Biodegradation of LAS Benzene Rings in Activated Sludge[1]

R. D. SWISHER, Inorganic Chemicals Division, Monsanto Company, St. Louis, Missouri

Abstract

Effluents from continuous flow and from fill and draw activated sludge cultures were analyzed quantitatively for benzene ring content by ultraviolet spectroscopy, with appropriate correction for interference by nitrate ion. C_{12} LAS mixture and two of its components in pure form, 3-phenyl- and 6-phenyldodecane-*p*-sulfonates, all showed approximately 90% ring biodegradation under a variety of conditions once acclimation had occurred. When feeds to 3-phenyl and 6-phenyl acclimated cultures were interchanged, the characteristic 100% primary biodegradation (methylene blue analysis) proceeded without interruption. However, ring degradation was impaired for 4–7 days until reacclimation was accomplished. Biochemical implications are discussed.

Introduction

LINEAR ALKYLBENZENE SULFONATE (LAS) has served as the surfactant in commercial detergent formulations in several countries during the past few years. It has replaced the poorly biodegradable tetrapropylene alkylbenzene sulfonate which was formerly used. The change has resulted in substantial decreases in the foaming properties and methylene blue active substances (MBAS) of sewage treatment effluents and the receiving rivers, because of the facile biodegradation of LAS by microorganisms.

It is regrettable that even before LAS had had a chance to prove itself—even before the change was completed—we began to hear calls for the detergent industry to make yet another change—calls to replace LAS by some "supersoft" surfactant. Perhaps we may anticipate a decline in these calls with continuing accumulation of evidence from the field showing the success of LAS. The present work may also contribute to that decline since it answers one particular criticism that has been made against LAS; namely, that in its molecular structure there is a benzene ring which for some reason might resist biodegradation.

First of all, it must be emphasized that such incompletely degraded fragments of the original LAS molecule cannot constitute any particular hazard even if they are present, because it has been shown experimentally that the fish toxicity of LAS disappears along with its MBAS response upon biodegradation (1). Fragments must necessarily be present during the biodegradation of any complex molecule; it does not fly apart into its component atoms in a single step, but rather undergoes a long sequence of consecutive reactions, each one changing the molecule slightly. LAS is no exception. Temporary presence of intermediate biodegradation products has been demonstrated (2). The biochemical reactions involved in their formation and subsequent disappearance have been investigated, and the evidence for the eventual complete degradation of the entire molecule has been reviewed (2,3).

[1] Presented at 22nd Annual Purdue Industrial Waste Conference, Lafayette, Indiana, May 4, 1967.

Bacterial attack on the LAS molecule begins at the end of the alkyl chain remote from the benzene ring, so the earlier intermediate degradation products must inevitably contain intact benzene rings. But these are destroyed at a later stage. This is not particularly surprising, since biodegradation of the benzene ring in general is well known—so much so that no references need be cited. A wide variety of benzene derivatives, including several of the amino acids and other molecules directly involved in life processes, are degraded and also synthesized by living organisms. There is also ample evidence for biodegradation of the ring in sulfonated benzene derivatives. This has been proved both by Warburg respirometry (4,5) and by ultraviolet (UV) spectrophotometry (6) for sodium benzene sulfonate, toluene sulfonate and other short chain derivatives. Ring degradation in LAS itself has been indicated by infrared (7) and UV spectrophotometry (8,9) and by isolation of a partially biodegraded fraction in which both spectrophotometry and elementary analysis indicated the absence of benzene rings (10).

The above work on LAS ring biodegradation was all done on commercial type mixtures containing from 5 to 20 or more isomers and homologs, leaving the possibility that in spite of the positive results perhaps a few of these components may have been resistant to ring degradation. Accordingly, the present work was undertaken to study two of these components in pure form—the sulfonates of 3-phenyl- and 6-phenyldodecane. These particular ones were chosen because earlier studies have shown that the position of the phenyl group along the chain is somewhat more important than is the length of the chain in affecting the speed of the primary biodegradation (11,12).

Preliminary studies on the biodegradation of these isomers in river water indicated extensive ring destruction in each case (13). The present work confirms this in activated sludge in a more quantitative manner. Similar results are also being obtained using the shake culture procedure after suitable provisions for acclimation, and will be presented at a later date (14).

Experimental Procedures

Preparation of Sulfonates

C_{12} LAS. Benzene was alkylated with α-dodecene to give a mixture of 2-, 3-, 4-, 5- and 6-phenyldodecane, followed by conversion to the Na sulfonate with no attempt at separation of isomers.

3-Phenyldodecane Sulfonate. Obtained by sulfonation of 3-phenyldodecane (Eastman 8060) (50 g) with 20% oleum (70 g) at 40–50C for 45 min. After adding 12.5 ml of water, the sulfonic acid upper layer was separated and neutralized in 80% isopropyl alcohol with NaOH, filtered to remove the insoluble Na_2SO_4 and the filtrate was evaporated to recover the crude sulfonate (68 g). This was boiled with 300–400 ml of acetone, leaving 3.35 g of acetone insoluble fraction which was predominantly the *para* sulfonate contaminated with sodium carbonate (13). The fraction termed "3øS," principally the *para* isomer, was ob-

tained by cooling with dry ice, filtering out and drying the resulting precipitate to 57 g of product. Microdesulfonation gave 3-phenyldodecane. Further information on these fractions has appeared elsewhere (13).

