Biodegradation Studies on a *p*,tert.-Octylphenoxypolyethoxyethanol[1]

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Abstract

Recent results have suggested that *p*,tert.-octylphenoxynonaethoxyethanol (OPE_{10}) has a high degree of degradability. These results have been confirmed and extended. The loss of response to cobalt thiocyanate under a wide variety of laboratory test conditions demonstrates that OPE_{10} will readily undergo bacterial attack. Significant loss of foaming and ethylene oxide in laboratory continuous and semicontinuous activated sludge units and septic tank-percolation field units demonstrate that the degree of degradation under reasonable conditions can be extensive. Tracer studies and oxygen consumption tests are used to support this conclusion. These results indicate that OPE_{10} should be adequately degraded in conventional sewage disposal systems.

Introduction

IN VIEW OF THE PRESENT concern about the biodegradability of tetrapropylene-based alkyl aryl sulfonates (ABS) and the present changeover to highly biodegradable linear alkylate sulfonates (LAS), the biodegradability of other widely-used surfactants is of interest. The ethoxylates of *p*,tert.-octyl phenol represent one class of nonionic surfactants which has found wide use in industrial and household applications.

A closely related type of surfactant in which the hydrophobe is obtained by alkylating phenol with tripropylene has been the subject of a number of investigations. The 10-mole ethoxylate of this hydrophobe is known to degrade in a highly concentrated bacterial suspension obtained from a sewage treatment plant (16). Infrared studies have suggested that the poly(ethylene oxide) chain is degraded in river water (8). On the basis of shake culture experiments, river water die-away tests and bench-scale continuous activate sludge unit experiments, Huddleston and Allred (12,13) concluded that this type of surfactant degrades slower than ethoxylates based on primary alcohols. Garrison and Matson (9) came to similar conclusions on the basis of shake culture experiments, river water die-away tests and Warburg respirometry.

Booman et al. (4) presented evidence concerning the biodegradability of the 10-mole ethoxylate of *p*, tert.-octyl phenol (OPE_{10}) which indicated that this surfactant had sufficient degradability that it would not cause a foaming problem when adequate sewage treatment practices were employed. This conclusion was based primarily on the results of experiments in continuous and semicontinuous activated sludge units. This work showed that acclimation of bacteria to OPE_{10} can and does occur in a reasonable length of time. An inoculum taken from a continuous activated sludge unit was reported to give high degradation by cobalt thiocyanate analysis in a shake culture test.

Some apparent discrepancies can be noted among the results of these investigators. The basis for much of this is the fact that acclimation of bacteria is necessary before extensive degradation of branched octyl and nonyl phenol ethoxylates can be achieved. Differences in laboratory test conditions can lead to differences in degree of acclimation and consequent extent of degradation. The results of Booman et al. (4), as well as those reported here, indicate that acclimation does occur under reasonable conditions—at least in the case of OPE_{10}.

In this paper we will discuss additional information which has been developed using shake flask tests. Also, shake flask data with fresh sludge will be presented to show that degradation of OPE_{10} apparently depends upon the nature of the nutrients available to the sludge microorganisms.

The rate and extent of acclimation and biodegradation of OPE_{10} in activated sludge units has been estimated by cobalt thiocyanate analysis, foam measurements and loss of radioactive carbon from the hydrophile. Comparison with ABS, LAS and a linear secondary alcohol ethoxylate has been made. For the anionics, only methylene blue analysis and foam measurements were used to follow the degradation. The degradability of OPE_{10} in the presence of either ABS or LAS has also been investigated.

The same analytical tools have been used to estimate the degradability of OPE_{10} in a model septic tank-percolation field system. LAS was tested in a parallel system.

Materials and Analytical Methods

The *p*,tert.-octylphenoxynonaethoxyethanol (OPE_{10}) used for this work was commercial grade TRITON X-100 (Rohm and Haas Company). The radio-tagged samples of OPE_{10} were prepared in the laboratory to the specifications for TRITON X-100. The samples contained molecules tagged with H^3 on the aromatic ring and molecules tagged with C^{14} randomly distributed in the poly(ethylene oxide) chain. The linear secondary alcohol ethoxylate used was Tergitol 15-S-9 (Union Carbide Corporation) a nine-mole ethylene oxide adduct of an 11- to 15-carbon alcohol. The alkyl aryl sulfonates were obtained from the Soap and Detergent Association. The LAS sample had the designation SDA-3S; the ABS sample, SDA-3.

