

Microbial Degradation of the Sulfonate of Dodecyl Benzene Sulfonate

MELVIN A. BENARDE, BERNARD W. KOFT, RAYMOND HORVATH, AND LOUIS SHAULIS

Department of Civil Engineering and Department of Bacteriology, Rutgers, The State University, New Brunswick, New Jersey

Received for publication 28 August 1964

ABSTRACT

BENARDE, MELVIN A. (Rutgers, The State University, New Brunswick, N.J.), BERNARD W. KOFT, RAYMOND HORVATH, AND LOUIS SHAULIS. Microbial degradation of the sulfonate of dodecyl benzene sulfonate. *Appl. Microbiol.* **13**:103-105. 1965.—It has been observed that the sulfonate portion of alkyl benzene sulfonate (ABS) will undergo microbial attack in certain pure and mixed cultures if an energy source, such as glucose, is available. The evidence for this is provided by the stoichiometric relationship between the reduction of ABS concentration and the appearance of inorganic sulfur compounds.

The surfactant, dodecyl benzene sulfonate, a component of domestic and industrial detergents, is generally accepted as a recalcitrant molecule, refractory to microbial metabolism. As a result, residues remain to build up in soil and water. The billows of foam observed on watercourses and in sewage plants need no additional documentation here. Public health implications of this accumulation have led to legislation in this country and abroad, curtailing the use of this compound.

For the most part, studies investigating the biological disruption of this molecule have been directed at total dissimilation of the carbon skeleton. Investigators' approaches have utilized natural systems such as river and lake water, activated sludge, and other sewage material as sources of microbial activity to achieve the proposed disruption (Lamb, 1953; Degens et al., 1950; Hammerton, 1955; McKinney and Symons, 1959; Wayman and Robertson, 1963). The approaches have been generally unsuccessful.

Essentially, all of these experiments can be considered as a selective enrichment-culture approach in which alkyl (dodecyl) benzene sulfonate (ABS) was to serve as both the carbon and energy source for the proposed selective isolation of specific microbial populations capable of metabolizing ABS. As these investigations have proven fruitless, they have given rise to the concept of "recalcitrance." Briefly, the theory supporting recalcitrance develops the idea that β -oxidation of the alkyl moiety is prevented by

the branched configuration. If this proposal is valid, then another approach is needed.

This surfactant possesses both hydrophobic and hydrophilic moieties, thus, it seemed reasonable to investigate the possibility of metabolizing the hydrophilic sulfonate structure. Interestingly enough, House and Fries (1956), seeking information on the interaction of ABS and activated sludge, noted the production of significant amounts of inorganic sulfur-35 compounds from sulfur-35-labeled ABS. In addition, Leclerc and Beaujean (1952) reported production of hydrogen sulfide by bacteria metabolizing ABS. As these investigators used commercial ABS (sulfate content as high as 40%) in their study, there is some question as to the origin of the sulfide sulfur reported. To our knowledge, these observations have not been pursued further. However, they tend to support the rationale that organisms capable of removing this sulfonate should occur in nature. Active metabolism of this sulfonate would eliminate the detergency character, water solubility, and, hence, foaming.

MATERIALS AND METHODS

Accordingly, enrichment culture systems were devised in which ABS was supplied only as the sulfur source, rather than a carbon and energy source. Glucose served this requirement in the media. ABS was purified by ether extraction to remove the inorganic sulfate. The enrichment methods consisted of continuously profusing columns of mixed soils and of Winogradski column isolation. ABS-glucose-basal salts medium (with-

out sulfates) was allowed to drip slowly through glass columns (61 × 5.1 cm) containing soil samples. The medium consisted of the following: ABS (purified to 100% activity), 0.5 g; glucose, 1.5 g; NH_4Cl , 1.0 g; Na_2HPO_4 , 1.0 g; KCl , 0.5 g; MgCl_2 , 0.1 g; distilled water, to 1 liter; agar, 12 g; $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 0.2 g. Effluents for further study were collected in flasks. The Winogradski column, containing ABS, pond mud, lake water, and shredded filter paper, was allowed to stand for 1 week before isolation-sampling began. Lead acetate-impregnated paper strips were hung in the effluent-containing flasks and Winogradski transfer flasks, and allowed to stand for observation of growth and hydrogen sulfide production.

Subcultures in ABS glucose media were made with material from flasks showing either H_2S production or reduction of ABS concentration as measured by methylene blue determination (American Public Health Association, 1960). From media showing the responses noted, samples were plated on ABS-glucose-iron agar for pure culture isolation. Discoloration of the medium around colonies as a result of iron sulfide formation was of particular interest. From these platings, many promising cultures were obtained. Four isolates proved to be significantly active in metabolizing ABS as indicated by the methylene blue procedure. These were all short, gram-negative, aerobic rods.

The methylene blue procedure for ABS analysis depends upon two primary factors: the presence of the sulfonate moiety, and, to some extent, the length of the alkyl side chain. To be certain that the sulfonate structure was being metabolized, it was necessary to augment the analysis of ABS with a procedure specific for sulfur. A modified sulfate determination as given in *Standard Methods* was used (American Public Health Association, 1960). This consisted of ether extraction of the ABS, followed by H_2O_2 oxidation of the inorganic sulfur compounds. A positive test would require the generation of inorganic sulfur compounds either as a sulfate or as those oxidizable to sulfate by addition of hydrogen peroxide. The correlation of these two tests would allow specific study of sulfonate removal.

