unlike linear or branched chain alkylbenzenesulfonates had no adverse effect on the digestion process (10).

Rate of Hydrolysis and Energy of Activation

Esters of α-sulfo fatty acids are surprisingly resistant to hydrolysis, possibly because the presence of the bulky sulfo group retards attack at the carboxylate linkage.

The rate for acid catalyzed hydrolysis, as shown in Table V, generally decreases with increase in molecular weight of the primary alcohol and is significantly less for esters of secondary alcohols.

The rate for alkaline hydrolysis again decreases with the number of methylene groups in the primary alcohol and is markedly less for esters of secondary alcohols. Apart from any other considerations this suggests that the isopropyl or secondary butyl esters would be the most suitable in the formulation of esters spray-dried in the presence of alkaline builders.

The rate of alkaline hydrolysis for sodium methyl α-sulfopalmitate and sodium methyl α-sulfostearate, at 60, 80, and 100C, plotted as log k vs. 1/T gave straight lines consistent with the Arrhenius equation. Calculation of the energy of activation gave values of 16.5 and 16.6 kcal per mole, respectively.

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Ultraviolet Spectroscopic Analysis for Following the Biodegradation of Hydrotropes[1]

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Abstract

The standard methylene blue colorimetric method for the analysis of ppm concentrations of anionic detergents is sensitive to the chain length of the hydrophobic portion of the molecule. The response to this method is not quantitative among alkylbenzene sulfonates when the alkyl chain is shorter than about eight carbon atoms, and becomes essentially zero with the sulfonates of benzene, toluene and xylene.

Although these low molecular weight aromatic sulfonates have few detergency properties, their function and substantial commercial use as solubilizers in detergent formulations lend some importance to a study of their biodegradability.

Ultraviolet spectroscopic analysis was successfully applied to the study of the biodegradation of these compounds both in a synthetic medium-sewage *inoculated* system and in the well-known river die-away procedure.

Sensitivity to below 1 ppm was obtained from absorption bands between about 220 and 230 mμ. Bands were also utilized near 260–270 mμ for those compounds having significant absorption in this region. Information concerning changes in functional group substitution and aromatic degradation was obtained from monitoring these two band systems during the biodegradation period.

Introduction

DURING THE PAST few years much attention has been focused on the problems of detergent biodegradation. In the search for more biodegradable detergent materials it was, of course, necessary to develop suitable biological test methods and the necessary analytical techniques to assay the results of the biological tests.

The river die-away, semicontinuous activated sludge, and shake flask systems are the most common biological test methods employed in detergent biodegradation studies. Warburg manometry and biochemical oxygen demand (BOD) tests are less frequently used in such studies.

The methylene blue colorimetric analysis (1) is by far the most common method of analysis for anionic detergents in the various biological test systems. The cobaltothiocyanate method (2) appears to be a satisfactory test for nonionic detergents in the various test systems. Surface tension and foam measurement methods also have been used to monitor detergent degradation, though these measurements do not provide sufficient sensitivity to detect small changes in detergent concentration.

However, with the exception of Warburg manometry and BOD tests, none of the analytical techniques mentioned above are suitable for monitoring the biodegradation of benzene, toluene and the xylene sulfonates. These compounds do not respond to the methylene blue test, and since they also neither foam nor significantly affect surface tension, these methods of analysis cannot be applied.

Warburg manometry could be used with these compounds, but the technique is somewhat difficult to apply, requires special equipment, and as normally occurs with such compounds, considerably less than 100% of the theoretical oxygen requirement for complete oxidation of the compound is obtained—which complicates a proper interpretation of the test result. BOD tests could be used; however, as ordinarily applied, the analysis method is slow and tedious and

[1] Presented at the AOCS Meeting, Houston, April 1965.

TABLE I

Composition of Medium

Compound	Wt. g
NH_4Cl	0.1500
K_2HPO_2	0.0750
$MgSO_4$	0.0125
KCl	0.0125
Yeast extract	0.0050
Deionized water	1.0 liter

also presents the same interpretive problems as the Warburg method. A recent paper by Kelly et al. (3) describes a special biodegradation test and chemical oxygen demand (COD) analysis using the AutoAnalyzer; however, this procedure still does not solve the problem of interpretation of results and thus the use of the test remains open to question.

Toluene and xylene sulfonate products commonly known as hydrotropes are used in the detergent industry primarily as solubilizing agents to increase the salt content in built heavy-duty liquid detergents. The most commonly used type is sodium xylene sulfonate. In a recent year production of this material amounted to 22.9 million pounds (4).

The present work utilized both a river water and a synthetic preparation as the biological test media for the hydrotrope biodegradation study.

Ultraviolet spectroscopic analysis was used to monitor the degradation of the hydrotropes, which included benzene, toluene and the xylene sulfonate isomers. The UV analyses were made using a Beckman DB spectrophotometer with associated recorder and a Beckman scale expander.