For most of the cobalt thiocyanate analyses the procedure of Crabb and Persinger (6) was used. When alkyl aryl sulfonates were also present, the analytical results have been corrected for the slight interference due to the methylene blue-active material. There was no interference due to OPE_{10} in the analysis for alkyl aryl sulfonates.

In part of the shake flask experiments the method of Gordon and Haines (10) was employed. This procedure involves separation of the surfactant by manual ether extraction at pH 10 to 11 followed by reaction with ammonium cobalt thiocyanate, forming a benzene-extractable complex. After evaporation of the benzene, the complex is decomposed with dilute

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sodium hydroxide solution and the thiocyanate determined using the Aldridge (1) thiocyanate reaction to give a deep red glutaconaldehyde dye (λ max. 532 mμ). The color test is sensitive to less than 0.5 μg. of thiocyanate and permits the determination of 0.5 ppm of OPE_{10}. In a few cases the cobalt thiocyanate procedure of Greff et al. (11) was employed to obtain comparative data. The methylene blue procedure of Longwell and Maniece as modified by Slack was used for the determination of alkyl aryl sulfonates (15,17).

Surface tensions were determined using a duNoüy ring tensiometer. Foam measurements were made by the method of Huddleston and Allred (12). In this procedure 50 ml of test solution are shaken by hand for 15 sec in a 100-ml glass-stoppered graduate. The foam is allowed to settle for 15 sec and its volume estimated by means of the cylinder calibration.

Results and Discussion

Shake Culture Tests

Bacterial attack of OPE_{10} can be readily demonstrated in the shake culture test of Allred and Huddleston (2). Flasks containing 30 ppm surfactant, organic nutrients and inorganic salts are inoculated with a bacterial culture. Following aeration by shaking for seven days, samples are removed for analysis. (Data are recorded in Table I.) Using a bacterial culture obtained from a laboratory continuous activated sludge unit and known to be acclimated to OPE_{10}, OPE_{10} will undergo 90% or greater degradation in seven days as indicated by three cobalt thiocyanate procedures. Similar results are obtained with two different feed media. When a small amount of an unacclimated culture of mixed liquor from an activated sludge sewage plant [2] is employed as inoculum, a lesser degree of degradation is observed. The 98 and 99% loss of response to the cobalt thiocyanate procedure of Gordon and Haines (10) demonstrates bacterial attack on essentially all the molecules. The degradation products, however, still show some response to the cobalt thiocyanate procedures of Crabb and Persinger (6) and Greff et al. (11). Following four seven-day transfers in the presence of OPE_{10}, sufficient acclimation of the culture is noted to produce a 96% loss to the Crabb and Persinger method. In this experiment, the method of Greff et al. was not applicable due to the formation of a precipitate during analysis. The fact that the loss of cobalt thiocyanate response is due almost entirely to degradation rather than adsorption on the new bacterial substrate is shown by addition of 30 ppm OPE_{10} to culture flasks aerated for seven days without surfactant being initially present. With continuous agitation for one-half hour to allow time for adsorption, recovery of the OPE_{10} was 80 to 99% by the three analytical procedures.

In addition to the above experiments, other types of shake flask tests were performed to determine the effect of medium on degradation when fresh unacclimated activated sludge was employed as the source of microorganisms. In all cases a basal salt-buffer of the following composition was used: $MgCl_2 \cdot 6 H_2O$—80 ppm; $FeCl_3 \cdot 6 H_2O$—10 ppm; $CaCl_2 \cdot 2 H_2O$—25 ppm; Na_2HPO_4–KH_2PO_4 (pH 7.2)—0.01 M. In addition, various nutrients were added to the medium.

The results are given in Table II. The various nutrients added to these media are listed in the first two columns. In all cases the initial OPE_{10} concentration (by analysis) was 75 to 100 ppm. For Experiment I the inoculum was prepared by centrifuging and washing fresh mixed liquor from an activated sludge sewage treatment plant (Levittown, Pennsylvania). It was then added to the prepared media. The suspension (305 ml total volume) was shaken in a one-liter flask on a rotary shaker at 30C. Periodically, the pH was adjusted to 7.1 to 7.2. Experiment II was similar to Experiment I but the inoculum used was one ml. of the 28-day-old culture from the preceding experiment. This inoculum was added to 250 ml of the fresh medium.

