Alkyl Benzene Sulfonate Effects on Stream Algae Communities

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ABS is toxic to fish and other aquatic organisms. It also is toxic at varying concentrations to lower life forms at all trophic levels within the aquatic environment. Algae represents the level of the primary producer. Those forms ranking above it are dependent upon it for their energy supply. A reduction in the productive ability of algal communities will result in a reduction of the total energy flow and a consequent reduction in the productive capacity of the entire community. The object of this investigation was to determine the effects of alkyl benzene sodium sulfonate on the productivity of stream algal communities.

The major types of synthetic detergents have diverse effects on a wide variety of organisms. Manganelli and Crosley (1) found that synthetic detergents appear to denature the cytoplasmic membrane and damage the cytoplasm of the protozoa and higher organisms in activated sludge. Tomcsik (2) indicated that surface active agents denature the cytoplasmic membrane of *Bacillus sp.* and damage protoplasts.

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Work on plants in relation to detergent effects is limited. Degence (3) observed no adverse effect when four water plants were grown in concentrations of 40 mg/l of anionic detergent. Jenkins, Klein, and McGauhey (4) found that ABS was concentrated in barley and sunflower roots when grown in water cultures. At 10 mg/l ABS the sunflowers were retarded 20 percent, and at 50 mg/l the plants were retarded 65 percent. Other sunflower plants in water cultures containing 10 mg/l ABS showed distinct signs of chlorosis. *Lupinus albus*, which was grown in fertilized soil with up to 20 mg/l ABS, was unaffected.

Hynes (5) showed that *Cladophora domerate* placed in shallow dishes containing 50 mg/l ABS suffered chloroplast destruction and chlorophyll loss into the water. Algae which had been placed in 25, 20, and 10 mg/l ABS died after three weeks. That which was placed in 5 mg/l ABS appeared unhealthy. In 2.5 mg/l and in the controls, the plants appeared unaffected. Both *Ranunculus pseudofluitan* and *Potomogeton pectinatus* were seriously affected by 2.5 mg/l ABS.

Experimental

Two types of algal communities were used in experiments. *Vaucheria* communities were obtained from the Logan River, a swift moving stream. *Cladophora* communities were obtained from a standing water aquarium. Both types of communities are found attached to rocks in relatively large mats. Parts of the algal mats were transferred to experimental containers. These samples were considered as the experimental unit. No attempt was made to obtain pure cultures. Attendent invertebrates and bacteria were considered part of the community.

The ABS used in all experiments was of the same type. The trade name for the compound is Nacconol NRSF. The compound is a propylene derivative and is 92.5 percent active ABS. A stock solution of 5,000 mg/l ABS was made for each experiment. Aliquots of the stock solution were added to the experimental containers to obtain desired concentrations.

The parameter measured was that of the rate of C^{14} uptake by the communities. The primary production in an aquatic ecosystem is dependent upon the rates at which photosynthetic organisms fix inorganic carbon and produce organic material. The use of C^{14} presents a comparatively simple procedure, and most important, is a very sensitive method for measuring production (6). Ryther (7) states that C^{14} uptake represents a measure of net production and not gross production. Strickland (6) says that C^{14} uptake experiments generally give a measure of photosynthesis that is somewhere between the net and gross values, possibly nearer the former. C^{14} has mostly been applied to work related to phytoplankton productivity. However, the same basic principles apply when used with benthic periphyton.

Conditions were varied by subjecting community samples to different concentrations of ABS for different periods of time. All experiments included completely randomized factorial arrangement of treatments. The basic response, counts per minute per milligram algae, was subjected to an analysis of variance to determine the effects of time and ABS concentrations.

All experiments included a 4 x 5 factorial arrangement of treatments. The experiments for both Vaucheria and Cladophora communities in high and low ranges of ABS concentrations consisted of five one-gallon glass containers, twenty-five 60 ml. glass bottles and a water trough employed as a constant temperature bath. The algal communities were placed in the gallon containers. Each container held one liter of water. Appropriate concentrations of ABS were added. Algal communities were subsampled and the samples were placed in 60 ml. bottles at four time periods: 12 hours, 24 hours, 48 hours, and 96 hours. Four samples were taken from each of the respective gallon containers. This represented four replications of each treatment or concentration of ABS. C^{14} was added in 0.25 uc aliquots to each bottle. Five of the 60 ml. bottles, one representing every ABS concentration, were covered with aluminum foil to estimate error due to surface adsorption of C^{14} by the algal communities and account for loss due to respiration in the dark. The bottles were placed in the trough and flooded with 700 foot-candles of light.

