Anionic and Nonionic Surfactant Sorption and Degradation by Algae Cultures

ERNST M. DAVIS and EARNEST F. GLOYNA,
Environmental Health Engineering, The University of Texas, Austin, Texas 78712

Abstract

Degradation of three surfactants has been determined by organic extraction procedures and infrared spectroscopy. Axenic cultures of five species of blue-green algae and three species of green algae which are common to waste stabilization ponds were test organisms. Analytical data are shown comparing the effects produced by the algae cultures and a heterogeneous microcosm. Linear alkyl sulfonate was the anionic surfactant compound tested. An alkyl polyethoxylate and an alkyl phenol polyethoxylate were the nonionic test surfactants. Sorption of the compounds by the algae usually was followed by release and degradation of up to 99% of some of the component parts of the surfactant molecule.

Introduction

Waste treatment facilities which have either the trickling filter or the waste stabilization lagoon will have characteristic algal populations throughout the year. Aside from the relatively high numbers of bacteria, the algae species usually are members of two divisions, namely the *Cyanophyta,* or blue-green algae, and the *Chlorophyta,* or green algae. Surfactant compounds used in household cleaners and laundry preparations and many other specialized cleaning operations are primarily of the anionic and nonionic types. Cationic surfactants are found in comparatively small concentrations in domestic waste treatment facilities and for this reason they were omitted in this investigation. The bulk of household detergent products contain either anionic surfactants of the linear alkyl sulfonate type or nonionic compounds of the alkyl polyethoxylate or alkyl phenol polyethoxylate types.

The methodology and tools available to the researcher for determining the degradation of surfactants is well documented (1,2). (Swisher, presented at the June 1963 AOCS Meeting, Princeton, N.J.). On the other hand the contribution that different species of algae and their associated bacteria make to this overall degradation has been largely unanswered. This investigation was undertaken to determine the effect of axenic cultures of algae on known surfactants. Secondarily, tests were made to establish sorption characteristics of these surfactants by the algae. This sorption phenomenon has been reported (3) but quantitative estimates of the mechanism have not been established. Additional information on the preferential breakdown of the structural components of each surfactant compound was sought. The question of whether the infrared spectrophotometric analyses could yield this type of data became an integral part of the investigation. Some doubt has existed as to the thoroughness of extraction of the surfactants by organic solvents prior to complexation in concentrations of and below 50 mg/liter. One report of degradation of a polyethoxylated alkyl phenol (4) was accomplished at the 20 mg/liter level but the isolation procedure was by ion exchange columns and foam stripping procedures.

Experimental Procedures

What has been termed a normal die-away test was performed on all series on this investigation. Preparation for the addition of the test compounds comprised the most important segment of the experimentation. The algae species were cultured in an almost totally inorganic growth medium (5,6) until they reached their early log growth phase. That point in time was approximately 7 to 14 days after inoculation into series of 250 ml erlenmeyer flasks containing 150 ml of the medium. The exact time depended on the species in question. The only organic compound present in the culture medium at the time of inoculation of the algae was EDTA at a level of 10 mg/liter. This caused no interference in the tests. Additions of sterile distilled water to the cultures were incorporated during the test period to correct for losses by evaporation. The algae were cultured at a temperature of 25 $\pm$ 1.1 C adjacent to banks of fluorescent lights which were on cycles of 16 hr on and 8 hr off. The amount of illumination on the cultures during these periods was 285 $\pm$ 7 foot-candles.