The results of Experiment I show that on the basis of cobalt thiocyanate analyses (Gordon and Haines), degradation of OPE_{10} can occur with fresh unacclimated activated sludge. It is only normal, therefore, that the culture enrichment that naturally occurs in activated sludge units finally leads to extensive degradation of OPE_{10} in these units. Degradation was somewhat slower in those flasks containing very high amounts of added organic nutrient. This was due presumably to the initial preference of the microorganisms for the organic nutrients which were present at an overwhelmingly high ratio compared to the surfactant. In total, these results indicate clearly that fresh sludge contains a significant number of microorganisms which will degrade OPE_{10}.

In Experiment II, similar media were inoculated with small volumes of the respective cultures from Experiment I. Good degradation occurred only in the flasks containing the glucose or glucose-nutrient broth supplements. Apparently, sufficient growth of those bacteria capable of degrading OPE_{10} did not occur

[2] Lower Bucks County Joint Municipal Authority Sewage Treatment Plant, Levittown, Pennsylvania.

TABLE I

Shake Culture Test with OPE_{10}

Inoculum source [a]	Medium [c]	Degradation after 7 days by cobalt thiocyanate analysis		
		Gordon & Haines	Crabb & Persinger	Greff, et al.
Acclimated culture [b]	A	99	97	90
Acclimated culture	B	99	96	91
Sewage plant	A	98	74	55
Sewage plant	B	99	....	....
Sewage plant [d]	B	98	96	ppt.
% recovery [e]		80	99	88

[a] Five milliliters of inoculum for 500 ml of solution.
[b] From a continuous unit started with mixed liquor from a sewage treatment plant and fed OPE_{10} for two weeks.
[c] A—300 ppm yeast extract; 3,000 NH_4Cl; 1,000 K_2HPO_4, 250 $MgSO_4 \cdot 7 H_2O$, 250 KCl, 2 $FeSO_4 \cdot 7 H_2O$.
B—Continuous activated sludge unit feed.
[d] Four transfers in shake flasks with OPE_{10} before using.
[e] Recovery of 30 ppm OPE_{10} added to seven-day flask grown without surfactant.

TABLE II

Effect of Medium on Degradation of OPE_{10} in the Shake Flask

Medium			Degradation, %[a]			
			Experiment I Fresh sludge from sewage treatment plant 4,400 ppm solids		Experiment II Media inoculated from I	
$(NH_4)_2SO_4$ conc., ppm	Organic supplement	Conc., ppm	5 days[b]	14 days[b]	5 days	14 days
100	None	.....	>97	100	0	....
100	Yeast extract	100	>97	100	0	9
1,000	Yeast extract	300	81	100	49	82
	glucose	10,000				
1,000	Glucose	10,000	89	100	94	97
	nutrient broth	10,000				

[a] Based on OPE_{10} concentration (75–100 ppm.) determined at day zero, after inoculation.
[b] Cobalt thiocyanate analysis by method of Gordon and Haines; others by method of Crabb and Persinger.

in the flasks containing little or no organic supplement. These results suggest that without acclimation, the microorganisms derived from fresh sludge cannot utilized OPE_{10} as a sole carbon source. In the presence of common nutrients, however, the necessary organisms can grow and degrade OPE_{10}. Results from other experiments reported below suggest that after more extensive acclimation, OPE_{10} can serve as a sole source of carbon and energy.

Bench-Scale Activated Sludge Tests

The operating parameters and operating procedures for continuous activated sludge units have been presented elsewhere (4). Since the earlier work was reported, the desirability of buffering the synthetic sewage feed for the units has been realized. Activity of microorganisms can be drastically affected by pH and maintenance of pH at a realistic level is important. The feed was also sterilized in order to avoid bacterial attack in the feed jar and feed tubes prior to entering the aeration chamber. The synthetic sewage used was a modification of that prescribed by German law (7). A 0.01-molar phosphate buffer has been added to the feed so as to maintain the pH at 7.2. The composition of this medium follows:

Nutrient broth, Difco (Difco Laboratories, Detroit, Michigan)		260 ppm
Yeast extract, Difco		20 ppm
Urea		27 ppm
$CaCl_2 \cdot 2\ H_2O$		5 ppm
$MgSO_4 \cdot 7\ H_2O$		25 ppm
$Fe_2(SO_4)_3$		8 ppm
Buffers (0.01 M)	KH_2PO_4	537 ppm
	Na_2HPO_4	1,084 ppm

The feed was sterilized with Millipore filters (Millipore Filter Corporation, Bedford, Massachusetts) and was never used for more than three days after filtering. The activated sludge source was mixed liquor from the Levittown sewage treatment plant.