The UV method of analysis provides several unique advantages over the other common analytical methods previously mentioned. A UV analysis can be made directly without any concentration or extraction steps, since with these compounds sensitivity is better than 1 ppm. Additionally, UV analysis provides information about the molecule—specifically, the aromatic system—and, therefore, during the biodegradation process it should be possible to note functional group changes as they might occur on or in the near proximity of the aromatic ring. Disappearance of UV absorption during the degradation is generally considered to indicate rupture of the benzene ring system.

Experimental

The samples under study were added to half-gallon fruit jars and diluted to 1 liter to give a 5 ppm concentration with either river water or, as in most of our studies, with the aqueous synthetic medium shown in Table I.

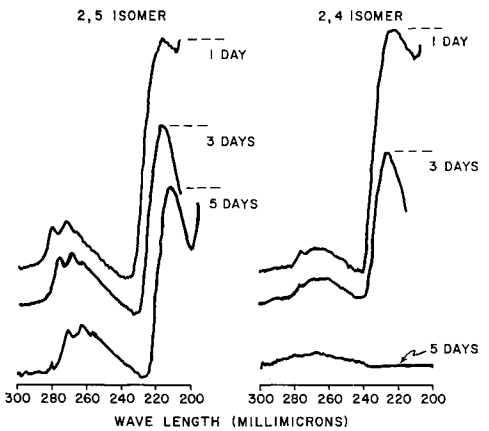

FIG. 1. UV spectra of 2,4 and 2,5 xylene sulfonate vs. biodegradation time. River water medium.

TABLE II

Ultraviolet Wave Length and Absorption Maxima of Some Aromatic Compounds

Compound	K Band mμ	E max	B Band mμ	E max
Benzene	200	7,000	255	220
Phenol	210.5	6,200	270	1,450
Benzaldehyde	244	11,400	280	
Benzoic acid	230	11,600	273	970
Benzene sulfonate	215	8,500	262	<800
p-Toluene sulfonic acid	220	12,000	260	<800
p-Xylene sodium sulfonate	216	9,000	271	1,500

In the synthetic system each jar was inoculated with 5 ml of raw sewage. After mixing the contents, the lids were loosened and the samples allowed to incubate on a bench top at room temperature. A control jar containing all ingredients except the sample, was prepared and inoculated in the same manner to serve as a reference medium for the UV analysis.

Aliquots were removed at intervals for the UV analyses which were made in 1 cm cells using the solution from the control jar as the reference solution in the spectrophotometer. The wave length region from 300 to 200 mμ was scanned at each sampling period, since these compounds exhibited two usable absorption peaks, one near 220 mμ and another generally near 270 mμ.

Results and Discussion

Benzene exhibits two intense absorption bands in the UV at about 180 mμ and 200 mμ, and a weak absorption band around 255 mμ. The molar absorptivity of these three bands is about 47,000, 7,000 and 220 $1/mole/cm^{-1}$, respectively. All three bands are associated with the π electron system of benzene and are affected by ring substitution. With the compounds included in this study we are concerned with two UV band systems near 220 and 270 mμ; these bands are the 200 and 255 bands of benzene shifted to the longer wave lengths by the ring substitutions. The shorter wave length band is often called a K, or primary, band and the longer wave length band noted as a B, or secondary, band. The B bands are the most characteristic of benzene and its derivatives, and in this band system one would expect to note changes in the UV spectrum if changes in functionality were introduced into the benzene ring during biodegradation. Table II shows the effect of ring substitution of some functional groups which presumably could be encountered in the biodegradation pathway of many of the compounds included in this study.

The data for the aromatic sulfonate compounds were calculated from our experimental work and are in good agreement with values reported by Weber et al. (5).

The major difficulty encountered in the UV analysis was the difficulty in maintaining a balance between the reference solution and the sample. Distilled water could not be used for the full UV spectrum since the synthetic medium itself contained a strong absorption beginning at about 250 mμ.

The synthetic medium control solution, however, was satisfactory as a reference solution generally for about 10–12 days of incubation. After this time an imbalance in the system would occur, shown in most cases by a strong increase in absorption below about 240 mμ in the control reference solution masking any sample absorption below this wave length.

