comparable detergent levels.

Analytical Deviation—AutoAnalyzer Methylene Blue Method. The accuracy and repeatability of the AutoAnalyzer methylene blue method was determined from replicate analyses of detergent in distilled water and also from triplicate analyses of each test jar in a standard river die-away study.

Table VI shows a summary of the analysis of distilled water solutions of linear alkylate sulfonate using the methylene blue AutoAnalyzer method.

The above findings generally agree with the standard deviations obtained by the standard manual methylene blue method, except the deviation at the 5 ppm level which is larger than expected.

The reproducibility of the river die-away test and also the analytical repeatability were studied in an experiment utilizing the methylene blue AutoAnalyzer method and samples of Arkansas River Water.

Five lots of water were drawn from the same point in the river at 10-min intervals to provide five different water samples. Three aliquots of water were removed from each of the five different water samples and added to Mason fruit jars for the typical river die-away test.

Thus a total of 15 sample jars was prepared from the five water samples, allowing a test to test comparison to be made between the three jars containing identical river water, and a study of water sample variation to be made by comparing the biodegradation patterns between the five different waters.

Analytical repeatability was obtained by a triplicate analysis of each test sample jar. Each of the five river waters was also analyzed in triplicate providing 60 samples for each analysis cycle and resulting in a total of 600 samples during the 10 days of the study.

In brief, the experiment consisted of triplicate *tests* (or jars) from each of the five different river water samples, each test (or jar) analyzed in triplicate.

TABLE VIII

Standard Deviation for Individual Observations of River Water, Test Replicate and Analytical Parameters in LAS Biodegradation

Incubation (days)	LAS concn (ppm)	River water[a]	Test[b]	Analytical[c]
1	8	0.29	0.25	0.22
4	8	0.34	0.32	0.32
5	5–8	0.83	0.83	0.24
6	0.4–6	2.3	2.3	0.14
8	0.3–0.6	0.12	0.07	0.03

[a] Different river waters; one test; one analysis for each water.
[b] Different test; one river water; one analysis for each test.
[c] Analytical deviation for one test and one river water.

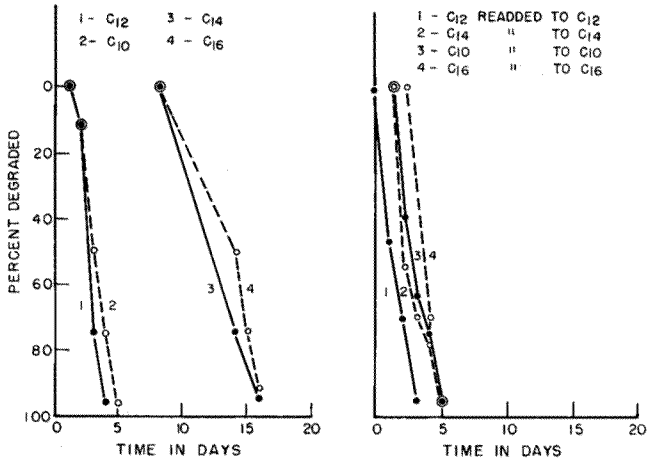

FIG. 1. Biodegradation of initial and subsequent readditions of straight-chain LAS in Mississippi River water.

Table VII shows the results of the fourth, fifth and sixth days of the river die-away study as described above.

A statistical summary of the data in Table VII is shown in Table VIII.

The 5- and 6-day values are shown to be identical for the river water and test columns since at these two points the variation between test jars in the same river water was greater than the variation between different waters, which is contrary to the statistical model used for the above calculations.

The above data again show large deviations in the active portion of the biodegradation curve even among replicates of the same water, as shown by the 2.3 value under the sixth test day, compared to the 0.14 analytical deviation for this period.

However, if the shape of the active portion of the curve is not of prime interest, the above data would be useful as a measure of disappearance of the detergent since at 12 days (not shown) all tests in each of the five waters had reached a disappearance level between 94 and 96%.

