Analysis for Sulfate Ion in the Biodegradation of Anionic Detergents[1]

2919

T. C. CORDON, E. W. MAURER, O. PANASIUK and A. J. STIRTON,
Eastern Regional Research Laboratory,[2] Philadelphia, Pennsylvania 19118

Abstract

A turbidimetric method is described for determining sulfate ion in parts per million formed during the biodegradation of surface-active organic sulfates or sulfonates. The procedure involves the precipitation of barium sulfate under controlled conditions, using a reagent consisting of 0.5% gelatin and 1% barium chloride in 50% ethanol. Gelatin stabilizes the barium sulfate suspension and helps prevent the formation of turbidity from barium chloride and detergent. Ethanol destroys any protective colloid action that may inhibit the precipitation of barium sulfate. Corrections were necessary for turbidity caused by the microorganisms and in a few instances for turbidity caused by the reaction of detergent and barium chloride. Absorbance was read against a blank at 420 mμ using 5 cm cells. Parts per million sulfate were read from a standard curve. Phosphates do not interfere in the determination.

Introduction

THE PROBLEM OF THE BIODEGRADATION of synthetic detergents in aerated water and sewage systems appears to have been largely solved by the substitution in commerce of linear alkylbenzenesulfonates (LAS) for the branched chain isomer mixtures formerly in use. However, linear alkylbenzenesulfonates are not biodegradable under anaerobic conditions and there is still considerable interest in the mechanisms of biodegradation of synthetic detergents. Evidence has been presented that in the case of alkylbenzenesulfonates attack occurs first on the alkyl side chain.

The biodegradation of alkylbenzenesulfonates to sulfate ion has been investigated by several workers (2,5,7). Primary alkylbenzenesulfonates have been shown to be more readily and completely degraded than secondary, and tertiary to be quite resistant (7). Secondary treatment of sewage has been found to convert 80–90% of branched chain alkylbenzenesulfonate (ABS), tagged with S^{35}, to inorganic sulfate (5). Biodegradation of ABS by pure and mixed cultures in the presence of glucose as an energy source showed that for each millimole of ABS that disappeared, as measured by methylene blue, an equivalent amount of inorganic sulfate was formed (2).

We were interested in following the formation of sulfate ion during the biodegradation of tallow-based detergents, using the Esso Research biodegradation test (3,6). A standard method for sulfates (1) which had been applied in biodegradation studies (2), was found to be unsuitable in the presence of detergents.

A turbidimetric procedure was developed for determining inorganic sulfates in the presence of anionic detergents and inorganic phosphate, based on the method of Dahlgren (4). A barium chloride-gelatin solution was used with apparently good results until it was discovered, with a known sample, that the presence of detergent inhibited the precipitation of barium sulfate. This may be due to protective colloid action. It was corrected by adding ethanol to the barium chloride-gelatin reagent.

Further investigation of the analytical procedure showed that some turbidity was formed by the reaction of barium chloride with less soluble detergents in the absence of sulfate ion. Methyl cellulose or glycerol were not as effective as gelatin in preventing this. Correction for turbidity due to the detergent can be made from data such as that in Table I, when detergent concentration is known. If detergent concentration changes, as it does during the Esso test, it may be estimated from COD (chemical oxidation demand) and MBAS (methylene blue active substance) values.

When sodium octadecyloxyethyl sulfate, which is not easily soluble at room temperature (Krafft point 46C), was run with this procedure high absorbance was obtained at the beginning of the test, due to the detergent. Absorbance decreased rapidly as the detergent was broken down, and then increased as inorganic sulfate was formed. We have no evidence that partially degraded detergents produce turbidity with the barium chloride-gelatin-aqueous ethanol reagent.

Experimental

The biodegradation tests (3,6) were carried out in 1-gallon wide mouth jars containing 3 liters of deionized water, 10 mg/liter of inoculum, nutrient salts, and 40 mg/liter of detergent. Contents were stirred continuously by magnetic stirrers to insure adequate aeration.

Nutrient Salts

Stock solution No. 1—Dissolve the following in one liter of deionized water:

$Mg(NO_3)_2 \cdot 6H_2O$	5.375 g
$Ca(NO_3)_2 \cdot 4H_2O$	2.925 g
$Fe(NO_2)_3 \cdot 9H_2O$	0.375 g
$CoCl_2 \cdot 6H_2O$	1.025 g

Stock solution No. 2—Dissolve the following in one liter of deionized water:

$(NH_4)_2HPO_4$	4.76 g
K_2HPO_4	36.6 g
KH_2PO_4	31.1 g

Use 1 ml of solution No. 1 and 5 ml of solution No. 2 in deionized water to make one liter of nutrient salts solution.