RESULTS AND DISCUSSION

Much of our present knowledge of sulfate metabolism has been derived from studies of the anaerobe *Desulfovibrio desulfuricans*. Peck (1962) has reviewed the work in this area and has proposed an energy-yielding metabolic scheme. Aerobic metabolism of sulfates has not yet reached this level of sophistication.

The results in Fig. 1 show the ABS dissimilating activity of a pure culture grown aerobically in a gyratory shaker at ambient temperature. For each millimole of ABS that has disappeared, an equivalent amount of inorganic sulfur has been produced. Approximately 70% of the ABS was

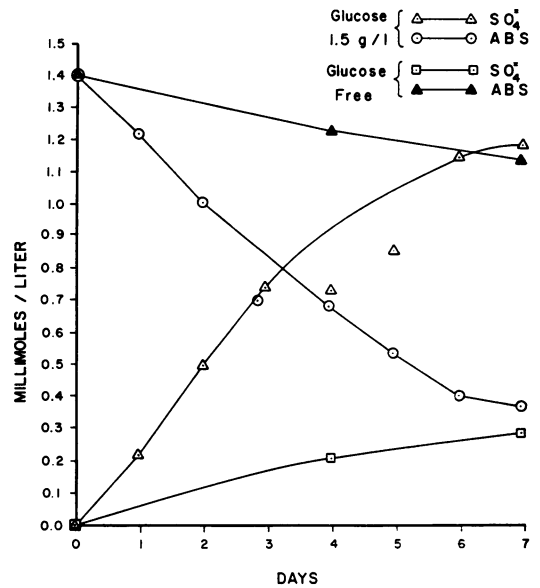


FIG. 1. Elaboration of inorganic sulfur (sulfate ion) during microbial metabolism of ABS.

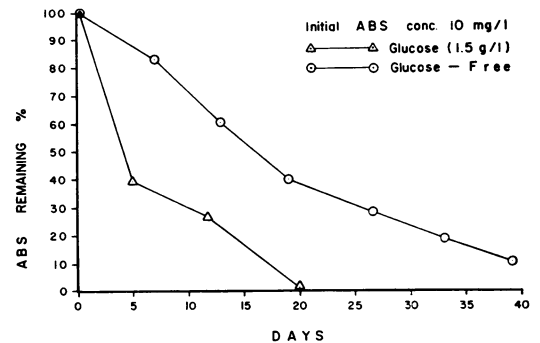


FIG. 2. Disappearance of ABS in lake-water aquariums.

removed in 7 days. This may be compared with the 1 to 2 months required to achieve 30 to 40% reductions, as reported in the references cited.

The addition of either cysteine, methionine, or sodium sulfate to the test system did not alter the rate or total amount of sulfonate metabolism.

The breakdown of ABS in an aquarium containing lake water is indicated in Fig. 2. ABS degradation studies were carried out in aerated aquariums containing approximately 20 gal of water taken from Lake Carnegie, Princeton, N.J. Two aquariums were set up. To one, only ABS and our organism were added; the other contained ABS, glucose (1.5 g per liter), and the organism. A similar pattern of disappearance of ABS occurs when this organism is present in a

mixed culture system. Again, a much shorter period of ABS dissimilation was observed than previously reported. Our values for ABS resemble Sharman's (1964) values for straight-chain biodegradable compounds.

From the data, we concluded that the metabolism of ABS can be carried out by organisms found in nature, and more rapidly than previously reported if an energy source other than the ABS molecule itself is supplied.

LITERATURE CITED

- AMERICAN PUBLIC HEALTH ASSOCIATION. 1960. Standard methods for the examination of water and waste water, 11th ed. American Public Health Association, New York.
- DEGENS, P. N., H. VANDER ZEE, J. D. KOMMER, AND A. H. KAMPHUIS. 1950. Synthetic detergents and sewage processing. V. Effect of synthetic detergents on certain water fauna. *J. Inst. Sewage Purif.* **1**:63-68.
- HAMMERTON, C. 1955. Observations on the decay of synthetic anionic detergents in natural waters. *J. Appl. Chem. London* **5**:517-524.
- HOUSE, R., AND B. A. FRIES. 1956. Radioactive ABS in activated sludge treatment. *Sewage Ind. Wastes* **28**:492-506.
- LAMB, C. 1953. Experiments on the effects of certain detergents on the biological oxidation of sewage. *J. Inst. Sewage Purif.* **4**:269-281.
- LECLERC, E., AND P. BEAUJEAN. 1952. Influence d'un détergent synthétique sur l'autoé puration d'ure eau d'égout. *Bull. Centre Belge Etude Doc. Eaux Liege* **17**:152-158.
- McKINNEY, R. E., AND J. M. SYMONS. 1959. Bacterial degradation of ABS. I. Fundamental biochemistry. *Sewage Ind. Wastes* **31**:549-556.
- PECK, H. D., JR. 1962. Symposium on metabolism of inorganic compounds. V. Comparative metabolism of inorganic sulfur compounds in microorganisms. *Bacteriol Rev.* **26**:67-94.
- SHARMAN, S. H. 1964. Extensive biodegradation of synthetic detergents. *Nature* **201**:704.
- WAYMAN, C. H., AND J. B. ROBERTSON. 1963. Biodegradation of anionic and nonionic surfactants under aerobic and anaerobic conditions. *Biotech. Bioeng.* **5**:367-384.