The concentrations of ABS used in the Cladophora-Vaucheria experiments were 0, 5, 25, 50 and 100 mg/1. In both of these experiments, the water used was from a standing water aquarium. In all other laboratory experiments tap water was used after it was passed through an active carbon, sand, and gravel filter. The chemical quality of the tap water in mg/1 included 42.0 Ca^{++}, 18.0 Mg^{++}, 4.0 K^{+}, 0.5 NH_4^{+}, 1.0 Cl^{-}, 8.0 $SO_4^{=}$, 209.0 HCO_3^{-}, 0.44 $NO_3^{=}$, 1.5 $PO_4^{\equiv}$, and a conductivity of 329 umhos. The aquarium water included 44.0 Ca^{++}, 85.0 Mg^{++}, 8.0 K^{+}, 0.3 NH_4^{+}, 10.0 Cl^{-}, 508.0 HCO_3^{-}, 44.0 $NO_3^{=}$, 95.0 $PO_4^{\equiv}$, and a conductivity of 924 umhos.

Concentrations of 0, 4, 8, 16 and 32 mg/l ABS were used in experiments involving "soft" and "hard" tap water. Vaucheria was used in both runs. "Soft" water contained 2 mg/l total hardness as calcium carbonate. "Hard" water contained 180 mg/l total hardness as calcium carbonate.

In all experiments, the algal communities were killed with a 4:1 solution of concentrated hycrochloric and glacial acetic acids. The samples were flushed with distilled water, dried at 90° C for 48 hours and weighed. The samples were finally pulverized and distributed evenly on planchetts.

All samples were counted for five minutes in a 2 pi proportional counter. The resulting response data was expressed as counts per minute per milligram algae.

Results and Discussion

The analysis of variance for the Cladophora experiment showed that ABS concentration, time, and their interaction had an effect on the uptake of C-14 by the community (Table 1). Both treatments have a negative influence on the assimilation of C^{14}. If the main effect of concentration is considered alone, 5 mg/l stimulates assimilation of carbon. The time factor contributed to the reduction of the productivity of the algal community.

The results of the Vaucheria experiment were slightly different: Only ABS concentration had an effect on C^{14} assimilation. Five mg/l ABS seemed to stimulate uptake.

TABLE 1

Treatment means and analysis of variance of the amount of C^{14} (counts per minute per mg) assimilated by Cladophora and Vaucheria in aquarium water treated with alkyl benzene sulfonate.

ABS Conc (ppm)	Species	Time of Exposure 12 hrs.	24 hrs.	48 hrs.	96 hrs.	Mean
0	Clado.	277.48	314.27	147.23	56.41	198.87
	Vauch.	65.28	79.45	71.06	107.81	80.90
5	Clado.	381.23	326.04	184.77	69.31	240.33
	Vauch.	56.82	93.40	96.22	103.75	87.55
25	Clado.	178.10	149.33	150.77	58.64	134.21
	Vauch.	16.10	12.85	6.30	5.51	10.19
50	Clado.	77.64	120.88	30.63	14.94	61.02
	Vauch.	15.25	10.85	2.96	2.77	7.96
100	Clado.	18.31	14.37	7.38	11.45	12.87
	Vauch.	6.39	3.76	1.85	2.43	3.61
Mean	Clado.	233.19	231.22	130.21	52.68	
	Vauch.	39.96	50.08	44.60	55.57	

Analysis of Variance

Source of Variation	D.F.	Cladophora Sum of Squares	Cladophora Mean Squares	Vaucheria Sum of Squares	Vaucheria Mean Squares
Replications	3	161.19		574.20	
Concentration	4	563870.59	140967.63*	114456.90	28614.23*
Time	3	290079.22	96693.07*	1765.25	588.42
Time x conc.	12	178282.49	14856.87*	8569.69	714.14
Experimental Error	57	134451.01	2358.78	24353.36	427.25
Total		1166844.50		149719.40	

*Significance at the 0.5 percent level.

It is interesting to note that maximum uptake for Cladophora was higher than maximum uptake for Vaucheria. Both experiments indicated that as concentration increased, assimilation of the C^{14} decreased. In the run utilizing Vaucheria, the effect of concentration over time did not change significantly.

The point estimating equation determined from the coefficients accounting for 80 percent of the variation was:

$$Y = 350.5 - 5.78X_1 + 0.02X_1^2 - 3.03\ X_2 + 0.003\ X_1X_2,$$

where Y is C-14 uptake in counts/minute/mg of community, X_1 is concentration of ABS in mg/l and X_2 is time of exposure in hours. A surface representation of the interactions involved indicate a linear drop in C^{14} uptake from a high point at 0 concentration and the first time period (Fig. 1). The surface displayed a low response at 75 mg/l and 96 hours.