This interference was a problem in our earliest

TABLE III

Biodegradation of Aromatic Sulfonates in Synthetic and in River Water Medium—UV Analysis

Sulfonate compound	Medium	Days of Incubation (percent remaining)													
		1	2	3	4	5	6	10	15	20	25	30	35	45	55
Benzene	Synthetic	100	37	8	5	5	0								
Toulene	Synthetic	75	0												
Ethylbenzene	Synthetic	100		100	97	57	0								
2,4 Xylene	Synthetic	100	92	84	6	0									
2,3 Xylene	Synthetic	100				100			100		100	93	25	0	
2,5-3,5 Xylene [a]	Synthetic	100				100			70		70	70	15		
2,5 Xylene	Synthetic	100				100			100		100		100	100	
2,5 Xylene	River Water	100								40					
2,5 Xylene	River Water	100													0

[a] Prepared by rearrangement (7) from 2,5 xylene sulfonates, NMR analysis 60% 3,5 isomer and 40% 2,5 isomer.

work with the river die-away medium, where measurement of the peak near 220 mμ was desirable to obtain its high sensitivity, since 1 cm cells without scale expansion were used at that time.

During the work with the river water medium it was observed that the river water reference control solution seemed to maintain a balance with the river water containing samples for an extended period, *if* sample biodegradation was not occurring. In many cases with the river water medium, once extensive or complete sample degradation had occurred the control reference solution would no longer balance the sample solution. In most cases this condition was again noted by a strong increase in absorption in the reference solution over the sample solution, causing a below baseline deflection of the recorder pen. It appears, therefore, that the sample has an influence on the system—perhaps preventing the formation of some UV absorbing material which *is* formed in the medium without added sample, or biodegradation of the sample also initiates degradation of organic matter in the sample medium at a more complete or a faster rate than in the control medium containing no sample. Either of these conditions could cause a relative increase in the absorption of the control solution.

This condition can be clearly shown in the river water biodegradation of 2,4 and 2,5 xylene sulfonates, as shown in Figure 1. Both samples were incubated on the same day under identical conditions. After five days the 2,4 isomer was apparently extensively degraded, with disappearance of the absorption near 270 mμ—however, a strong imbalance in absorption in favor of the reference solution was obtained at 230 mμ in the manner previously described, while at the same five-day check the 2,5 isomer absorption below 230 mμ (at 220 mμ) was nearly the same as the first day, indicating little degradation and no change in the sample versus control solution balance.

This condition was not obtained with all samples in the river water medium—some compounds degraded completely and an essentially flat baseline UV absorption was obtained from 300 to 200 mμ. Some compounds also gave useful absorption at 220 mμ even after several weeks of incubation using the regular control medium as a reference. However, the difficulty as shown in Figure 1 was very frequent and led to the development and adoption of the synthetic medium system.

As previously mentioned, in the synthetic medium system an apparently perfect balance between control and sample solution was maintanied for about 10 days—all samples degrading within this period yielding an essentially baseline UV spectrum. Samples requiring longer incubation were satisfactorily continued by monitoring the band system around 270 mμ using a scale expander or preferably a longer cell path to increase the sensitivity. This "B" band system is the most characteristic of benzene and its derivatives and could be used exclusively in degradation studies except at very low concentrations or among the aromatic sulfonate compounds which have very low absorption in this UV region.

Table III shows the results obtained from the UV analysis of a variety of aromatic sulfonates.

The data in Table III show that all of the aromatic sulfonates listed were degraded in the synthetic medium system, except 2,5 xylene sulfonate. With additional time this isomer also would undoubtedly be degraded, since it was 100% degraded in the river water medium. Results are shown for the 2,5 isomer in the river water medium, since this isomer has sufficient absorption in the interference-free 270 mμ region to yield quantitative results at the concentration employed.

In a readdition study, a 7.5 ppm quantity of 2,5 xylene sulfonate was added to a river water medium in which a 5 ppm charge of a commercial xylene sulfonate had been 85% degraded in 6 days' incubation time. A 100% degradation was obtained in 17 days, showing a very significant response of the 2,5 isomer to simple adaptation.

Nevertheless, the differences in nonadapted biodegradability of the xylene sulfonate isomers, particularly the 2,4 and 2,5 isomers, is a very interesting finding, and further work is in progress in an attempt to explain *this phenomenon.*

Figure 2 shows the recorded UV spectra of benzene, toluene and 2,4 xylene sulfonate during the biodegradation of these compounds.

The disappearance of essentially all UV absorption between 200 and 300 mμ is considered to indicate cleavage of the benzene ring, though not necessarily indicating complete oxidation to CO_2 and H_2O, since

