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(Biodegradation)
EE & PC-Div.
Envir. Assess.
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CURRENT OBJECTIVE:

To evaluate the susceptibilities of FC-95 and FC-143 to microbial decomposition.

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 biodegradation study is described which allows the evaluation of the susceptibility of FC-95 and FC-143 to aerobic microbial degradation. The culturing procedures used in this study are modeled after the Soap and Detergent Association's presumptive (shake culture) test for the determination of ABS/LAS biodegradability. Microbial inocula were obtained from activated sludge collected at Chemolite, Decatur and Metro waste treatment plants. Analytical procedures included GLC, TLC, ^{14}C-scintillation counting and analysis for released fluoride. Degradation of reference compounds demonstrated the suitability of the biodegradation test conditions.

Information Liaison Initials: SKW

SUMMARY

Fluorochemicals FC-95 and FC-143 were shown to be completely resistant to biodegradation in a 2½-month shake culture biodegradation study. The mixed microbial test cultures used in this study were derived from activated sludge inocula obtained from three waste treatment systems (Chemolite, Decatur, & the Twin Cities Metro plant). The cultures were maintained in dilute yeast extract-basal salts media supplemented with the hydrogen analog of the respective fluorochemicals. Test cultures also contained FC-95 or FC-143. Phenol and 1-dodecene-derived linear alkyl sulfonate (LAS) were used as reference compounds. Their degradation demonstrated that biodegradation could occur under the test conditions. All cultures were transferred 15 times over the 2½-month period, and temperature was controlled at 25° C. during the latter half of the experiment.

In the final growth period, degradation products of ^{14}C-labeled fluorochemicals were assayed for by thin-layer chromatography (TLC) and gas liquid chromatography (GLC). Chemicals separated by TLC were visualized by TLC-autoradiograph. Methylated and nonmethylated culture extracts separated by GLC were detected by electron capture. No degradation products were detected. Scintillation counting showed that all radioactivity associated with the labeled fluorochemicals remained in the culture medium.

In all but the final growth period, fluorocarbon biodegradation was monitored simply by measuring the initial and final fluoride concentration in the media. No increase in fluoride concentration was observed indicating that if biodegradation did occur, it did not result in the release of fluoride. Control cultures supplemented with fluoride showed that fluoride is not lost from the media under the experimental conditions used.

While this study cannot rule out the possibility that conditions could be found that would allow the biodegradation of these compounds, the results of this study suggest that these chemicals are likely to persist in the environment for extended periods unaltered by microbial catabolism.

INTRODUCTION

The fluorochemicals selected for this study, FC-143 and FC-95, have perfluorinated carbon chains and are chemically stable. The perfluorinated portion of fluorocarbons have not been found to be susceptible to biological degradation (1). Therefore, biodegradation studies were conducted on these compounds primarily for the sake of completeness. Without such testing, it could not be said with certainty that these compounds would resist microbial modification.

Since biodegradation was unlikely, the best feasible test conditions for biodegradation were selected. Inocula were obtained from areas considered likely to contain acclimated microorganisms. Long acclimation periods were used in an attempt to select and develop populations of microbes capable of degrading these compounds, and hydrogen analogs of the fluorocarbons were added to try to select organisms that might gratuitously "cometabolize" the fluorocarbons.

METHODS AND MATERIALS

Chemicals

FC-95, FC-143, the hydrogen analog of FC-95, ammonium octanoate (the hydrogen analog of FC-143), carbon-14 labeled FC-143, and carbon-14 labeled FC-95 were obtained from Commercial Chemicals Division. These chemicals were used as received unless designated otherwise (Arthur Mendel-Report in Progress).

Standard linear alkylate sulfonate prepared for use as a reference compound for biodegradation studies was obtained from the US/EPA Laboratory in Cincinnati, Ohio. Except where noted, all other compounds were reagent grade.

Culture Media

The control medium used in these studies had the composition shown in TABLE 1.