6-Phenyldodecane Sulfonate. Prepared from Bader 6-phenyldodecane in the same manner as the 3-phenyl isomer (69 g). The fraction insoluble in boiling acetone amounted to 3.4 g, and was predominantly the *para* sulfonate contaminated with sodium carbonate (13). The fraction termed "6øS," largely the *para* isomer, was obtained by cooling the hot acetone filtrate with ice water, filtering out and drying the resulting precipitate to 46.4 g of product; desulfonation gave 6-phenyldodecane.

A small portion of 6øS was further purified by dissolving 3.5 g in 30 ml of boiling acetone and cooling in ice water, yielding 3.0 g of recrystallized product; this was recrystallized once more in the same manner to give the sample termed "6øR," also predominantly the *para* isomer.

The main acetone filtrate from 6øS was cooled in dry ice and gave a further crop which filtered and dried to 12.1 g. Crystals resembling 6øS and 6øR could not be obtained on attempted recrystallization from acetone. The entire 12.1 g was separated into five fractions of increasing solubility in acetone, all of which gave infrared spectra substantially identical with that of 6øR. The fraction termed "6øX" was one of the most soluble of the five, amounting to about 1 g. Its apparent molecular weight by Epton titration was about 360, significantly higher than the theoretical 348, and the Epton endpoint was slow and indistinct, suggesting the possible presence of disulfonate. Further information on some of these fractions has appeared elsewhere (13).

1-Phenyldodecane Sulfonate. Sulfonation as above using 1-phenyldodecane (Eastman 6816). The sulfonation mixture was dissolved in about 1000 ml water, neutralized with NaOH. After standing overnight at room temperature, the crystals of *para* sulfonate were filtered out and then recrystallized by dissolving in about 500 ml of hot water (containing a small amount of isopropyl alcohol to reduce foaming and viscosity), yielding 48.7 g of product termed "1øP." This product has been described more fully elsewhere (17).

Sulfophenylundecanoic Acid Disodium Salt. Mol Wt 386, Termed "SøU." Prepared and characterized previously (1) via sulfonation of phenylundecanoic acid (Eastman 5352).

Parasulfobenzoic Acid Monopotassium Salt. Mol Wt 240, Termed "PSB." Eastman P1647, practical grade.

Biodegradation

Continuous Activated Sludge. Cultures were maintained in the glass units (Fig. 1) described earlier (1). Working volume of the aeration section was 300 ml, of the settling section 75 ml, air flow rate around 100 ml/min. The synthetic sewage was made up from city tap water by adding: Nutrient broth,* 150 mg/liter; K_2HPO_4, 50 mg/liter; NaCl, 30 mg/liter; $MgSO_4$, 10 mg/liter; surfactant, 50 mg/liter (0 in blank feed), and was introduced at 100, 50 or 20 ml/hr, giving aerator residence times of 3, 6 or 15 hr. Mixed liquor suspended solids levels were maintained near 4000 mg/liter by occasional discard of solids; during operation at 15 hr residence time the solids level held at 2500–3500 mg/liter and discard was not necessary. The sludge cultures had originally been obtained from a municipal sewage treatment plant several years previously. They had been used in the laboratory under similar conditions since that time for surfactant biodegradation research, receiving a daily inoculum of about 5 ml of fresh sludge in efforts to maintain diverse microbial populations.

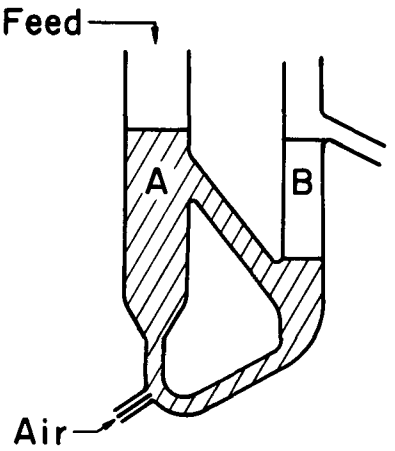


FIG. 1. Continuous flow activated sludge unit. A = Aerator, working volume 300 ml (shaded section). B = Settler, working volume 75 ml.

Semicontinuous Activated Sludge. The confirmatory test procedure of the Soap and Detergent Association was used (15), except that the sludge had already been acclimated to LAS by several months use in the laboratory. Briefly, the SDA procedure calls for fill and draw operation using 1500 ml of mixed liquor, suspended solids around 2500 mg/liter with 23 hr aeration, ½ hr settling and ½ hr for withdrawing one liter of settled effluent and refilling with one liter of fresh feed. Feed is made from tap water by adding: glucose, 130 mg/liter; nutrient broth,* 130 mg/liter; beef extract, 130 mg/liter; K_2HPO_4, 130 mg/liter; $(NH_4)_2SO_4$, 25 mg/liter; surfactant, 20 mg/liter (0 in blank feed).

Methylene Blue Analysis. Feeds and effluents were analyzed by the modified Hellige method previously described (1).

Sludge Extraction Procedure. Examination of sludge for possible presence of adsorbed intermediates was carried out by methanol extraction. The mixed liquor sample (100–200 ml) was suction-filtered and the unwashed wet cake was oven dried at 110–120C. The dried cake was weighed (0.3–1 g) and then extracted by boiling with four 50–100 ml portions of methanol, decanting each portion successively into a beaker where the extracts were evaporated almost to dryness. Water was added (10–20 ml) and evaporation was continued to a volume of 2–5 ml to remove the remainder of the methanol. The aqueous residue was then diluted to 50 ml with water and examined in the same manner as the effluents.

