

TECHNICAL REPORT SUMMARY

Date
8/25/81

TO: TECHNICAL COMMUNICATIONS CENTER - 201-2CN

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Division Environmental Laboratory (EE & PC)		Dept. Number 0535
Project Commercial Chemicals Division		Project Number 9970012600
Report Title Biosorption of CS-2151 (CS-2070)		Report Number
To D. R. Ricker, Commercial Chemicals Division, 53-4N		
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Notebook Reference None (see Lab Request 6457 S)		No. of Pages Including Coversheet
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KEYWORDS:
(Select terms from 3M
Thesaurus. Suggest other
applicable terms.)EE & PC DIV
(ENV. LAB)
Biosorption
Bioconcentration
Microorganisms
Fluorochemical
Surfactants
Waste Treatment

CURRENT OBJECTIVE:

To measure the biosorption partition coefficient of CS-2070.

REPORT ABSTRACT: (200-250 words) This abstract information is distributed by the Technical Communications Center to alert 3M'ers to Company R&D. It is Company confidential material.

A 0.125 mg/l solution of CS-2070 (the solids portion of CS-2151) was shaken for 1 hour at 25°C in the presence of 2,000 mg/l of viable or heat killed activated sludge. After removing solids by centrifugation, the test material concentration was determined as the difference between the TOF* concentration of the test and control cultures. The results showed that most of the CS-2070 shaken in the presence of viable activated sludge remained in the solution. On the other hand, heat killed sludge apparently adsorbed CS-2070.

*TOF is Total Organic Fluorine.

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BIOSORPTION OF CS-2151 (CS-2070)

Introduction:

This report describes tests performed on CS-2151 to evaluate its biosorption to the volatile portion of activated sludge mixed liquor. Since CS-2151 is not likely to biodegrade, this testing indicates whether the subject material is likely to be removed in waste treatment through sorption to sludge. This testing is also useful in predicting whether CS-2151 is likely to accumulate through sorption to the organic portion of soil and aquatic sediments.

Methods and Materials:

The Environmental Laboratory received the test sample on 11/4/80. The sample was a light yellow liquid. It was reported to contain 25% solids in water. The solid portion of this product is designated CS-2070. Greg Vraspir performed this work in accordance with the attached Environmental Laboratory Protocol. L. D. Winter of the Commercial Chemicals Laboratory performed the analytical work. Since a sufficiently sensitive specific analytical technique was not available, the final CS-2070 concentration was determined by measuring the difference between the total organic fluorine (TOF) concentration in the CS-2070 containing sample and identically treated control samples not containing CS-2070.

Results and Discussion:

Results of this work are shown in Table 1 below:

Table 1.

Biosorption of 0.125 mg/l of CS-2070(1) on 2,000 mg/l of heat killed and viable activated sludge,

Condition of Sludge	TOF ⁽²⁾ Expressed as mg/l CS-2070		Final CS-2070 Concentration (mg/l) (by difference)
	Test	Control	
Heat Killed	1.1	1.1	0
Viable	.97	.84	.13
Sludge Free	.15	<.02	.15
Sorption Control ⁽³⁾	.10		

Footnotes:

(1) CS-2151 is a 25% aqueous solution of CS-2070.

(2) TOF is total organic fluorine.

(3) The sorption control is a CS-2070 solution that was neither centrifuged nor exposed to the plastic centrifuge tubes.

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After exposure to heat killed sludge the TOF concentrations of the test and control centrifugates were identical. This suggests that CS-2070 is absorbed by heat killed organisms. Viable organisms on the other hand, did not absorb CS-2070. This difference between the sorption of the test material by heat killed and viable cells was not totally unexpected. In all the bioadsorption studies reported by Smith et al. (1, 2) in which they measured the biosorption of hydrocarbons by both heat killed and viable cell culturals they found higher sorption coefficients with heat killed cells. The reason for this difference is not known.