TABLE 1

CONTROL MEDIUM COMPOSITION

1) Basal salts solutions:

   1.0 g/l - NH_4Cl
   2.0 g/l - K_2HPO_4
   0.25 g/l - $MgSO_4 \cdot 7H_2O$
   0.002 g/l - $FeSO_4 \cdot 7H_2O$

2) Well water - 25 ml/l

3) Yeast extract - 0.3 g/l

4) Hydrogen analogs of either FC-95 or FC-143 - 20 mg/l

Media were prepared from stock solutions which were combined and brought to volume just prior to each culture transfer. A fresh solution of $FeSO_4 \cdot 7H_2O$ was prepared and dry yeast extract was used in media preparation at each transfer. The pH of all media was adjusted to 7.5 with 1.0 N HCl and if overshot adjusted back with 1.0 N NaOH. The well water was added to insure an adequate supply of trace elements. Analyses of the well water made during the 12-month period prior to the initiation of this study showed its calcium hardness to range from 92 to 144 mg/l expressed $CaCO_3$. Any precipitate resulting from the addition of well water was removed by filtration through a #54 Whatman filter.

The purified hydrogen analogs of FC-95 and FC-143 were used in biodegradation test media and controls. These compounds were included in an attempt to select a microbial population likely to degrade the fluorocarbons. Enzymes capable of catalyzing defluorination reactions are frequently identical to enzymes involved in carbon-hydrogen bond cleavage (1). Additional components of other specific media are listed in TABLE 2.

TABLE 2

GROWTH MEDIA FORMULATIONS

Media	Components
FC-95	FC-95 Control Medium + 50 mg/l FC-95
FC-143 Test	FC-143 Control Medium + 50 mg/l FC-143
Phenol Controls	FC-95 or FC-143 Control Medium + 30 mg/l Phenol
LAS Controls	FC-95 or FC-143 Control Medium + 30 mg/l Standard Linear Alkylbenzenesulfonate (LAS)
Fluoride Controls	FC-95 or FC-143 Control Medium + 33.2 mg/l NaF (15.0 mg/l F^-)
^{14}C-FC-95 Test	FC-95 Control Medium + 50 mg/l ^{14}C-FC-95
^{14}C-FC-143 Test	FC-143 Control Medium + 50 mg/l ^{14}C-FC-143
FC-95 + LAS	FC-95 Control Medium + 30 mg/l LAS + 50 mg/l FC-95
FC-143 + LAS	FC-143 Control Medium + 30 mg/l LAS + 50 mg/l FC-143

Culturing Procedures

The initial growth period was started by inoculating 49 ml of each medium with 1 ml of activated sludge supernatant. The activated sludge used was a mixture of two sludges collected on the day of inoculation. The sludge was obtained from the Metropolitan Waste Control Commission's Metro plant in Saint Paul, Minnesota, and the Chemolite Waste Treatment Plant in Cottage Grove, Minnesota.

Following inoculation, the cultures in polypropylene Erlenmeyer flasks were shaken at 200 rpm on rotary shakers at room temperature (4).

At the end of each growth period, each culture was transferred to identical fresh media using a 1% inoculum from the preceding culture (i.e., 0.5 ml of existing culture to 49.5 ml of identical new medium).

The growth period between transfers varied as is noted in TABLE 3. A 10 ml sample was taken from each culture at 10 minutes after inoculation or culture transfer and at the end of each growth period. Samples were centrifuged for 10 min. at 17,000 x g prior to analysis of the centrifugate. Deviations from this culturing procedure are noted in TABLE 3.

The final growth period differed from preceding periods. Media were prepared with Carbon-14 labeled FC-95 and FC-143. One hundred ml cultures were grown in flasks on a rotary shaker in a growth chamber controlled at 25° C. ± 1. Twenty ml samples were taken at 10 min., 2 days and at 7 days.