The test compounds were a linear alkyl sulfonate (S & D A Standard Lot 1–1) and two nonionic compounds, alkyl polyethoxylate (AE 7.4) and alkyl phenol polyethoxylate (ABE 9.5). The numbers 7.4 and 9.5 indicate the theoretical number of moles of ethylene oxide per mole of compound. Analysis for the compounds was essentially similar to extraction techniques which have been proven to be reliable in this type of investigation (4,7). The extraction of the nonionic surfactants was accomplished by increasing the ionic strength of the test solution with 8% NaCl followed by chloroform separation. Three extractions of 20 ml chloroform each were used routinely. After extraction from a 100 ml sample of centrifuged and membrane filtered filtrate, the chloroform was evaporated over a steam bath, the residue was dried at 65 C and, after cooling, the residue was redissolved in 5 ml carbon tetrachloride. An aliquote of this solution was placed in a 1 mm NaCl window cell opposite a carbon tetrachloride reference cell and scanned from 900–3400 cm^{-1} with a Beckman Model IR-7 spectrophotometer. Anionic isolation (LAS) was by complexation in phosphate buffered acid solution with *n*-octylamine followed by chloroform extraction. Similarly three 20 ml extractions of chloroform were used followed by steam, evaporation, drying and dissolution with carbon tetrachloride. An aliquote was then scanned from 900–3400 cm^{-1}. Characteristic absorption bands for AE 7.4 appear at 1040 cm^{-1} (primary hydroxide), 1087–1149 cm^{-1} (polyethylene oxide) and 2900 cm^{-1} (aliphatic C–H). The most useful bands in this investigation were the 1087–1149 cm^{-1} and 2900 cm^{-1} (polyethylene oxide) and 2900 cm^{-1}. ABE 9.4 ex-

TABLE I
Per Cent Decrease of Surfactant Components in Microcosm Filtrates at Beginning of Test Period

Test algae	LAS		AE 7.4		ABE 9.5		
	1136 cm^{-1}	2900 cm^{-1}	1087–1149 cm^{-1}	2900 cm^{-1}	1087–1149 cm^{-1}	1520 cm^{-1}	2900 cm^{-1}
Anabaena variabilis	4.1	1.0	13.1	14.7	26.1	41.1	43.2
Anacystis nidulans	7.1	4.1	26.6	20.0	17.8	7.5	20.7
Oscillatoria borneti	[a,b]	[a,b]	16.3	17.1	0.8	[a,b]	1.9
Oscillatoria formosa	[a,b]	[a,b]	0.7	0.6	1.3	0.6	7.0
Phormidium faveolarum	9.2	10.0	30.7	24.6	0.7	[a,b]	1.1
Ankistrodesmus braunii	11.1	9.2	41.4	54.8	36.6	25.3	28.1
Chlorella pyrenoidosa	2.5	1.1	[a,b]	[a,b]	28.6	11.7	23.6
Scenedesmus obliquus	0.4	1.0	30.0	16.9	31.4	2.8	1.1
Pond microcosm	1.1	14.1	43.4	39.0	64.7	11.7	53.8
Algal contaminants	[a,b]	0.3	[a,b]	0.4	[a,b]	[a,b]	[a,b]

[a] Indicates component decrease based on total value for that component recoverable in filtrate from 50 mg/liter total compound concentration of blank.
[b] No change.

hibits absorption peaks at 1087–1149 cm^{-1} (polyethylene oxide), 1220–1250 cm^{-1} (alkyl phenol polyether group), 1520 cm^{-1} (aromatic C–H) and 2900 cm^{-1} (aliphatic C–H). Of these, the 1087–1149 cm^{-1}, 1520 cm^{-1} and 2900 cm^{-1} bands were the most reliable due to the comparatively low concentrations of surfactant used in the tests. Absorption bands used for LAS were the 1136 cm^{-1} (sulfonate) and the s900 cm^{-1} (aliphatic C–H). Typically, LAS should produce other peaks at 1010–1042 cm^{-1} (LAS), 1180–1235 cm^{-1} (sulfonate) and 1460–1470 cm^{-1} (aliphatic C–H).

Standard curves were plotted as optical density versus the concentration of the compound for each of the components of the compounds. For example, curves were plotted for the polyethylene oxide band and the aliphatic C–H band for AE 7.4. From these curves the change of corresponding concentration of adducts was calculated as per cent decrease or increase compared to that which was found at the beginning of each run. Each transmission value from the IR scan was analyzed by the tangent baseline technique (8) and converted to optical density values for analysis. Changes in concentrations of the surfactant component are reported as decreases or increases from that portion found at the beginning of the run because it was found that sorption by the algae occurred very rapidly. The values represent the filtrate concentration changes.