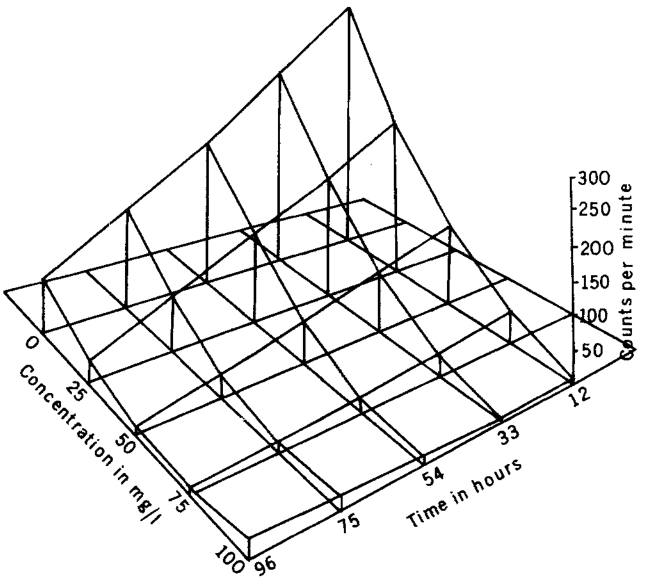

Figure 1. C^{14} uptake in counts per minute per mg of Cladophora community as related to time of exposure to ABS concentration.

The soft water experiment with ABS concentrations of 0, 4, 8, 16 and 32 mg/l indicated significance due to ABS concentration, time, and their interaction. Response differences due to time and the concentration x time interaction were found at the 0.5 percent level of confidence. The differences due to concentration were found at the 10 percent level (Table 2).

TABLE 2

Treatment means and analysis of variance of the amounts of C^{14} assimilation by Vaucheria in "soft" and "hard" water.

ABS Conc. (ppm)	Water	Time of Exposure 12 hrs.	24 hrs.	48 hrs.	96 hrs.	Mean
0	Soft	102.47	56.91	29.57	68.48	64.35
	Hard	186.78	55.21	18.49	20.38	70.20
4	Soft	66.94	81.20	38.90	36.51	55.88
	Hard	91.33	42.04	22.64	14.65	42.66
8	Soft	44.18	41.94	52.82	61.98	50.09
	Hard	106.06	24.46	32.44	21.35	47.07
16	Soft	25.93	85.63	41.34	47.74	50.16
	Hard	87.15	28.83	14.48	6.81	34.31
32	Soft	51.68	58.01	33.43	12.84	38.99
	Hard	21.05	14.64	15.14	3.89	13.68
Mean	Soft	72.80	80.92	48.88	56.88	
	Hard	98.67	33.63	20.63	13.41	

Analysis of Variance

Source of Variation	D.F.	Soft Water Sum of Squares	Soft Water Mean Squares	Hard Water Sum of Squares	Hard Water Mean Squares
Replications	3	2329.43		5583.44	
Concentration	4	5504.58	1376.14***	26768.50	6692.12*
Time	3	8191.85	2730.61**	90957.22	30319.07*
Time x Conc.	12	22093.46	1841.12*	35147.04	2928.92*
Experimental Error	57	34370.45	609.30	36542.94	641.10
Total	79	72849.77		194999.14	

*Significance at the 0.5 percent level.
**Significance at the 1.0 percent level.
***Significance at the 10.0 percent level.

The analysis of variance for the "hard" water experiment indicated significance due to differences in concentration, time, and their interaction (Table 2). There was an apparent stimulation of the uptake of C^{14} at low levels of ABS. Maximum counts in this experiment were comparable to those in the previous experiment with Vaucheria communities. The point estimating equation accounting for 58 percent of the variation was found to be:

$$Y = 116.79 - 3.99X_1 + 0.032X_1^2 - 4.09X_2 + 0.026X_2^2 + 0.032X_1X_2 \text{ (Fig. 2).}$$

The decrease due to the main effect of ABS concentration exhibited a linear relationship. The decrease due to the main effect of time showed a quadratic relationship, inferring a slight recovery in response over time. The interaction in this experiment included both linear and quadratic terms. However, there was a general decrease from the high point at 0 concentration and 12 hours to a low point at an ABS concentration of 32 mg/l and 54 hours.