Chemical Analysis

Fluoride ion concentrations were measured using a fluoride ion electrode (Orion ion analyzer fluoride electrode model 96-09), and a standard curve drawn from the results of measurements of accurately prepared fluoride standards. The concentrations of these fluoride standards bracketed the concentrations present in the experimental samples. Fluoride curves were set up at each sampling period, except for transfer 1. For the analyses following this transfer, a 1.0 ppm fluoride standard was used to calibrate the instrument with the assumption that the slope of the previous fluoride curve remained constant.

Phenol analysis was done according to Standard Methods for the Examination of Water and Wastewater, 14th Edition, 1975. Linear alkylbenzenesulfonate (LAS) was analyzed for by the methylene blue, chloroform extraction method described in the 14th edition of Standard Methods (3), except in transfers 8-14, LAS was analyzed by a modification of this method. In this modified method, the samples was diluted to 100 ml in a separatory funnel. Also added to the separatory funnel were 25 ml of Standard Methods methylene blue solution and 100 ml of chloroform. This mixture was shaken for 30 seconds, allowed to settle, swirled, and the chloroform drawn off through glass wool into a 2.5 cm diameter, spec 20 curvette. Percent transmittance was read at 652 nm and compared to a standard curve prepared with surfactant samples of known concentration treated in the same manner.

TABLE 3

SUMMARY OF CULTURING PROCEDURES USED
IN THE SHAKE FLASK BIODEGRADATION STUDY OF
FC-95 AND FC-143

Transfer #	Culture Growth Period (days)	Notes
0	3	Used activated sludge inoculum from Metro and Chemolite.
1	3	FC-143-hydrogen analog added to 143 cultures and controls.
2	4	
3	3	At the time of culture transfer, 1 ml of Decatur sludge supernatant added to cultures.
4	3	LAS replaced phenol as a reference compound. LAS media was inoculated with a mixture of control culture and Chemolite and Decatur sludge supernatant.
5	3	
6	3	The use of fluoride control was discontinued.
7	6	Shaker was inadvertently turned off, possibly for 5 days, during this growth period.
8	3	½ ml of Metro sludge supernatant was added to all cultures.
9	6	
10	4	
11	4	In this and subsequent growth periods, cultures were grown in a reciprocating shaker-water bath at 100 strokes per min. and 25° C.
12	6	
13	6	
14	8	
-	+6 (2)	
15	7	

78 days = Total Enrichment Period

Carbon 14 Counting Techniques

Scintillation counting was performed on 1 ml samples of culture centrifugate added to Aquasol(R), and counted with an internal standard quench correction. The radioactivity of these samples was compared to known weight samples of ^{14}C-FC-95 or ^{14}C-FC-143 added directly to Aquasol.

Solid samples were collected directly onto millipore HA 0.45 µm filters composed of cellulose acetate and cellulose nitrate. The filters were then washed with deionized water and placed into paper combustion cones, wet with Combustaid(R), and combusted in Agrichem's Packard(R) combustion equipment. The $^{14}CO_2$ resulting from combustion was trapped in a scintillation fluid containing an organic amine and counted in Agrichem's Packard scintillation counter. Samples were recounted with an internal standard for quench correction.

Thin-Layer Chromatography (TLC)

Thin-layer chromotography was performed to detect radioactive metabolites of ^{14}C-FC-95 and ^{14}C-FC-143. Ten ml culture samples were collected and immediately frozen. These samples were stored frozen for about 1 month. The samples were extracted immediately after thawing with 10 ml of ethyl acetate. The samples were then centrifuged at 17,000 x g to ensure the separation of the ethyl acetate, water, and solids phases. The water phase and portions of the ethyl acetate phase were evaporated to dryness under N_2. The dried samples were resuspended in a 9:1 hexane:ethyl ether mixture. (Some samples which evaporated to dryness in air before spotting were resuspended in methanol.) The resuspended samples were spotted on E. Merck silica gel GF_{254}. Small spots of solids residue were also applied directly to these plates. All samples were referenced against a mixture of ^{14}C-FC-143 and ^{14}C-FC-95. The plates were developed with 10% ethanol in ethyl acetate and visualized by exposing Kodak no-screen x-ray film on the plates for one week.