Results and Discussion

Rates of decreases of recoverable concentrations of surfactants from the filtrates of aquatic systems will vary as to the speed that they disappear and the kinds of organisms comprising the system. This is apparent from the results obtained during the duration of the test periods. Not only did the axenic cultures of blue-green algae differ between species of the same genus and between genera regarding sorption and degradative capabilities but differences between them and the green algae can also be seen from the results of this investigation.

Sorption of organic compounds such as surfactants by algal cell well be responsible for a lack of complete understanding of the true quantities of surfactants present in a wastewater treatment facility environment. The per cent decreases of the 50 mg/liter equivalent of surfactant compound added to each algae culture are presented in Table I. These amounts were sorbed by the biomass. These data represent the differences in the component band transmission spectra compared to the algal growth medium blank containing the same amount of active surfactant compound. Sorption must have occurred rapidly because the length of time between addition of the surfactant and extraction in all cases was well within 2 hr. The most striking data in Table I is the apparent lack of sorption of the anionic surfactant components by both species of *Oscillatoria*. Also, the bacterial suspension of the blue-green algae contaminants showed little ability to selectively remove any of the components from the medium. Fortunately, because of this, the degradation data shown in Tables II and III is more meaningful. The highest sorption capacity, with few exceptions, was evidenced by *Ankistrodesmus braunii* and that oc-

TABLE II
Per Cent Change of LAS and AE 7.4 Component Concentrations in Culture Filtrates From That Present at Beginning of Test Period

Test algae	LAS 1136 cm^{-1}			LAS 2900 cm^{-1}			AE 7.4 1087–1149 cm^{-1}			AE 7.4 2900 cm^{-1}		
day	7	14	21	7	14	21	7	14	21	7	14	21
Anabaena variabilis	[a]−39.9	−82.1	−85.8	−27.2	−66.4	−72.5	+21.5	+16.0	−69.8	+11.2	+29.0	−82.1
Anacystis nidulans	−18.6	−81.0	−86.2	−41.6	−98.0	−98.0	+13.0	+65.0	−45.2	+102.0	+51.0	−74.6
Oscillatoria borneti	−29.0	−84.0	−87.0	−30.6	−99.0	−99.0	−14.0	−34.0	−68.2	−06.7	−37.0	−71.4
Oscillatoria formosa	− 7.7	−33.0	−20.1	−24.6	−71.5	−62.0	−16.9	−24.0	−62.9	−17.0	−22.0	−64.7
Phormidium faveolarum	−22.5	−30.0	−59.0	−44.2	−66.7	−75.7	+27.7	−19.0	−66.4	−31.2	−47.0	−89.0
Ankistrodesmus braunii	−17.3	−54.5	−54.2	−12.7	−18.0	−49.9	+28.5	−44.0	−66.2	−21.1	−62.0	−68.1
Chlorella pyrenoidosa	− 8.3	− 6.7	−14.1	−34.7	−55.8	−66.8	− 4.4	− 5.0	−40.6	−10.7	−41.0	−79.0
Scenedesmus obliquus	− 7.8	−59.0	−58.5	−10.6	−50.0	−51.4	−15.4	−55.0	−72.0	−14.0	−61.0	−79.2
Pond microcosm	−25.8	−18.0	−92.1	+12.1	− 1.4	−91.1	−47.1	−88.0	−99.0	−82.1	−96.0	−97.5
Algal contaminants	−12.5	−19.8	−22.9	−23.8	−34.1	−39.8	−16.0	−19.2	−37.5	−10.9	−16.2	−28.1

[a] − Indicates component decrease based on total value for that component recoverable from 50 mg/liter total compound concentration of blank. + Indicates similar component increase in concentration.

curred with the hydrophile group of all three test surfactants. Differences in recovery of functional groups are shown in Table I. Hydrolytic or other enzymatic activity may have been responsible for those differences. It is entirely possible that both absorption and adsorption mechanisms were also active.