Spectrophotometry

A Cary Model 14M double beam recording spectrophotometer was used. To minimize adsorption of surfactant on the cell walls, all measurements were made in the presence of 0.009 molar KH_2PO_4 (16) by mixing 5 ml of sample (settled effluent, unfiltered) with 45 ml of 0.01 molar KH_2PO_4. Cleaning of cells between measurements was found to be unnecessary; the cell was rinsed six times with the solution to be

* Through a clerical error, N Broth was used instead of nutrient broth. N Broth consists of Tryptose (peptone), 20 g; NaCl, 5 g; K_2HPO_4, 4 g; KH_2PO_4, 1.5 g; Sodium Desoxycholate, 0.1 g.

measured before the final filling. One-centimeter silica cells were used so that the effective path length corresponded to 1 mm of the original sample before dilution.

Spectra were obtained by scanning downward from 300 mμ at 0.5 mμ/sec, against 1 cm of 0.01 M KH_2PO_4 in the reference beam. Measurements at a specific wavelength (e.g. 300, 223, 210, 193 mμ) were made over a period of at least 30 sec, averaging to minimize effects of electronic noise. On each occasion, a second reference cell (0.01 M KH_2PO_4) was immediately measured in the same manner to obtain a baseline reading and to guard against instrumental drift or instability. The baseline reading was subtracted to give the actual net absorbance.

Development of Ring Analytical Method

Spectra of LAS and Model Biodegradation Products

Free benzene has three characteristic UV absorption bands with maxima around 180, 200 and 256 mμ. These bands are also shown by compounds containing the benzene ring, with maxima shifted to longer wavelengths depending on the nature of the substituents (18). LAS shows these three bands at around 193, 223 and 260 mμ; SøU is almost identical and PSB is only slightly different (Fig. 2). SøU is a model of intermediate compounds formed in the early stages of LAS biodegradation, with introduction of a carboxyl group at the end of the chain. The simplest possible ring-containing intermediate is PSB. Possibly this is the stage in biodegradation immediately before attack on the benzene ring, after the chain has been entirely oxidized to a carboxyl group at the ring. All other ring-containing biodegradation intermediates should fall somewhere in between the extremes of SøU and PSB.

It will be most convenient to express benzene ring analytical results in terms of parts per million (ppm) of the equivalent LAS. Thus "1 ppm" of rings would be the amount of rings present in a 1 mg/liter (2.88 μM) solution of LAS, mol wt 348, corresponding to 0.225 mg/liter of rings calculated as free benzene, C_6H_6.

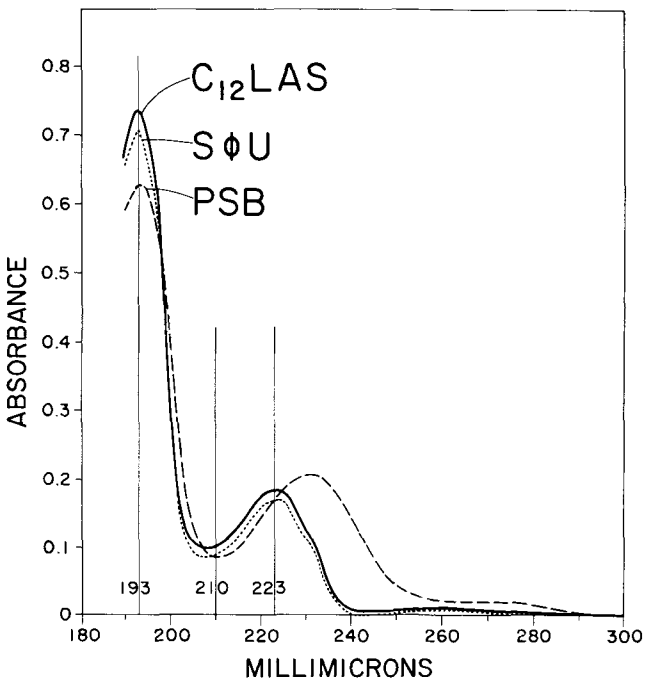


FIG. 2. UV spectra of 50 ppm benzene rings in the form of C_{12} LAS (50 mg/liter), SφU (55.5 mg/liter), PSB (34.5 mg/liter); 1 mm equivalent light path. (Solutions were diluted with 9 volumes 0.01 M KH_2PO_4, measured against 0.01 M KH_2PO_4 as reference in 1 cm cells.)

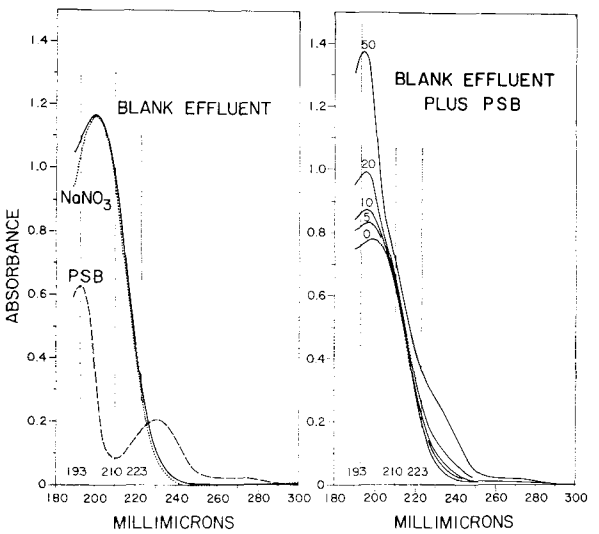


FIG. 3. (a) UV spectra of activated sludge blank effluent (solid line), sodium nitrate (100 mg/liter = 16.5 ppm nitrogen, dotted line), PSB (34.5 mg/liter = 50 ppm rings, dashed line); 1 mm equivalent light path. (Diluted as in Figure 2.) (b) UV spectra of activated sludge blank effluent spiked with PSB corresponding to 0, 5, 10, 20 and 50 ppm of rings; 1 mm equivalent light path. (Diluted as in Figure 2.)