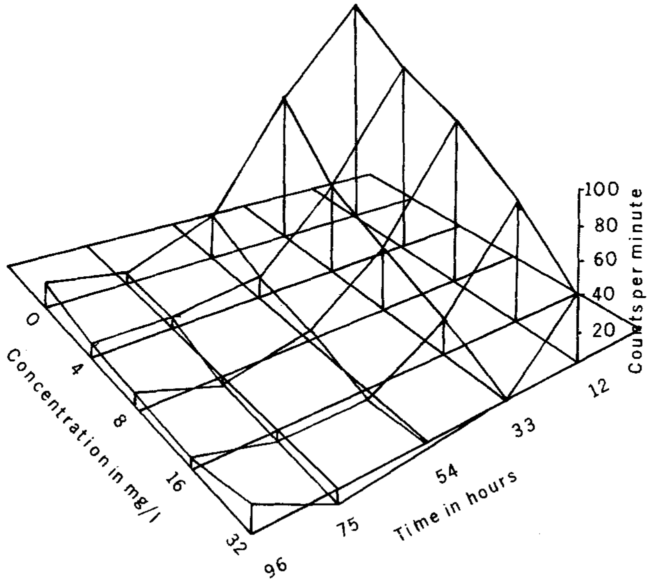

Figure 2. C^{14} uptake in counts per minute per mg of <u>Vaucheria</u> community in hard water as related to hours exposed to ABS concentrations.

The soft water experiment also displayed significant concentration, time and interaction effects (Table 2). The response surface was not easily defined. The best fit with linear and quadratic terms accounted for only 37 percent of the variance. An empirical plot of the surface, however, still is sufficiently revealing to merit description (Fig. 3).

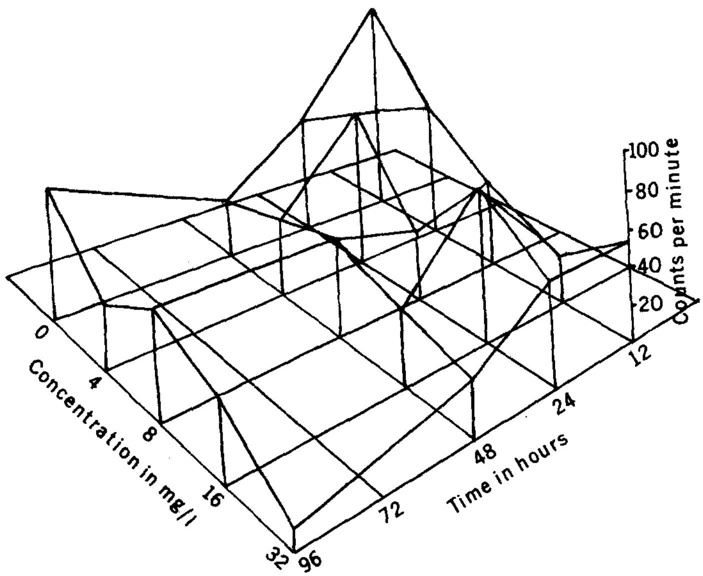

Figure 3. The empirical response surface of C^{14} uptake (counts per minute per mg) of Vaucheria communities in soft water treated with ABS concentration.

Upon examination of the tables of means and the figures, three effects become apparent: (1) ABS has a negative effect on C^{14} uptake for both algae communities, (2) the communities appear to partly recover their ability to assimilate C^{14} at extended exposures to high concentrations, and (3) a slight stimulation of C^{14} uptake appears to occur at abbreviated exposures to low concentrations.

A reduction in productivity is alluded to by the decrease in C^{14} assimilation. Ample evidence exists that the photosynthetic mechanism is affected by detergents, e.g., detergents fraquent chloroplasts (8) as well as make the chlorophyll-protein complex water soluble (9). Chlorophyll leaching from the cells was observed during the course of the experiment.

The apparent increase in C^{14} assimilation at extended exposures to high concentrations may be a function of the heterogenity of the community. Mortality of the algae may provide the basis for proliferation of reducer organisms which are less sensitive to the ABS concentrations. Such organisms as Sphaerotilus natans which exist on organic material are present in the community.

The apparent stimulation at low concentrations is a phenomenon which also has its parallels. Robinowitch (10) points out that most inhibitors appear to have a stimulating effect at low concentrations. Manganelli and Crosley (1) noted stimulated "slime" growth in low concentrations of detergent.

The differences that existed between Cladophora and Vaucheria communities (Table 1) illustrate species or adaptation differences. The endemic environment of both communities was changed for the experiment. Vaucheria was taken from a flowing water environment with a water quality similar to the tap water and placed into aquarium water without a velocity. The Cladophora community was subjected to a temperature change from 27° C. to 10° C.

Acknowledgement

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