Two additional parallel controls were run in this experiment, a sludge free control and a control to aid in determining whether CS-2070 sorbed to the plastic centrifuge tubes. These controls measured the CS-2070 concentrations in the absence of interfering organic fluorine from the activated sludge, and thus showed the precision of the analytical technique. The measured CS-2070 concentration in the sludge free control was 0.025 mg/l greater than that initially added, while the sorption control concentration was 0.025 mg/l less.

The precision of the measurements is too low to allow the calculation of biosorption coefficients for CS-2070. However, the data do indicate that CS-2070 is likely to remain in aqueous solution during passage through a wastewater treatment system. They also suggests that CS-2070 is not likely to adsorb to microorganisms or other organics in the aquatic or soil environments.

References:

- 1 Smith et al, Environmental Pathways of Selected Chemicals in Freshwater Systems, Part I, SRI International, EPA Contract #68-3-2227, EPA-600/7-77-113, October, 1977, NTIS #PB-274 548.
- 2 Smith et al, Environmental Pathways of Selected Chemicals in Freshwater Systems, Part II, EPA-600/7-78-074, May, 1978.

METHOD FOR MEASURING BIOSORPTION TO ACTIVATED SLUDGE

INTRODUCTION

In their report on experimental procedures for studying environmental pathways of chemicals in freshwater systems (5.1), Smith et al state that biosorption is an important parameter since it may affect biomagnification up the food chain, and the available concentration for biodegradation. They indicated that this phenomenon may also affect the viability of microorganisms that participate in the compounds biodegradation.

Still another reason for performing such tests is that biosorption may be a significant mode of removal of chemicals during its passage through a wastewater treatment system. The land disposal of the sludge from these treatment systems may be a significant mode of transporting the chemical to terrestrial ecosystems. Subsequent uptake and accumulation by plants may move the chemical into the human food chain.

This procedure is a simple method of measuring biosorption. It is modeled after that of Smith et al (5.1, 5.2) but differs in that it calculates the biosorption partition coefficient (K_p) based on the volatile suspended solids (VSS) concentration in activated sludge. The method of Smith et al uses a mixture of laboratory-grown pure cultures. This use of a naturally developed mixed population may cause some variability in results due to variation in the population structure of different activated sludge samples, but this variability should be small.

Smith et al interpreted data they generated (5.1) to indicate that the value of K_p for a specific type of environmental sample should not vary by more than a factor of 3.

Small variability will not affect the major purposes of the test described above, thus the precision requirements of this method don't justify the maintenance of pure cultures in the laboratory.

The method assumes that all sorption is to the volatile portion of the activated sludge. The validity of this assumption is substantiated by the fact that most sorption to soil can be attributed to its organic content (5.3). If the small organic content of soil (typically 2%) is responsible for most sorption to soil, it's reasonable to assume that the great majority of sorption to activated sludge will be due to its large organic content (typically 70%).

This method is suitable when analytical test methods are available for the chemical. It is not appropriate for readily biodegradable or hydrolyzed materials. However, the test can be done on stable degradation products.

The test measures biosorption at a mixed liquor suspended solids* (MLSS) concentration of 2,000 mg/l, and, if possible, at a test concentration of 1/2 mg/l or 1/2 saturation, whichever is smaller. If test concentrations larger or smaller than 1/2 mg/l must be used, MLSS concentrations should be adjusted up or down proportionately.