Tables II and III relate the per cent changes of each recoverable component of the three compounds tested at weekly intervals for 21 days. It should be noted that the changes of apparent concentrations of components are relative to the amounts sorbed as reported in Table I. The question of whether the bacteria, which are unavoidable and continual contaminants of blue-green algae laboratory cultures, contributed significantly to the overall degradation of the surfactants was partially answered by this investigation. As indicated by the last row in Tables II and III, degradation of all components occurred at fairly constant rates during the test period. Comparison of these data with that for all of the axenic blue-green algae cultures would indicate the strong possibility of a symbiotic relationship between the bacteria and blue-greens themselves. With few exceptions the amounts of surfactant components remaining at 21 days in the algae cultures were lower than in the algal contaminant series. Specifically those exceptions were the sulfonate radical of LAS with *Chlorella pyrenoidosa* and *Oscillatoria formosa,* the ethoxyl chain of ABE 9.5 with *Ankistrodesmus braunii,* and the aromatic radical of ABE 9.5 with *Chlorella pyrenoidosa.* The bacteria which were found as contaminants of the bluegreen cultures were consistently of two genera, namely *Flaveobacterium* and *Brevibacterium.* Washed cultures of these bacteria were added to the algal growth medium in concentrations of approximately 10^4/ml. Comparatively few ($\leq$100/ml) bacteria were present at any time in the axenic green algae cultures and for this reason a similar analysis of their contribution to the degradation was omitted.

Throughout the data in Tables II and III several points appear which add credence to the hypothesis that sorption of the surfactants precedes a release to the immediate aqueous environment. Some examples of this are the following: *Oscillatoria formosa* with LAS at 21 days; sulfonate of LAS with *Chlorella pyrenoidosa* at 14 days; aliphatic groups of AE 7.4 with *Anabaena variabilis* and *Anacystis nidulans* at 14 days; ethoxyl group of ABE 9.5 with *Anacystis nidulans* at 7 days. The other instances of increased concentrations of components are evident by increases (+) or smaller decreases (−) than occurred at an earlier date in the test period. What would appear to be errors in component concentration increases appear at the following points: ethoxyl group of AE 7.4 at 7 and 14 days and the aliphatic group at 14 days with *Anabaena variabilis*; ethoxyl group of AE 7.4 at 14 days and the aliphatic group at 7 and 14 days with *Anacystis nidulans*; aliphatic group of ABE 9.5 at 7 and 14 days with *Oscillatoria formosa;* aliphatic group of ABE 9.5 at 14 days with *Phormidium faveolarum;* the ethoxyl group of ABE 9.5 at 14 days, the aromatic group at 7 and 14 days, and the aliphatic group at 14 days with *Chlorella pyrenoidosa.* These data indicate that greater amounts of the particular chemical components were released to the medium and recovered from same. Additional analyses were conducted on these algae to insure that errors were not made in the amounts of active surfactant added and in the infrared spectrum interpretations themselves. The exact cause for what would appear to be higher concentrations of components in the medium than were actually added is unknown. It is entirely possible though that the algae in question secreted compounds to the medium which were chemically reactive or similar to those being analyzed. Also, it is possible that the components themselves were enzymatically hydrolyzed, remaining in a sorbed condition and later released. This would account for the differences in rates of decrease and degradation of the components which is in evidence in Tables II and III.

Overall, considering the degradation of the three different surfactant compounds, it is apparent that the laboratory acclimated stabilization pond water and its associated heterogeneous microcosm was capable of greater reduction of all components than individual species. This might be anticipated because of the very nature of that system itself. The data in Table III shows, by comparison to that in Table II a slightly greater amount of the ethoxyl and aliphatic groups of ABE 9.5 present at the termination of the test period than was present for LAS and AE 7.4. The implication would appear to be that the alkyl phenol polyethoxylate molecule was either inhibitory to certain groups of organisms in the heterogeneous microcosm or that it simply was

TABLE III

Per Cent Change of ABE 9.5 Component Concentrations in Culture Filtrates From That Present at Beginning of Test Period