TLC was repeated on the remaining portion of the solvent samples. The solvent was allowed to evaporate to dryness in air, and the residue resuspended in methanol. These plates were spotted more heavily, developed as before, and visualized with x-ray film for 2 weeks.

Gas-Liquid Chromatography (GLC)

Ethyl acetate extracts were prepared as described in the thin-layer chromatography methods. Control solutions were made by dissolving ^{14}C-FC-95 and ^{14}C-FC-143 in ethyl acetate. Portions of the ethyl acetate extract samples and the ethyl acetate control solutions were also methylated. Aliquots of the methylated and nonmethylated ethyl acetate extracts and controls were injected onto the 5713 Hewlett Packard gas chromatograph with electron capture detector. Methylated samples were injected within 3 hrs. of their methylation. The chromatographic column was 12 ft. x 1/8" O.D. stainless steel packed with 20% DC 200 (12,500 CS) on 10% Bentone 34 and 70% 80/90 mesh Anakrom P.A. The injection port temperature was 250° C., and the detector temperature 300°C. The column temperature was programmed to hold for 4 min. at 75° C., to rise to 180° C. at 8° C. per min., and to hold at 180° C. The flow rate was adjusted to 35 ml/min. of Argon/methane, 95/5.

Methylations were performed by adding a 20 µl aliquot of a 1 µg/ml $C_9F_{19}COOH$ solution, as a reference compound to each sample. Diazomethane was then added until a yellow color persisted. The samples were then loosely capped, swirled and allowed to stand for 15 minutes. Nitrogen was blown over the samples until the yellow color disappeared, and the sample was returned to its original volume with ethyl acetate.

RESULTS AND DISCUSSION

Fluoride Release

In all but the final growth period, degradation of FC-95 and FC-143 was monitored only by analysis of fluoride concentration at the beginning and end of each culture period. It was assumed that if the fluorochemical portions of these molecules were degraded, fluoride ion would accumulate in the media. To ensure that fluoride was not lost from the culture by absorption, precipitation or volatilization, control cultures were grown with 15 mg/l of fluoride. This fluoride concentration is approximately what would result if FC-95 or FC-143 underwent degradation with 50 percent fluoride release. The results of the fluoride analyses conducted on different days showed considerable variation. This was due to the variable and very sluggish response of the fluoride electrode. TABLE 4a shows the results obtained at each transfer. TABLE 4b shows the results obtained when the same samples, which had been stored in polyethylene containers, were analyzed together after the termination of the experiment. Despite the variability due to the analytical technique, the results indicate that fluoride, if released to the media through biodegradation, would not be lost from the media.

The results of the fluoride analysis on fluorocarbon-containing cultures and controls are shown in TABLE 5. The results show that no biodegradation with fluoride release occurred.

TABLE 4a

INITIAL AND FINAL FLUORIDE CONCENTRATION (mg/l) OF FLUORIDE SUPPLEMENTED CONTROLS MEASURED BY SPECIFIC ION ELECTRODE AT THE TIME OF TRANSFER

Transfer #	FC-95 Fluoride Control		FC-143 Fluoride Control	
	Initial	Final	Initial	Final
0	21	23	20	21
1	23	22	21	20
2	20	22	18	21
3	26	17.5	25	16.5
4	16.5	19.2	16	17.3

TABLE 4b

INITIAL AND FINAL FLUORIDE CONCENTRATION (mg/l) OF FLUORIDE SUPPLEMENTED CONTROLS MEASURED BY SPECIFIC ION ELECTRODE MEASURED COLLECTIVELY AT END OF STUDY

Transfer #	FC-95 Fluoride Control		FC-143 Fluoride Control	
	Initial	Final	Initial	Final
0	16.4	16.2	15.7	17.0
1	15.6	16.2	15.7	15.0
2	15.6	16.2	14.5	16.4
3	16.2	16.2	16.4	15.6
4	15.6	15.7	15.7	17.0
5	19.3	17.0	15.0	16.4