1. Materials

1.1 Apparatus

- 1.1.1 60-70 mesh steel sieve
- 1.1.2 6 - 500-ml Erlenmeyer flasks
- 1.1.3 Boiling water bath
- 1.1.4 High-speed centrifuge
- 1.1.5 Spec 20
- 1.1.6 12-250-ml plastic centrifuge tubes
- 1.1.7 7 - 250-ml glass jars with Teflon or aluminum-lined caps. Note: Do not use Teflon-lined caps for evaluations of fluorochemicals unless otherwise stated by requestor.
- 1.1.8 Pipettes
- 1.1.9 2-liter volumetric flask
- 1.1.10 Rotary shaker
- 1.1.11 25°C incubator
- 1.1.12 ASTM thermometer
- 1.1.13 Graph of MLSS VS %T at 600 nm (attached)

1.2 Chemicals and Solutions

- 1.2.1 Deionized (DI) or distilled water.
- 1.2.2 4 liters activated sludge from an aeration basin at the Metro Wastewater Treatment System. Use within 48 hrs. of collection. Keep refrigerated (<5°C) until use.

*MLSS is the concentration of suspended solids, which are mostly microorganisms, in activated sludge mixed liquor.

- 1.2.3 Reagent grade KOH
- 1.2.4 Reagent grade KH_2PO_4
- 1.2.5 Prepare a 0.05% potassium phosphate buffer by adding 1 g of KH_2PO_4 to 2 liters of water and adjusting to pH 7 with KOH solution.
- 1.2.6 Prepare a 1 mg/l solution (or a saturated solution, whichever concentration is less) of the test chemical in the 0.05% phosphate buffer (Step 1.2.5). Know the concentration of this solution to 3 significant figures.

2. Procedure

- 2.1 Pass the activated sludge through a 60-70 mesh stainless steel sieve.
- 2.2 Wash and adjust sludge concentration.
 - 2.2.1 Place sludge in 250-ml plastic centrifuge tubes. (Remember to balance weight of opposite centrifuge tubes!) Centrifuge at 14,000 x G (10,000 rpm in IEC B-20A centrifuge with 872 head) for 10 minutes.
 - 2.2.2 Resuspend sludge in 0.05% potassium phosphate buffer (1.2.5) and centrifuge again as above.
 - 2.2.3 Again, resuspend the sludge by shaking with fresh phosphate buffer (1.2.5) and adjust the MLSS concentration to approximately 4,000 mg/l by making dilutions and using the attached graph (Appendix 1) which compares MLSS absorbance at 600 nm of a 2.5 cm light path.
 - 2.2.4 Determine the total suspended matter and total volatile solids in 2 samples of the washed homogeneously resuspended sludge in accordance with Standard Methods (5.4).
- 2.3 Place 100.0 ml of homogeneously suspended washed sludge (2.2) into each of four (4) 500-ml Erlenmeyer flasks.
- 2.4 Prepare killed cells.
 - 2.4.1 Place 2 of the flasks from Step 2.3 in a boiling water bath for 15 min. to kill cells.

- 2.4.2 Bring killed cells back to 25°C in cold water bath or refrigerator before using.
- 2.5 Add 100.0 ml of the test chemical solution (1.2.6) to one (1) of the 2 flasks containing the killed cells (2.4) and to one (1) of the 2 remaining flasks containing the viable cells (2.3).
- 2.6 Add 100.0 ml of the phosphate buffer (1.2.5) to the remaining flask of viable cells and to the remaining flask of killed cells.
- 2.7 Add 100.0 ml of the test chemical solution 1.2.6) and 100 ml of the phosphate buffer (1.2.5) to a 5th 500-ml Erlenmeyer flask.
- 2.8 Add 200 ml of the phosphate buffer (1.2.5) to a 6th and final 500-ml flask.

The 6 flasks have the following composition:

- (1) 0.5 mg/l (measured to 3 significant figures) test compound + approximately 2,000 mg/l killed activated sludge.
 - (2) approximately 2,000 mg/l killed activated sludge in buffer.
 - (3) 0.5 mg/l test compound + approximately 2,000 mg/l viable activated sludge.
 - (4) Approximately 2,000 mg/l viable activated sludge in buffer.
 - (5) 0.5 mg/l test compound in buffer.
 - (6) Buffer.
- 2.9 Shake the 6 flasks, prepared in Steps 2.3-2.8, for 1 hour at 25°C.
 - 2.10 Transfer the contents of the flasks to six (6) 250-ml plastic centrifuge tubes. Centrifuge for 10 min. at 14,000 x g (10,000 rpm in the IEC B-20A centrifuge with 872 head).
 - 2.11 Immediately after the centrifuge stops decant or pipette off the clean supernatant without disturbing the cell pellet. Transfer to glass jars with aluminum foil or Teflon-lined caps. To a 7th jar add 100.0 ml of test chemical solution (1.2.6) and 100.0 ml of buffer (1.2.5) that has not been exposed to the plastic centrifuge tube.