All three spectra in Fig. 2 were made from 144 μM solutions, 50, 55.5 and 34.5 mg/liter respectively, with "50 ppm" of rings in each. The absorbances are approximately equal for both the 193 and 223 mμ maxima as well as for the minimum between them at 210. Table I shows these absorbances calculated as the molar absorptivity. It also includes the individual components 3øS and 6øS, which were so similar to the C_{12} LAS mixture that they could not be included clearly in Fig. 2. All of the entries in Table I agree reasonably well and it seemed possible that by assuming average values for the molar absorptivities of the rings it should be possible to estimate semiquantitatively the ring content of a biodegraded solution from its absorbance.

Nitrate Interference and Correction

Direct observation of these benzene ring absorption bands in activated sludge effluents proved to be impossible because of a very intense absorption, peaking at 200 mμ throughout the entire region (Fig. 3a). Nevertheless, it proved possible to calculate the approximate ring content of such effluents by an algebraic method, since the interfering absorption came predominantly from a single source: nitrate ion. The major contribution of the nitrate is evident from the spectrum of pure sodium nitrate shown in Fig. 3a—it follows the curve of the blank effluent very closely except at the extreme lower wavelengths.

If the spectrum of an effluent specimen originates

TABLE I
Molar Absorptivity
Absorbance of 1 molar solution, 1 cm path

	193 mμ	210 mμ	223 mμ
C_{12} LAS	51,000	7000	12,700
3øS	50,500	7300	12,700
6øS	49,000	6500	13,100
SøU	49,000	6300	11,700
PSB	43,500	5900	11,800

only in its nitrate and ring content, and if Beer's law is obeyed, the amount of each can be calculated by measuring the absorbance at two different wavelengths and solving two equations in two unknowns. The most sensitive wavelengths to use are those of an absorption maximum, as at 193 mμ, and an adjacent minimum, as at 210 mμ. In the present work both maxima, 193 and 223 mμ, were used in conjunction with the minimum at 210, thus providing data for two independent calculations of ring content.

The spectrum of a ring compound in pure form at known concentration, as in Fig. 2, provides us with a factor, K, which when multiplied by the absorbance, B, at 1 mm equivalent path length and at a given wavelength, will give R, the ppm of rings present:

$$KB = R$$

From the pure nitrate spectrum a similar factor, J, can be determined which when multiplied by the corresponding absorbance, C, will give N, the ppm of nitrate nitrogen:

$$JC = N$$

Values of K and J so determined are shown in Table II. The K's for the rings were arbitrarily taken as as the average of SøU and PSB. Table II indicates that these values would give results somewhat too high for undegraded LAS, if present.

Considering now a solution containing both rings and nitrate, its total absorbance as measured is the sum of the ring absorbance and the nitrate absorbance. At wavelengths 1 and 2, these will be:

$$A_1 = B_1 + C_1 = R/K_1 + N/J_1$$
$$A_2 = B_2 + C_2 = R/K_2 + N/J_2$$

which lead to:

$$R = K_1K_2(J_1A_1 - J_2A_2)/(K_2J_1 - K_1J_2)$$
$$N = J_1J_2(K_1A_1 - K_2A_2)/(K_1J_2 - K_2J_1)$$

Thus the ring and nitrate nitrogen content of the solution can be calculated from the absorbances at the two wavelengths and the corresponding K and J factors.

The assumption was made that the rings and nitrate were the only UV absorbers in the system. This is not quite true, since the spectrum of the blank effluent does deviate somewhat from that of pure nitrate at the shorter wavelengths. Furthermore, upon calculation in the above manner the blank effluent does show several ppm of apparent rings. This indicates the presence of natural absorbers, which may or may not be actual benzene rings. This interference can be minimized by subtracting the blank effluent values from the others to give the net ring content, assuming that it is present to the same extent in all the effluents.

Another minor source of interference is the turbidity or flocculence which may remain in the settled effluent to varying degrees (filtration should be avoided because of possible losses to the filter medium by adsorption). Such material gives a slight general absorption which seems to be reasonably constant across the whole spectrum range. Since the absorbance of the solutes is essentially zero at 300 mμ, the absorbance actually measured at that wavelength (0.01 to 0.02) was assumed to be due to the particulate matter and was subtracted from the values determined at the shorter wavelengths.

TABLE II

K and J Factors

Factor × 1 mm absorbance = ppm rings

	Type	193 mμ	210 mμ	223 mμ
C_{12} LAS	K	68.2	495	273
3øS	K	69.0	477	275
6øS	K	70.8	532	266
SøU	K	71.2	555	298
PSB	K	80.0	589	294
Rings	K	75	572	296
Nitrate	J	15.8	16.6	56.5

Computation

Data obtained on a typical blank effluent (Culture B-5, Feb. 22, 1967) are presented as an example. Five parts of effluent were mixed with 45 parts of 0.01 M KH_2PO_4 and the absorbance was measured in comparison with 0.01 M KH_2PO_4 in the reference beam, using 1 cm cells (corresponding to 1 mm of undiluted effluent). R and N were calculated from the equations, using K and J values from Table II.