TABLE 5

INITIAL AND FINAL FLUORIDE CONCENTRATION (mg/l) OF FC-143 AND FC-95-CONTAINING CULTURES AND OF NONSUPPLEMENTED 95 AND 143 CONTROL CULTURES

	FC-95 Test		95 Control		FC-143 Test		143 Control	
Transfer #	Init.	Final	Init.	Final	Init.	Final	Init.	Final
0	0.46	0.51	0.31	0.33	<0.1	<0.1	<0.1	<0.1
1	0.50	0.46	0.36	0.36	<0.1	<0.1	<0.1	<0.1
2	0.42	0.66	0.34	0.56	<0.1	<0.1	<0.1	<0.1
3 (5)	1.75	1.6	1.75	1.5	.83	..	.81	1
4	0.73	0.71	0.68	0.60	<0.1	<0.1	<0.1	<0.1
5	0.72	0.78	0.61	0.68	<0.1	<0.1	<0.1	0.56
6	0.73	0.8	0.63	0.70	<0.1	<0.1	<0.1	<0.1
7	0.14	0.17	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
8	0.90	0.84	0.66	0.66	<0.1	<0.1	<0.1	<0.1
9	0.84	0.73	0.72	0.60	<0.1	<0.1	<0.1	<0.1
10	0.72	0.81	0.60	0.68	<0.1	<0.1	<0.1	<0.1
11	0.81	0.80	0.69	0.62	<0.1	<0.1	<0.1	<0.1
12	0.74	0.73	0.66	0.62	<0.1	<0.1	<0.1	<0.1
13	0.73	0.84	0.64	0.66	<0.1	<0.1	<0.1	<0.1
14	0.81	0.78	0.66	0.64	<0.1	<0.1	<0.1	<0.1

Reference Compounds

Reference compounds were used to demonstrate that the biodegradation test conditions used were suitable to degrade compounds known to be somewhat resistant to degradation.

In the first four growth periods, 30 mg/l phenol was added to two cultures which were identical to the test cultures, except that they lacked fluorocarbons. Analytical problems prevented the measurement of phenol concentration during the first three growth periods. In the fourth growth period, phenol was found to degrade to less than 1.3 mg/l, the limit of sensitivity of the method as applied. This demonstrated that the test conditions were suitable for the biodegradation of phenol.

In the fifth through final growth periods, reference linear alkyl sulfonate (LAS) was used as the reference compound. This compound is a standard reference material used in the Soap and Detergent Association's biodegradation test method for anionic surfactants(6). This material is considered to be relatively easily degraded. In the Soap and Detergent Association's shake flask biodegradation test, the results are considered invalid if the removal of 1-dodecene-derived LAS is not nearly complete.

The data showing the extent of degradation of LAS in surfactant supplemented controls are depicted in TABLE 6. The data showing the equivalent amount of methylene blue active substances in the controls not supplemented with LAS are depicted in TABLE 7. Little LAS degradation occurred during the first few adaptive transfers. Three transfers were required before the majority of the LAS began to degrade in the surfactant supplemented control for FC-95. Five transfers were required for LAS degradation in the 143 control. Therefore, it appeared that organisms capable of degrading 1-dodecene-derived LAS were not initially present in sufficient numbers for LAS degradation. The test condition allowed for enrichment of these organisms, but enrichment occurred at a slower rate than had been anticipated. Consequently, changes were made in the procedure to increase the rate and likelihood of acclimating organisms capable of degrading the fluorochemicals. Growth periods were extended from 3 to 4-6 days, and temperature was raised from room temperature ($<20^{\circ}$ to $22^{\circ}C$) to a constant temperature of $25^{\circ}C$. Results of LAS degradation in the final growth period are shown in TABLE 8.