If immediate analysis is not possible, store sample at 4°C to minimize biodegradation.

- 2.12 Analyze the water phase of all test and control samples by appropriate methods to determine the concentration of the test compound.

3. Calculations

Calculate the biosorption partition coefficient (K_p) between the volatile portions of the sludge and buffer as follows:

$$K_p = \frac{\text{ug test chemical/g VSS}}{\text{ug Substrate/ml Supernatant}}$$

3.1 Total chemical in water.

$$T_W = (W_T) \times (C_W)$$

T_W = Total mass of chemical left in water at sorption equilibrium, ug.

W_T = Total water used in adsorption study, ml (usually 200 ml).

C_W = Concentration of chemical in water at sorption equilibrium minus the concentration measured in the chemical-free control water (from flask 6), ug/ml.

3.2 Total chemical in sludge at sorption equilibrium (assuming no breakdown).

$$T_S = (C_C \times W_T) - T_W$$

T_S = Total quantity of chemical sorbed to solids, ug.

C_C = Final concentration of chemical in sludge free control (from flask 5), ug/ml.

W_T = Total water used in adsorption study, ml (usually 200 ml).

T_W = Total quantity of chemical left in water, ug (3.1).

3.3 Total Volatile Solids used in Adsorption Study.

$$S_T = \frac{(VSS) \times (V_S)}{1000}$$

S_T = Total volatile solids used in adsorption study, g.

VSS = Volatile suspended solids concentration of sludge added to biosorption study, mg/l (2.2.4).

V_S = Volume of sludge added to biosorption study, liter (usually 0.1 liter).

3.4 Biosorption Coefficient (K_p)

$$K_p = \frac{T_S/S_T}{C_W}$$

K_p = Biosorption partition coefficient

T_S = Total quantity of chemical sorbed to solids, ug (3.2).

S_T = Total volatile solids used in adsorption study, g (3.3).

C_W = Concentration of chemical in water at sorption equilibrium minus the concentration measured in the chemical-free control, ug/ml.

4. Presentation of Data

4.1 Test substances

4.2 Initial concentration (concentration in Jar #7 Step 2.11)

4.3 Final concentrations of supernatants from flasks 1 through 6.

4.4 Temperature, °C during shaking (2.9)

4.5 Report K_p (biosorption coefficient (3.4)) for both the viable sludge and heat-killed sludge.

- 4.6 Source of sludge, MLSS used in test (usually approximately 2,000 mg/l), VSS used in test (usually 1/2 of that measured).
- 4.7 pH of all samples
- 4.8 Time of incubation of test chemical with viable or heat-killed cells (usually 1 hr.).

5. References

- 5.1 Smith et al, Environmental Pathways of Selected Chemicals in Freshwater Systems, Part I, SRI International, EPA Contract #68-3-2227, EPA-600/7-77-113, October 1977, NTIS #PB-274 548.
- 5.2 Smith et al, Environmental Pathways of Selected Chemicals in Freshwater Systems, Part II, EPA-600/7-78-074, May 1978.
- 5.3 ASTM Draft 7, Standard Practice for Determination of Sorption Constants in Soil and Sediments.
- 5.4 Standard Methods for the Examination of Water and Wastewater, 14th Edition, American Public Health Association, 1976.

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8-19-75 JAP
(graphed by WAS, 12-16-80)

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