	193 mμ	210 mμ	223 mμ	300 mμ
Sample absorbance	1.267	1.097	0.360	0.036
Baseline absorbance	0.046	0.030	0.028	0.028
Net absorbance	1.221	1.067	0.332	0.008
300 mμ Net absorbance	0.008	0.008	0.008	
Corrected absorbance, A	1.213	1.059	0.324	
ppm Apparent rings, R	8.7		4.5	
ppm Nitrate nitrogen, N	17.3		17.4	

Measurements on effluent from culture B-1, fed 20 ppm of 6øR, were made and computed in the same manner, and the blank values were subtracted to give the net rings:

	193 mμ	223 mμ
B-1 Gross rings, ppm	10.0	5.9
B-5 Blank apparent rings	8.7	4.5
B-1 Net rings	1.3	1.4

Probable Accuracy

Calculations from the R and N equations show that an error of 0.01 (1 division on the chart paper) in the absorbance at 193 or 210 mμ will lead to an error of about 1 ppm in the rings, and at 223 mμ to about 3.5 ppm. The nitrate nitrogen is much less sensitive with corresponding error of only about 0.1 ppm. Actually, the absorbance can be estimated to within a few tenths of a division, and reproducibility is ordinarily within this range also. Although the absolute accuracy of the instrument may be somewhat poorer than the reproducibility, this factor is not important since it should affect all measurements about equally and thus is subtracted out with the blank.

As an experimental check on the method, a sample of blank effluent was spiked with PSB corresponding to 5, 10, 20 and 50 ppm of rings. The resulting spectra, shown in Fig. 3b, upon computation as above checked to within one or two ppm:

	Net rings found	
Rings added	193 mμ	223 mμ
5	4.5	4.6
10	8.1	9.0
20	17.5	18.6
50	46.8	50.4

A qualitative indication for the presence of rings is evident also in Fig. 2b: with increasing amounts the initially smooth nitrate peak is progressively deformed and shifted from 200 toward 193 mμ.

The assumption is made that the non-nitrate UV absorbers are present to the same extent in all effluents including the blank. Although this has not been explicitly proved, there has been no contrary indica-

tion throughout this work. The apparent ring content of the blank effluents measured at 223 mμ averaged about 4 ± 1 ppm for the continuous cultures and 5 ± 0.5 ppm for the semicontinuous, over the entire period of this work. Measured at 193 mμ it was about 3.5 higher. All LAS-fed effluents showed a similar difference between the gross ring content measured at 223 and 193 mμ, ranging from 2 to 4 ppm in the averages, with standard deviations ranging from 0.5 to 2 ppm. This pattern of results suggests that there is little difference in the background absorption from culture to culture or from time to time.

It should be noted that nitrite, if present, could cause serious error. Its UV spectrum resembles that of nitrate, but the absorption is somewhat weaker and the maximum is near 210 mμ instead of 200. Each ppm of nitrite nitrogen present would reduce the 193 mμ ring value by about 1 ppm and increase the 223 mμ ring value by 4 ppm. Since the gross ring values actually measured were higher at 193 than at 223 by 3–4 ppm, it seems likely that very little it any nitrite was present.

In view of the close agreement between the 193 and 223 mμ net ring values and the standard deviations, of approximately 1 ppm, consistently obtained throughout this work, it seems likely that the probable error is not much more than 1 ppm. This conclusion as well as the conclusion that it was actually benzene rings being measured are both supported by the consistent pattern of the experimental results: a) The straightest, purest compound, 1øP, showed 0 ppm remaining in the semicontinuous effluent. b) Under the same conditions, the 3øS effluents consistently averaged about 1.5 ppm rings remaining. c) The pure 6øR, with 2 ppm rings remaining, was definitely lower than the impure fraction 6øX (5 ppm remaining). d) Typical acclimation patterns were exhibited, with ring content of the effluent rising after an appropriate change in feed and then falling again as acclimation progressed.

The figure of 1 ppm suggested for the probable error of this method does not take into account the rather remote possibility that some ring-containing biodegradation intermediates may be formed which have K factors differing widely from the averages arbitrarily chosen in Table II. Nor does it include biological variations which may introduce much larger discrepancies upon occasion, originating for example in acclimation phenomena or in divergent population distributions in mixed cultures.

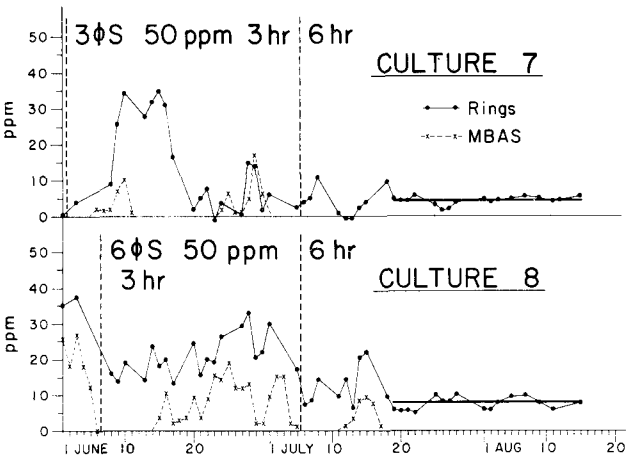


FIG. 4. Net ring content of continuous flow activated sludge effluents. Surfactant feed level raised to 50 mg/liter from 35 mg/liter on June 1 and June 6 respectively. MBAS = methylene blue active substance. Residence time 3 hr, 6 hr.

Ring Biodegradation Results

Results obtained on the six samples are summarized in Table III, while Fig. 4, 5 and 6 give the day-to-day record of several typical runs. Net ring values are those calculated from the 223 mμ absorption band; values from the 193 band agreed to within 1 ppm.