Microbial Adaptation. Another very important parameter of the test is the effect of microbial adaptation to the test substance. Major differences in initial biodegradability between some surfactants can become insignificant following a period of microbial adaptation. Some evidence that microorganisms adapted to linear alkylate sulfonates are simultaneously adapted to lower mol wt homologs but not to higher mol wt homologs poses an interesting question and merits

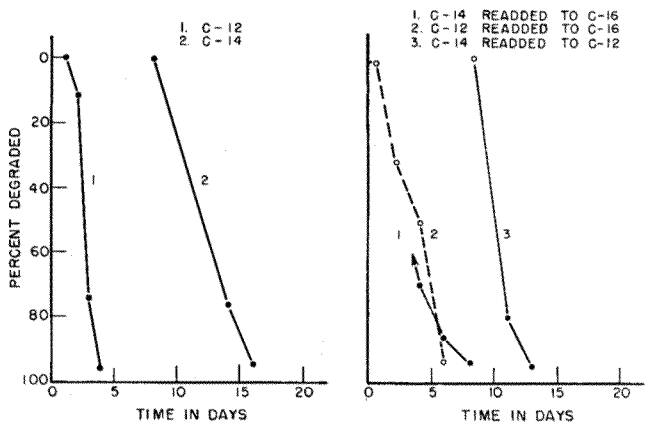

FIG. 2. Degradation of initial and subsequent cross-additions of straight-chain LAS in Mississippi River water.

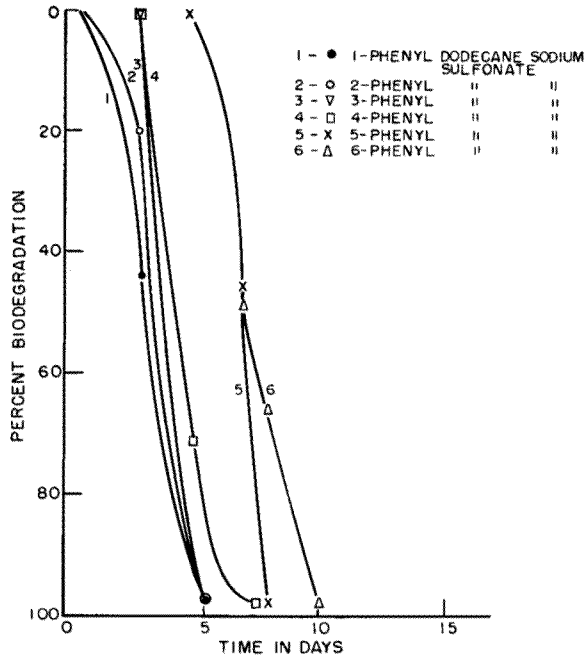

FIG. 3. Biodegradation of six possible dodecylbenzene sulfonate phenyl position isomers in Mississippi River water.

further study of microbial adaptation to such structures.

A mol wt effect is usually evident in a regular river die-away study similar to that shown in Table V where the data indicates the 344 MW sulfonate began degrading in three days, while 10 days were required before the initiation of an attack on the 367 MW sulfonate. Readdition of these two detergents to the same jars shortened the induction time of the 344 MW compound by only about one day, but a very large effect was observed with the 367 MW material. The induction time of the latter compound was reduced to about two days, practically equivalent to the 344 MW sulfonate.

This phenomenon was further investigated utilizing an isomeric mixture of straight-chain decyl, dodecyl, tetradecyl and hexadecyl alkylbenzene sulfonates. These compounds were prepared by alkylation of

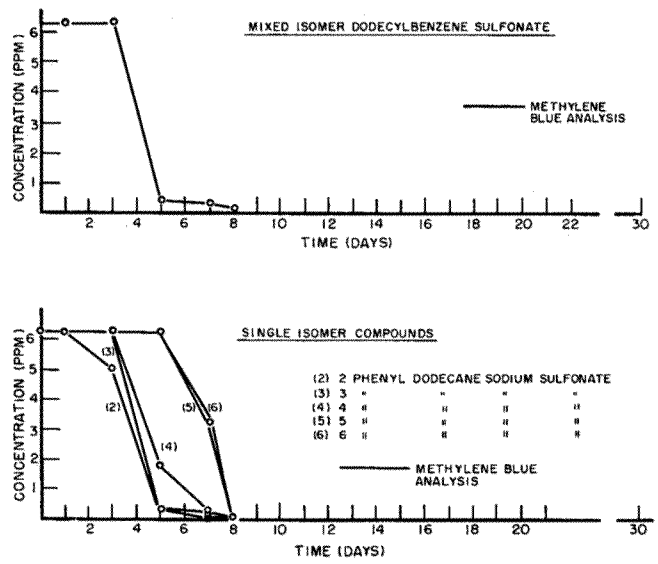

FIG. 4. Biodegradation of the mixed isomer (α-olefin derived dodecylbenzene sulfonates and of the 2,3,4,5 and 6 phenyl dodecane isomers.

benzene with a pure α-olefin, followed by sulfonation, neutralization and purification. This method of preparation produces all possible phenyl positional isomers except the 1-phenyl alkane. The latter compound was prepared by a special organic synthesis.