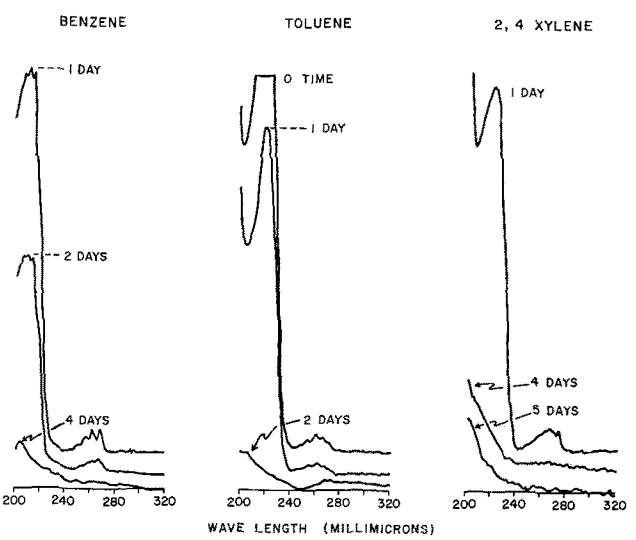

FIG. 2. UV spectra of benzene, toluene and 2,4 xylene sulfonate versus biodegradation time.

some organic residue transparent to UV absorption could be present.

The data obtained in this study indicate that benzene ring biodegradation is obtained with all of the aromatic sulfonates shown in Table III.

Foster et al. (6) have reported that benzene ring biodegradation occurs with commercial LAS compounds. This data, coupled with our results on hydrotrope degradation, indicate that benzene ring residual material is not a problem with biodegradable aromatic sulfonates.

During the biodegradation of these hydrotrope compounds the expected changes in the UV spectrum indicating formation of biological intermediates was not generally observed. It is probable that the transition from initiation of oxidation to ring cleavage is very rapid—some evidence for this was shown in Figure 2 where toluene sulfonate dropped from a level of 75% of its original concentration to zero concentration with an essentially transparent UV spectrum, in one day. It is also quite possible that biological intermediates might not be released from the oxidizing microorganisms after their formation. Cell extracts will be examined in future studies to explore this possibility.

A special effort was made to detect intermediate compounds by UV analysis in an experiment using a flowing cell arrangement with the DB spectrophotometer. The sample contained in a glass fruit jar was pumped into a second jar from which the solution flowed by gravity through the UV cell and returned to the sample jar. This arrangement prevented the pumping surge through the cell which caused large recorder deflections.

The DB spectrophotometer was allowed to operate continuously through its full cycle, which required about 18 min. The UV span between 300–200 mμ occupied 2 min of this 18-min cycle time.

A reference solution containing medium and sewage inoculum without added detergent was placed in the reference beam of the spectrophotometer using a standard 1 cm cell.

A sample of toluene sulfonate was prepared, inoculated with sewage microorganisms previously adapted to the toluene sulfonate, and the system placed in operation as described above.

The UV spectra indicates a change occurred in 3 hr, and a slight additional change in 20 hr. However, some sample turbidity had developed causing background interference, and since the change between 3 hr and 20 hr appeared to be relatively small, the experiment was discontinued. This study is described principally to indicate this potential application of UV spectrophotometry for the study of transient intermediate compounds.

Dagley et al. (7) mention a biological mechanism involving replacement of an aromatic functional group with hydrogen. If this were the mechanism of biodegradation with these hydrotrope compounds, the resulting hydrocarbons would probably not be detected in the UV analysis at the concentrations employed in this study, due to the decreased absorptivity of both the K and B band systems and the shift to a very short wave length of the sensitive K band.

However, this mechanism is considered to be unlikely with these compounds. When sulfonate group cleavage does occur, it is expected that the mechanism would follow one of the well-known aromatic pathways involving hydroxylation, and subsequent ring cleavage.

It does appear that the biodegradation of the compounds reported in this study provide little or no extracellular build-up of an aromatic biological intermediate, indicating a quick benzene ring cleavage once bio-oxidation has been initiated, or a retention of the intermediates by the microorganisms.

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Performance Evaluation of Selected Fabric Softeners

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Abstract

Some methods and variables of importance in softener evaluation were examined. A subjective, paired-comparison panel method is recommended for softening measurement, and a dye wicking method seems most reliable for rewettability measurement. Use of these methods shows that softening tends to decrease and rewettability to increase with unsaturation and introduction of ether groups in bridging radicals and with reduction of alkyl chain length, in a word with increasing hydrophilicity of various radicals. Rewet data are particularly sensitive to these effects. For excellent softening, two long chain alkyl groups are apparently required, and these should be at least C_{16} and more preferably C_{18} in length.

Introduction

CATIONIC SOFTENERS are in widespread use for the treatment of fabrics in commercial laundries and hospital institutions, and are now available in household products. These agents are added to the rinse water during normal laundering to impart softness, fluffiness, and antistatic qualities to the fabric (1,2,3). Agents may be included in softener products to change antibacterial effect (4). Cationic softeners are usually quaternary ammonium compounds such as dialkyldimethylammonium chloride (DADMAC), where the alkyl group is derived from hydrogenated tallow, ethoxylated analogues and imidazolinium salts. These compounds are highly substantive on cotton and rayon fabrics and when absorbed, produce desired effects (5).

Excluding germicidal properties, the criteria of