Continuous Cultures

After preliminary experiments had indicated a substantial amount of ring degradation during a 6-hr residence time, attempts were made to get data for 3-hr residence. The units were started with 10 mg/liter of surfactant in the feed (C_{12} LAS, 3øS or 6øS) and then increased to 20, 35 and finally 50 mg/liter each time the effluent methylene blue analysis dropped to zero. In past experience, such units have been run for long periods with mixed LAS with no particular difficulty, but in the present case smooth operation could not be achieved. Accordingly all units were slowed down to 6-hr residence, whereupon they soon stabilized to a steady state.

TABLE III
LAS Ring Biodegradation in Activated Sludge

Sample	Continuous (6 hr, 50 mg/liter)			Semicontinuous (24 hr, 20 mg/liter)		
	Run, days	Remaining net ppm	Degraded %	Run, days	Remaining net ppm	Degraded %
C_{12} LAS	26	10.7 ± 1.7	78.6	8	1.9 ± 0.4	90.5
				10	1.6 ± 0.5	92.0
3øS	26	4.2 ± 1.2	91.6	18	1.5 ± 0.4	92.5
	9	4.6 ± 0.9	90.8	8	1.7 ± 0.4	91.5
	18	4.7 ± 1.6	90.6	13	1.4 ± 1.0	93.0
	20	4.0 ± 0.7	92.0			
	16[a]	2.9 ± 0.8[a]	94.2[a]			
	21[a]	4.4 ± 1.6[a]	91.2[a]			
6øS	26	8.0 ± 2.4	84.0	15	3.0 ± 0.6	85.0
	9	6.1 ± 2.1	87.8	15	2.2 ± 0.6	89.0
	18	7.9 ± 1.5	84.2	13	1.3 ± 0.8	93.5
	20	8.1 ± 1.5	83.8	9[b]	11.3 ± 3.0[b]	72.2[b]
	16[a]	4.4 ± 0.7[a]	91.2[a]			
	21[a]	4.9 ± 1.1[a]	90.2[a]			
6øR				8	1.9 ± 0.4	90.5
				10	2.1 ± 0.8	89.5
				14	1.7 ± 0.6	91.5
				14	0.9 ± 0.6	95.5
				12[b]	10.4 ± 2.7[b]	74.0[b]
6øX				18	5.4 ± 0.4	73.0
1øP				14	0.2 ± 0.9	99.0
				15	0.0 ± 0.7	100.0
				13	−0.1 ± 1.1	100.5

[a] 15 hr, 50 mg/liter feed.
[b] 24 hr, 40 mg/liter feed.

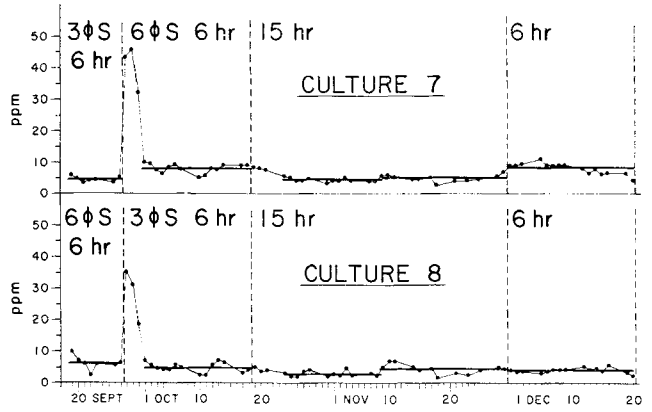


FIG. 5. Net ring content of continuous flow activated sludge effluents. Residence times 6 hr, 15 hr. Surfactant feed level 50 mg/liter. MBAS content of effluents was zero throughout.

Results from two of these cultures, operating on 3øS and 6øS, are shown in Fig. 4, starting shortly after the feed level was raised to 50 mg/liter from 35. Erratic operation at the 3-hr rate is indicated by the methylene blue analyses, which should have remained at zero. Culture 7 did run close to zero for a week or more, during which time there was a large upset in ring degradation. After the subsequent recovery the ring degradation leveled off at approximately 90%, about as extensive as it was after slowing down to the 6-hr rate. Culture 8 also experienced about a week of smooth operation during which only 15–20 ppm of rings showed up in the effluent (60–70% ring degradation), but then it upset, the methylene blue analysis indicating that a substantial amount of intact 6øS was appearing in the effluent. After switching to 6-hr operation one further upset occurred, during the second week, but things went smoothly thereafter, averaging about 85% ring degradation.

Subsequent experiments with these cultures are shown in Fig. 5. On September 27, the feeds were switched, with resultant upset in ring degradation although the effluent methylene blue analyses remained at zero throughout. By the fourth day, however, the cultures had adapted to their new feeds and ring degradation of each compound was proceeding to the same extent that had previously been achieved by the other culture. In other words, the extent of degradation was characteristic of the compound, not the culture.

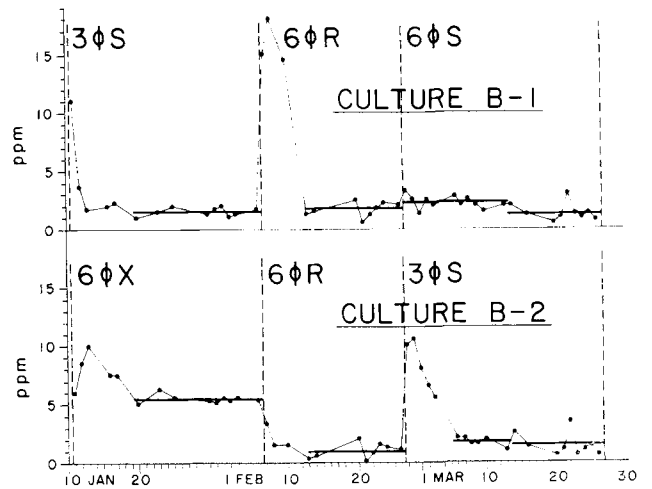


FIG. 6. Net ring content of semicontinuous activated sludge effluents. Residence time 24 hours. Surfactant feed level 20 mg/liter. MBAS content of effluents was zero throughout.