Test algae	Day	1087–1149 cm^{-1}			1520 cm^{-1}			2900 cm^{-1}		
		7	14	21	7	14	21	7	14	21
Anabaena variabilis		[a]+26.0	+ 5.2	−31.2	+22.5	−12.5	−29.7	+37.8	+ 5.4	−40.8
Anacystis nidulans		+ 1.0	+ 1.9	−28.7	− 1.0	− 2.1	−34.4	− 2.6	− 9.3	−70.8
Oscillatoria borneti		− 0.6	−10.0	−39.8	− 1.0	−15.0	−47.9	− 1.1	− 1.1	−85.9
Oscillatoria formosa		− 0.9	−28.0	−40.0	−29.2	−37.5	−39.8	+12.1	+25.0	−67.7
Phormidium faveolarum		−17.1	−18.0	−28.9	−22.1	−56.5	−67.0	−11.1	+17.0	−86.2
Ankistrodesmus braunii		− 1.0	− 9.8	−14.6	−14.0	−52.0	−54.3	− 6.1	−12.0	−71.1
Chlorella pyrenoidosa		+11.7	+34.0	−22.5	+27.0	+14.0	−19.8	+23.5	+29.6	−46.7
Scenedesmus obliquus		−10.0	−18.0	−27.7	− 7.5	−29.0	−30.5	−12.2	−15.0	−80.0
Pond microcosm		+24.3	−80.0	−89.9	+11.1	−27.5	−60.8	+15.7	−86.0	−81.0
Algal contaminants		−11.1	−16.4	−20.7	− 2.5	−19.8	−21.6	−18.3	−22.0	−24.9

[a] − Indicates component decrease based on total value for that component recoverable from 50 mg/liter total compound concentration of blank. + Indicates similar component increase in concentration.

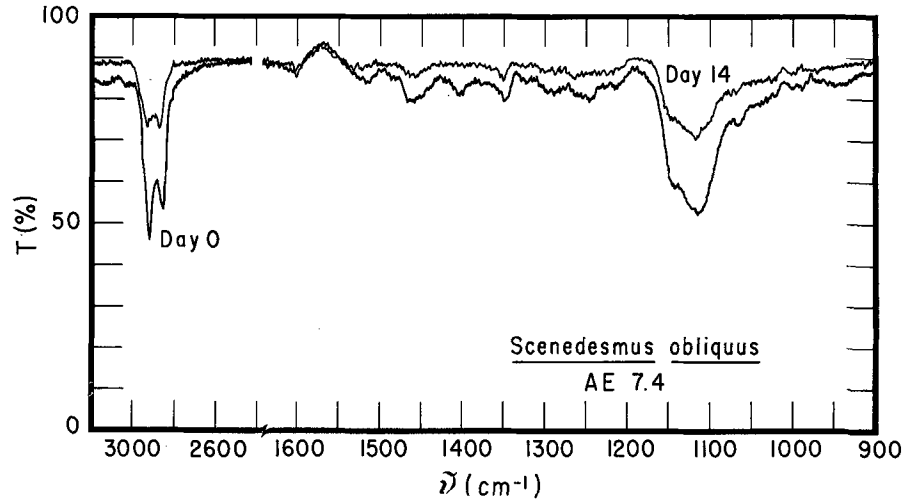

FIG. 1. IR Spectra of 50 mg/liter AE 7.4 extracted from filtrate of *Scenedesmus obliquus* immediately after addition and at 14 days.

more difficult to degrade. Some inhibition of cultures was observed during the tests and has been reported earlier (6,9). Note should be taken that a release of all components of ABE 9.5 into the aqueous medium occurred at 7 days when in the presence of *Anabaena variabilis, Chlorella pyrenoidosa* and the pond microcosm.