In the growth periods following transfers 11 and 12, an experiment was done to determine if 50 mg/l of FC-95 or FC-143 inhibited the degradation of LAS. These results are shown in TABLE 9. FC-95 appears to have an inhibiting effect on the microbial degradation of LAS. However, its presence was not completely inhibitory. Comparison with TABLES 8 and 6 shows that the presence of 50 mg/l of FC-95 inhibited LAS degradation by 18% and 23% during these two test periods. On the other hand, within the limits of the precision of our method, FC-143 did not appear to have a significant effect on LAS degradation.

In the final growth period, 50 mg/l of carbon 14-labeled FC-95 and FC-143 were used as test substrates in place of the nonlabeled fluorochemicals. Both FC-95 and FC-143 cultures were prepared in triplicate. The concentrations of the radioactive fluorocarbons present in the aqueous phase as determined by scintillation counting are shown in TABLE 10. The initial FC-95 concentration is much lower than expected. This low value could have resulted from a systematic error in the collection of the initial FC-95 samples. It is also possible that FC-95 had not completely dissolved in the cultures when the first sample was taken, but this seems unlikely, since the initial values for FC-95 concentration from all 3 parallel cultures were almost identical (30.3, 29.8 and 30.4 mg/l). Nevertheless, the remaining data show that the radioactivity associated with FC-95 and FC-143 remained in solution during the entire 7-day degradation test period. Analysis of the biological solids showed some binding of radioactive material, but the vast majority remained in the liquid phase.

TABLE 6

CONCENTRATION OF LAS (mg/l) IN SUPPLEMENTED CONTROLS AND % LAS REMOVED

	95 - Surfactant Control			143 - Surfactant Control		
Transfer #	Init.	Final	LAS % Removal(7)	Init.	Final	LAS % Removal
4	31.5	26.8	18.4	35.5	29.5	19.4
5	28.3	27.5	0.1	32.8	25.8	21.2
6	29.8	25.5	15.5	27.0	24.0	7.0
7	25.0	12.0	91.1	25.0	30.6	-2.0
8	31.2	3.75	89.8	37.0	35.8	11.1
9	33.0	3.17	95.1	38.9	13.7	94.6
10	32.7	2.33	95.9	42.7	12.8	89.7
11	31.0	2.0	95.0	39	13.7	93.8
12	31.3	2.33	96.5	41.3	19.7	77.9
13	31.7	2.5	93.5	41.3	18.0	88.3
14	31.3	3.0	92.8	40.3	13.7	90.7

TABLE 7

CONCENTRATION OF METHYLENE BLUE ACTIVE SUBSTANCE (mg/l) IN NONSUPPLEMENTED CONTROLS

Transfer #	95 - Control		143 - Control	
	Init.	Final	Init.	Final
4	1.0	1.9	4.5	4.5
5	1.15	.38	4.5	3.5
6	0.50	.75	7.1	5.50
7	5.25	10.2	5.0	10.2
8	3.0	0.88	9.0	10.9
9	5.75	1.83	10.9	12.2
10	4.17	1.17	12.3	9.67
11	4.33	0.67	11.5	12.0
12	3.0	1.33	12.3	13.3
13	3.67	0.67	11.3	14.5
14	3.67	1.0	14.5	11.3

TABLE 8

CONCENTRATION OF MBAS (mg/l) IN SURFACTANT SUPPLEMENTED AND NONSUPPLEMENTED CONTROLS DURING FINAL GROWTH PERIOD

Time	FC-95 Controls			FC-143 Controls		
	#1 LAS Suppl.	#2 LAS Suppl.	Non-Suppl.	#1 LAS Suppl.	#2 LAS Suppl.	Non-Suppl.
Initial	28.7	29.3	1.0	34.0	36.7	6.5
Day 2	13.0	26.0	1.0	8.0	22.3	5.3
Day 7	1.67	2.0	.7	6.3	6.3	4.0
% LAS Removal (7)	96.5	95.4	-	91.6	92.3	-