On October 18 the feed rate was slowed to a 15-hr residence time. Degradation of the 6øS rings was noticeably increased, while the 3øS, already almost complete, was improved only slightly. Subsequent increase of the flow rate back to 6 hr gave the original results again.

Semicontinuous Cultures

In a preliminary experiment on 6øS, fed at the standard 20 mg/liter, the ring content of the daily effluent averaged about 3 ppm for 2 weeks. This corresponded to 85% ring biodegradation during the 24-hr cycle, the same as had been achieved in 6-hr in the continuous culture fed at 50 mg/liter. The 6øS feed level was increased to 40 mg/liter from 20 in order to match the continuous run more closely. As a result, the effluent ring level rose to 11 ppm, corresponding to a drop in ring degradation from 85% to 72%. Methylene blue analyses were zero throughout.

Switching the feed from 6øS to 6øR, still at 40 ppm, gave little effect, with the average holding at 10 ppm, but when the feed level was cut back to 20 ppm, the daily effluent was down to 2 ppm rings within four days, and held there.

Subsequent experiments, depicted in Fig. 6, were designed primarily to study acclimation effects. The impure fraction 6øX gave rise to about 5 ppm of rings in its daily effluent. Switching to 6øR resulted in immediate lowering to around 1 ppm, with no indication of any requirement for further acclimation. This is consistent with the view that the major component of 6øX is 6-phenyldodecane-p-sulfonate, same as in 6øR. Subsequent switching of the feed to 3øS on February 26 did lead to a disturbance of ring degradation, and the culture required about a week to learn to utilize the 3øS rings. Culture B-1, acclimated to 3øS, experienced a similar difficulty on switching to 6øR on February 5, and it too required about a week to reorganize its metabolism. The further switch to 6øS had no such effect; since the two samples had substantially the same identity and purity, none would be expected.

Throughout all of the semicontinuous culture experiments, switching feeds and all, the methylene blue analysis of the effluents remained at zero.

Methanol Extraction of Sludge

To determine whether adsorption onto the sludge may have played some part in the removal of the LAS rings, samples of the sludge were analyzed from time to time by examination of the UV spectra of methanol extracts. In no case was there any indication of the ring bands at 193 and 223 mμ. Calculation showed that 10 mg of rings per gram of sludge should be readily detectable, if present, and this was experimentally verified by addition of PSB at that level to a wet sludge filter cake before drying and extraction.

All of the sludge extracts showed the same general pattern, with absorbance dropping from around 1.0 at 190 mμ to a minimum of 0.1–0.2 around 240 mμ, then leveling off or rising slightly as a broad shoulder or peak in the 260 mμ region and finally dropping to around 0.05 at 300 mμ. When run with a blank sludge extract in the reference beam, the differential spectra were substantially flat, with no indication that the ring bands at 193 and 223 were present.

Nitrification

The nitrate nitrogen content of the effluents was in the range of 5 to 20 ppm. Continuous effluents usually ran 8–12 ppm, semicontinuous 12–16 ppm. No correlation with presence or absence of LAS in the feed was evident.

Discussion

Extent of Ring Degradation

The experimental data in Table III lead to the conclusion that the benzene rings of LAS are indeed susceptible to biodegradation, and that almost complete removal can be accomplished by activated sludge under a variety of conditions.

The removal was not a result of adsorption onto the sludge since rings were not detectable upon extraction of the sludge. Adsorbed rings would have been detected easily at the level of 10 mg/g of sludge, if present, which is quite insignificant in comparison with the amount fed. For example, the continuous cultures each received 60 mg/day of rings, but the amount of rings adsorbed on its 1.2 g of sludge was less than 10 mg, if any.

It is evident from Table III that although complete degradation of the rings was accomplished in the case of 1-phenyldodecane-*p*-sulfonate, this was never quite achieved with the 3-phenyl or 6-phenyl isomers—there was a small residual UV absorption corresponding to a small fraction of the initial rings. Three possible factors may be involved. First, sufficient time to complete the degradation may not have been given. This appears to be the case with the 6øS, since ring degradation was more complete with 15-hr residence time than with 6 hr, but the effect was not nearly as pronounced with the 3øS. Second, there is always a possibility that the residual material originates in small amounts of impurities present in the samples. The 1-phenyl isomer is extremely insoluble in cold water, so that recrystallization is a very efficient means of purifying it. This is not the case with the other two isomers. The 3øS is particularly suspect because of the large recovery obtained in its recrystallization from acetone, achieved by cooling in dry ice, and presence of impurity is of course very obvious in 6øX. Third, there is also a possibility that two biodegradation pathways are involved, a major one leading to complete destruction, but also a minor pathway, taken by a small percentage of the original molecules, terminating in a dead end somewhere short of complete degradation.

Whatever its cause, this effect is of minor consequence, since ring degradation does proceed to the extent of 85 to 90% or more with either isomer, as well as with the C_{12} LAS mixture of five isomers.