No clear pattern is evident when comparing the degradation capabilities of the two algae groups (blue-green vs. green algae). Apparently though, the green algae species had a lesser capacity to degrade the ethoxyl hydrophile than the blue-greens. The aliphatic hydrophobe surfactant molecule component was comparatively amenable to the degradative of algal (and to some extent bacterial) metabolism. Attempts were made to subject a series of laboratory acclimated activated sludge samples to the same analytical procedures as described herein. Difficulty was encountered in the filtration step and the results were erratic and nonreproducible. Sufficient interference was encountered in the IR spectra to justify dismissal of the data. An approximation of the degradation of both the sulfonate group of LAS and ethoxyl groups of AE 7.4 and ABE 9.5 was established despite the difficulty encountered. Approximately 80% of the sulfonate (LAS) had been degraded (was not recordable) within the first 2 days of exposure to the activated sludge and over 70% of the ethoxyl group of AE 7.4 and ABE 9.5 was degraded within the first 7 days.

The 50 mg/liter concentrations of active surfactant compounds which were used in this study were comparatively low for infrared spectrophotometric analyses by conventional extraction methodology. Occasionally domestic wastewaters will have concentrations at or above 50 mg/liter. The anticipated range is ordinarily 10–35 mg/liter. Resolution was surprisingly good using the techniques described herein, however quite erratic results were obtained after the 21 day period. This was due to interferences from algal protoplasmic constituents because the cultures had entered the stationary or declining growth phases. Figures 1 and 2 show typical scans of filtrate extract of two of the test microcosms, *Scenedesmus obliquus* and acclimated stabilization pond water. The good resolution of the ethoxyl band (1087–1149 cm^{-1}) and CH band (2900 cm^{-1}) in both figures is evident. What might have constituted serious interference for the comparison between the single species and the pond microcosm extracts were the multiple bands which are shown in Figure 2. The period of acclimation of the pond water which was most suitable for this investigation was 7 days prior to the addition of the

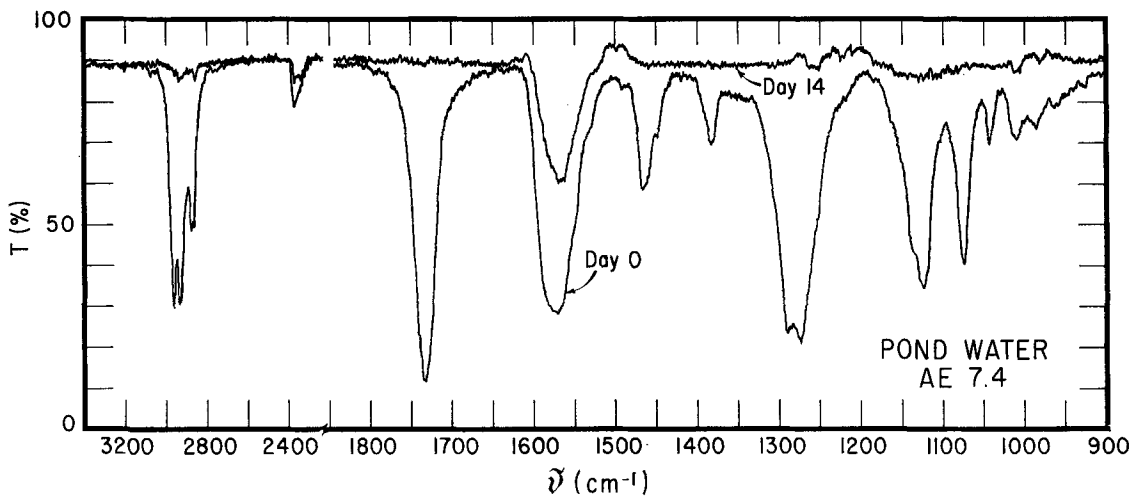

FIG. 2. IR Spectra of 50 mg/liter AE 7.4 extracted from filtrate of pond water immediately after addition and at 14 days.

surfactant compounds. Attempts to obtain good resolution before 7 days resulted in high interference. As is shown quite vividly in Figure 2 by the reduction in concentration of the unknown components (bands other than 1087–1149 cm^{-1} and 2900 cm^{-1}), the microorganisms were utilizing the chemicals as nutrient sources.

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