TABLE 9

EFFECT OF FC-95 AND FC-143 ON THE BIODEGRADATION OF LAS ANALYZED FOR AS MBAS

	FC-95 + LAS Culture			FC-143 + LAS Culture		
Transfer #	Init.	Final	% LAS Removal(8)	Init.	Final	% LAS Removal(8)
11	58.0	34.7	73.6	53.7	27.3	97.8
12	64.3	40.3	78.9	53.7	34.0	71.4

TABLE 10

CONCENTRATION OF ^{14}C-FC-95 OR ^{14}C-FC-143 IN THE CENTRIFUGATE OF TEST CULTURE DURING THE FINAL GROWTH PERIOD

	^{14}C-FC-95 Cultures		^{14}C-FC-143 Cultures	
	Concentration	Standard Deviation	Concentration	Standard Deviation
Init.	30.1 mg/l	0.3 mg/l	46.2 mg/l	0.9 mg/l
Day 2	52.8	0.5	48.0	0.3
Day 7	53.5	3.1	49.7	0.4

Thin-layer chromatography did not reveal the presence of radioactive metabolic products of either FC-143 or FC-95. Likewise, gas liquid chromatography of the same culture extracts, both before and after methylation, showed no products that were not initially present or not also present in controls. From the combination of these results, it can be concluded that no biodegradation of these fluorochemicals occurred.

REFERENCES AND FOOTNOTES

(1) Goldman, Peter, Enzymology of Carbon-Halogen Bonds. Degradation of Synthetic Organic Molecules in the Biosphere, Nat. Acad. of Sci., Washington, DC (1972).

(2) There was a six-day period before the onset of the final growth period during which the test cultures were shaken at 25° C. in the presence of FC-95 or FC-143.

(3) Standard Methods for the Examination of Water and Wastewater, 14th Edition, American Public Health Association (1975).

(4) Daytime temperatures were observed to range between 20 and 22° F. Night temperatures were not measured during that part of the study in which cultures were shaken at ambient temperature (see TABLE 3). However, measurement made near the termination of this 2½-month study, in January, showed that nighttime temperature frequently drops to 17° C.

(5) At this transfer, Decatur sludge was added which contained a high fluoride concentration.

(6) Subcommittee on Biodegradation Test Methods of the Soap and Detergent Association, A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulfonate and Linear Alkylate Sulfonate. J. of the American Oil Chemists' Society, 42:986 (1966).

(7) Percent LAS removal was calculated as:

$$\% \text{ Removal} = \frac{(MBAS_{SI} - MBAS_{CI}) - (MBAS_{SF} - MBAS_{CF})}{MBAS_{SI} - MBAS_{CI}} \times 100$$

Where:

$MBAS_{SI}$ = The initial methylene blue active substances (MBAS) concentration of the surfactant supplemented culture.

$MBAS_{CI}$ = The initial MBAS concentration of the nonsupplemented control (TABLE 7).

$MBAS_{SF}$ = The final MBAS concentration of the surfactant supplemented culture.

$MBAS_{CI}$ = The final MBAS concentration of the nonsupplemented control (TABLE 7).

(7) The percent LAS Removal was calculated as:

$$\% \text{ Removal} = \frac{(MBAS_{STI} - MBAS_{CI}) - (MBAS_{STF} - MBAS_{CF})}{(MBAS_{SI} - MBAS_{CI})} \times 100$$

Where:

$MBAS_{STI}$ = The initial methylene blue active substances (MBAS) concentration of the culture supplemented by both LAS surfactant and either FC-95 or FC-143.

$MBAS_{CI}$ = The initial MBAS concentration of the nonsupplemental control (TABLE 7).

$MBAS_{STF}$ = The final MBAS concentration of cultures supplemented with surfactant and fluorocarbon.

$MBAS_{CF}$ = The final MBAS concentration of the nonsupplemental control (TABLE 7).

$MBAS_{SI}$ = The initial MBAS concentration of the surfactant supplemental culture (TABLE 6).

It was assumed that MBAS concentration due to FC-95 or FC-143 was not reduced by the biodegradation or other loss of these compounds.

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