About 10 ppm of each of these compounds were added to a portion of Mississippi River water and the biodegradation allowed to proceed to ca. 95% completion. At this point, 10 ppm of fresh sample was readded to its original jar and the degradation monitored as before. The results are shown in Figure 1.

The adaptation step had a moderate effect on the C_{10} and C_{12} compounds, but a very large effect on the C_{14} and C_{16} compounds, shown in the latter two cases by a much shorter induction time and a reduction in the 95% degradation time from 16 to 5 days! Thus, an apparently very large difference between C_{10} and C_{12} compounds and C_{14} and C_{16} compounds shown by the usual river die-away test was found to be an insignificant difference when microbial adaptation was considered.

A number of cross additions were also studied as shown in Figure 2. Data in Figure 2 show the regular degradation pattern of a C_{12} and a C_{14} sulfonate. Curves also are shown in Figure 2 from cross additions of C_{12}, C_{14} and C_{16} straight-chain compounds after initial degradations had proceeded to 95% or more.

When a C_{14} compound was added to a jar previously containing a C_{16} compound, the induction time of the C_{14} was greatly reduced and its 95% degraded point reduced from 16 to ca. 8 days.

The C_{12} added to a jar adapted to a C_{16} compound resulted in a slight loss in reaching the 95% degraded point; from four days initially to about six days after addition.

The addition of the C_{14} compound to a jar adapted to a C_{12} compound gave no improvement in the C_{14} induction period and only a modest improvement in the 95% degraded point—from 16 to 13 days.

These limited data indicate that microorganisms adapted to a particular carbon chain length are simultaneously adapted to lower mol wt homologs but not to higher molecular weight homologs.

Effect of Phenyl Position on Biodegradation. The importance of the distance between the terminal alkyl methyl group and the benzene sulfonate group in regulating microbial degradation of linear alkylate sulfonates has been cited by several workers (1,2,5,7). This river die-away study confirms these observations.

When the 2-phenyl alkane isomers of octyl, decyl, dodecyl and hexadecyl benzene sulfonates were subjected to river water degradation, all were rapidly decomposed as measured by the standard methylene blue method. Mid-chain phenyl positional isomers of the same sulfonates were degraded considerably slower.

River water biodegradation patterns of the six possible phenyl position isomers of a C_{12} linear alkylate sulfonate are shown in Figure 3. The data indicate that among these C_{12} compounds the greater the distance between the terminal alkyl carbon and the benzene sulfonate group, the more rapid the biodegradation. However, it appears that the presence of mid-chain phenyl position isomers in a mixture containing all possible isomers does not significantly retard degradation of the mixture.

Figure 4 shows nearly complete degradation of the mixed isomers dodecylbenzene sulfonates in five days, while at this time level all of the single compounds 5- and 6-phenyl dodecane sulfonate and 35% of the 4-phenyl isomer remained undegraded. The 4-, 5- and 6-phenyl dodecane content of the mixed isomer dodecylbenzene sulfonate is ca. 50% of the total mixture.

However, biodegradation studies involving analysis by desulfonation-GLC of mixed isomer phenyl dodecane sulfonates containing the 2-, 3-, 4-, 5- and 6-phenyl isomers indicate that the order of biological attack is still in the same order as shown in Figure 4, with the 2-phenyl the most readily attacked and the 5- and 6-phenyl the most slowly (7).

Two workers (6) have recently reported that during degradation of the 4- and 5-phenyl isomers of dodecylbenzene sulfonate, intermediates were produced that appeared toxic to a soil isolate used in their study. In our study using river water which contains a mixed microbial culture, bacterial counts were found to be essentially identical following degradation of each of the six C_{12} isomers. This would indicate the absence of linear alkylate sulfonate toxicity to the mixed microbial population normally found in nature. It should perhaps be mentioned that inability of a microorganism to grow on a particular substrate does not necessarily constitute substrate toxicity.

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