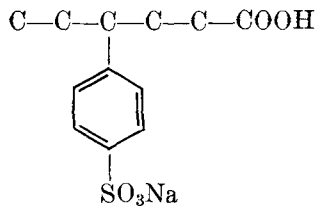
Effectiveness of Continuous vs. Semicontinuous Treatment

It is of interest to note in Table III that the extent of ring degradation in the semicontinuous cultures at 24 hr is not much better than in the continuous at 6 hr, and that it is actually worse at comparable feed concentrations. This may arise from an inhibiting action of the surfactant on the bacteria, since the surfactant would be present at a much higher level in the semicontinuous culture immediately after the daily feeding than when fed continuously. In any case, these results have bearing on an assumption that is often made—that a 24-hr semicontinuous activated sludge culture must obviously be a more vigorous biodegradation system than a continuous flow culture with a shorter residence time. This assumption is false in the present case, and may be in others.

Biodegradation Pathways

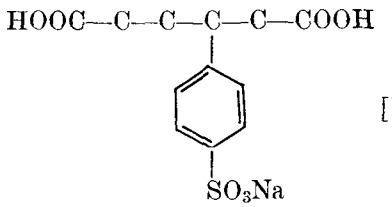
The information on acclimation obtained in the cross-feeding experiments is by itself insufficient for much further illumination of the details of the LAS biodegradation process. Nevertheless, some tentative inferences can be drawn.

Previous work (2,3) has shown that there is a pause in the biodegradation of 3-phenyldodecane sulfonate at an intermediate stage, γ-sulfophenyl caproic acid (I), which accumulates temporarily



[I]

in the medium and later disappears. From the same work it furthermore can be inferred that biodegradation of the 6-phenyl isomer probably proceeds via the dicarboxylic acid β-sulfophenyl adipic acid (II).



[II]

The 3øS-acclimated cultures readily accomplished the primary biodegradation of the 6-phenyl isomer, presumably bringing it to the dicarboxylic state (II), but could not degrade the ring without further acclimation. This indicates that the β-sulfophenyladipic acid is not formed in the biodegradation of 3-phenyldodecane sulfonate, and hence that the attack on the γ-sulfophenyl caproic acid does not proceed by oxidation of its terminal methyl group to a carboxyl, since that would form (II).

Two other possibilities remain—attack on some other portion of the chain, as by continued oxidation from the carboxyl end, or direct attack on the ring. Inasmuch as the two molecules in question are identical except for the methyl vs. carboxyl at one end of the chain, the inability of the 3øS-sludge to carry out such an operation on (II) the same as it can on (I) suggests that the extra carboxyl group in (II) is in some way incompatible with the (I)-enzyme. By similar reasoning it may be inferred that the methyl group in (I) is incompatible with the (II)-enzyme.

Environmental Effects

Field results shows conclusively that LAS is readily biodegradable, at a rate comparable to the other biodegradable organic components of sewage, and have given no indication of adverse effects on our environment. Direct experimental investigations have shown that effluents from the biodegradation of LAS are nontoxic at LAS levels of 100 mg/liter or more, much higher than can occur in the field (1). This present work shows that no significant amounts of molecular fragments remain after the biodegradation, and thus indicates an even greater margin of safety.

ACKNOWLEDGMENTS

R. V. Bethel, R. G. Schwartz and F. M. Smith were responsible for the daily care and operation of the activated sludge units and for the methylene blue analyses.

REFERENCES

1. Swisher, R. D., J. T. O'Rourke and H. D. Tomlinson, JAOCS *41*, 746–752 (1964).
2. Swisher, R. D., J. Water Pollution Control Federation *35*, 1557–1567 (1963).
3. Swisher, R. D., Chem. Eng. Progr. *60*(12), 41–45 (1964).
4. Bogan, R. H., and C. N. Sawyer, Sewage Ind. Wastes *27*, 917–928 (1955).
5. Symons, J. M., and L. A. del Valle-Rivera, Purdue Univ. Eng. Bull. Ext. Ser. No. *109*, 555–571 (1962).
6. Setzkorn, E. A., and R. L. Huddleston, JAOCS *42*, 1081–1084 (1965).
7. Ryckman, D. W., and C. N. Sawyer, Purdue Univ. Eng. Bull. Ext. Ser. No. *94*, 270–284 (1957).
8. Foster, D. J., and R. R. Fields, Soap Chem. Specialties *40*(8), 49–52 (1964).
9. Krüger, R., Fette, Seifen, Anstrichmittel *66*, 217–221 (1964).
10. Wickbold, R., Proc. Intern. Congr. Surface Activity, 4th, Brussels, 1964, paper C/VII 9.
11. Huddleston, R. L., and R. C. Allred, Develop. Ind. Microbiol. *4*, 27–38 (1963).
12. Swisher, R. D., J. Water Pollution Control Federation *35*, 877–892 (1963).
13. Swisher, R. D., presented at XXXVIth International Congress on Industrial Chemistry, Brussels, September 1966.
14. Swisher, R. D., presented at 24th annual meeting, Society for Industrial Microbiology, London, Ontario, August 1967.
15. Soap and Detergent Association Subcommittee on Biodegradation Test Methods, JAOCS *42*, 986–993 (1965).
16. Weber, W. J., Jr., J. C. Morris and W. Stumm, Anal. Chem. *34*, 1844–1845 (1962).
17. Gray, F. W., J. F. Gerecht and I. J. Krems, J. Org. Chem. *20*, 511–524 (1955).
18. Jaffe, H. H., and M. Orchin, Theory and Applications of Ultraviolet Spectroscopy, Wiley, New York, 1962, Chapter 12.

[Received May 10, 1967]