Reagents

The following reagents and standard solutions were prepared.

Gelatin-Ethanol Solution. Gelatin, 5.0 g, was placed in 500 ml deionized water and warmed to 50C with stirring. The solution was cooled to room temperature and made to 1 liter with absolute

[1] Presented at the AOCS Meeting, Chicago, October 1967.
[2] E. Utilization Res. Dev. Div., ARS, USDA.

TABLE I

Turbidity as a Function of Detergent Concentration, in the Absence of Sulfate Ion

Detergent	Absorbance at 420 mμ			
	40 ppm	30 ppm	20 ppm	10 ppm
Sodium octadecyl-oxyethyl sulfate $C_{18}H_{37}OC_2H_4OSO_3Na$	0.192	0.115	0.093	0.003
Sodium 2-hydroxy-octadecanesulfonate $C_{16}H_{33}CHOHCH_2SO_3Na$	0.193	0.091	0.048	0

ethanol. This solution may be used for at least 3 weeks. Not all brands of gelatin are satisfactory and we have used Gelatin, Bacteriological Granular supplied by the Amend Drug and Chemical Company. It is made by the partial hydrolysis of collagen derived from the skin, white connective tissue and bones of domestic animals used for food by man.

Barium Chloride-Gelatin-Ethanol. An aqueous gelatin solution, 500 ml of 1%, in deionized water, was prepared by warming to 50C. Barium chloride dihydrate, 25.0 g, was added, the mixture was stirred until dissolved and then made to 1000 ml with absolute ethanol. This solution should stand at least 2 days before use. Stir the solution well, drain the buret and reservoir and refill with freshly stirred solution immediately before use. This solution will keep at least 3 weeks.

Standard K_2SO_4 Solution. A stock solution, 1 ml = 0.75 mg SO_4^{--}, was prepared by dissolving 1.361 g of dried, reagent grade K_2SO_4 in 1 liter of nutrient salts solution. Solutions of concentration 3, 6, 9 and 12 ppm SO_4^{--} were prepared by adding 4, 8, 12 and 16 ml of stock from a buret to a 1-liter flask and diluting to volume with nutrient salt solution.

Turbidity Corrections

A correction for turbidity of the test solution due to microorganisms, in the absence of barium ions, can be made as follows:

1) Pipet a 25-ml sample of the test solution into a 50-ml volumetric flask, add 1 ml 85% H_3PO_4 and 5 ml of gelatin-ethanol solution, and make to volume with absolute ethanol.

2) Prepare a blank, pipetting 25 ml of nutrient solution, 1 ml 85% H_3PO_4 and 5 ml of gelatin-ethanol solution into a 50 ml volumetric flask. Make to volume with absolute ethanol.

3) Fill 5-cm cells with the respective solutions and determine the absorbance of the sample solution against that of the blank at 420 mμ. A Beckman Model B is a suitable type of spectrophotometer.

A correction for turbidity due to reaction of barium chloride with the detergent can be made by running the detergent in question through the analysis for sulfate at concentrations to be expected in the samples. If significant absorbance is found, values are recorded at several concentrations. The concentration of detergent at any stage in the biodegradation test can be estimated from COD and MBAS values and the appropriate correction made.

Analysis for SO_4^{--}

Analysis for SO_4^{--} at any stage in the biodegradation test is made as follows:

1) Place 25 ml of the test solution, 1 ml 85% H_3PO_4, 5 ml of gelatin-ethanol solution and 13 ml of absolute ethanol in a 50 ml volumetric flask. Mix after the addition of each reagent.

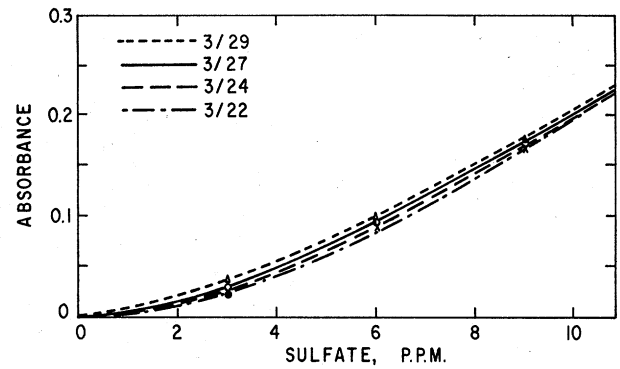

FIG. 1. Standard curve relating absorbance and ppm SO_4^{--}. Same $BaCl_2$-gelatin-ethanol reagent used over a period of 1 week.

2) Add 5 ml of the $BaCl_2$-gelatin-ethanol solution at the rate of 5 ml/2 min with constant uniform stirring (magnetic stirrer). Use a 5- or 10-ml reservoir type buret with needle valve control. Make to volume with absolute ethanol without correction for the presence of the magnetic stirrer.

3) Prepare a blank in the same manner using 25 ml of nutrient solution. Read the absorbance of the sample against the blank at 420 mμ in 5-cm cells.

4) Make three corrections to the absorbance reading by subtracting (a), the absorbance reading from the turbidity due to microorganisms, and (b) the absorbance reading from the blank (a run that contains the nutrient salts and inoculum but no detergent). This correction is necessary because some materials in the inoculum, probably sulfates, react with $BaCl_2$. In applicable cases subtract also (c), the absorbance due to detergent. From the remaining absorbance determine ppm SO_4^{--} from a standard curve.

The Standard Curve

Absorbance readings can be translated to ppm SO_4^{--} making use of the standard K_2SO_4 solution.

Place 25 ml of the 3, 6, 9 and 12 ppm SO_4^{--} solutions, measured with a buret, in 50 ml volumetric flasks. Form the $BaSO_4$ and determine the absorbance as directed by the analytical procedure, and plot ppm SO_4^{--} against absorbance.

There is some variation in standard curves run from day to day, and standard curves run with different lots of $BaCl_2$-gelatin-ethanol may not be identical. It is necessary, therefore, to prepare standard curves frequently and for most accurate results a standard curve should be made for each

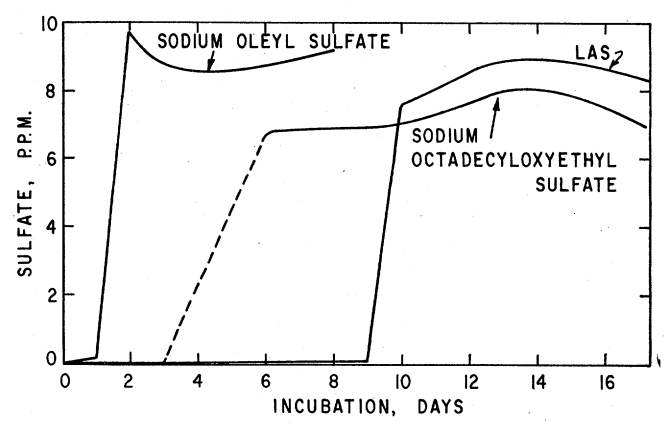

FIG. 2. Biodegradation of anionic detergents measured by formation of sulfate ion.

run. The variability of standard curves from one lot of $BaCl_2$-gelatin-ethanol over a 1-week period is shown in Fig. 1.

Results and Discussion

Sulfate analysis during the biodegradation of linear alkylbenzenesulfonate (LAS), sodium octadecyloxyethyl sulfate and sodium oleyl sulfate showed a maximum of 9.1, 8.2, and 9.7 ppm SO_4^{--} compared to theoretical values of 11.0, 9.2, and 10.4. This corresponds to 83%, 89%, and 93% degradation to sulfate ion, in 13, 13 and 2 days, respectively. Sulfate ion formation was followed daily except for weekends and there are no values for sodium octadecyloxyethyl sulfate for the fourth and fifth days. The results are shown in Fig. 2. Sulfur present in the detergent as organic sulfate or sulfonate may not be completely recoverable as SO_4^{--} because it is used in some form, in small amount, by the microorganisms in their metabolism.

REFERENCES

1. American Public Health Association, "Standard Methods for the Examination of Water and Wastewater," 11th Ed., American Public Health Association, New York, 1960.
2. Benarde, M. A., B. W. Koft, R. Horvath and L. Shaulis. Appl. Microbiol. *13*, 103–105 (1965).
3. Cordon, T. C., E. W. Maurer, M. V. Nuñez-Ponzoa and A. J. Stirton. Appl. Microbiol. *16*, 648–652 (1968).
4. Dahlgren, S. E., Acta Chem. Scand. *14*, 1279–1287 (1960).
5. House, R., and B. A. Fries, Sewage and Ind. Wastes *28*, 492–506 (1956).
6. Konecky, M. S., R. J. Kelly, J. M. Symons and P. L. McCarty, presented at the 36th Annual Meeting, Water Pollution Control Federation, Seattle, Washington, Oct. 6–10, 1963.
7. Ryckman, D. W., and C. N. Sawyer, Proceedings, 12th Industrial Waste Conference, Purdue University, Lafayette, Ind., p. 270–284 (1957).