In Figure 1, data (obtained by cobalt thiocyanate analysis) indicating acclimation of bacteria to OPE_{10} and the consequent removal of OPE_{10} from the synthetic sewage are presented.

The data in Figure 1 are for a unit operating at a retention time of 6 hr and a unit which was placed in operation with a retention time of 6 hr and then changed to three hours. Both units were started at the same time and initially were operating as duplicate units. Starting with fresh sludge, acclimation was attained in 5 to 11 days, and a high degree of degradation was maintained over a prolonged period of time at both 6- and 3-hr retention times.

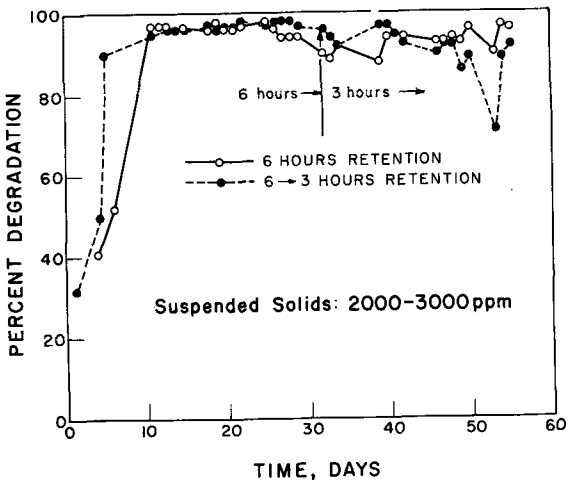

FIG. 1. Continuous activated sludge units: biodegradation of OPE_{10} at retention times of 3 and 6 hr.

A resumé of the performance of these two units in degrading OPE_{10} is presented in Table III, as are the results for a linear secondary alcohol ethoxylate and ABS. All units were started on the same day with the same source of washed Levittown sludge and received surfactant in the synthetic sewage at 20 ppm. The averages presented in this table were calculated from the data obtained from the 10th to 55th day of operation, inclusively. On the basis of the high loss of response to cobalt thiocyanate analysis (Crabb and Persinger), the foam reduction and surface tension increase, OPE_{10} and the linear secondary alcohol ethoxylate undergo a similar degree of degradation in the bench-scale activated sludge units at 3 and 6-hr retention time. Degradation of ABS was very poor at 6-hr retention by methylene blue. No foam measurements were made for the ABS effluent as the addition of defoamer to the aerator was continually required to maintain satisfactory operation.

Duplicate bench-scale activated sludge units were also fed 10 ppm each of OPE_{10} and LAS or ABS since these surfactants would be found in the presence of each other in practice. The results for a unit fed an OPE_{10}-LAS mixture are presented in Figure 2. Degradation of the surfactants was followed by cobalt thiocyanate and methylene blue analyses. A few major upsets occurred during the operation of these units so that the average degradation observed is somewhat low; however, Figure 2 does show that between upsets, a high degree of degradation for both surfactants was attained. In the case of OPE_{10} the results for duplicate units were 88% and 89% degradation. For LAS in the same units, the observed degradation was 90% and 85% during the same period of operation.

In another set of units which contained OPE_{10} and ABS, OPE_{10} degraded 90% and 95%. At the same time the ABS degraded 15% and 18%. It appears, therefore, that in a continuous activated sludge unit OPE_{10} can be degraded in the presence of ABS and that both OPE_{10} and LAS can be degraded simultaneously. By cobalt thiocyanate analysis (Crabb and Persinger), OPE_{10} degrades to as great an extent as LAS as measured by methylene blue analysis, and they are both much more degradable than hard ABS. Analytical considerations preclude the operation of the experiment at more realistic surfactant concentrations such as 10 ppm. alkyl aryl sulfonate and one to two ppm OPE_{10}.

TABLE III

Continuous Activated Sludge Test

Surfactant	Retention time, hr	Percent loss of response to: Cobalt thiocyanate [a]	Percent loss of response to: Methylene blue	Foam loss [b], %	Surface tension [c], dynes/cm
OPE_{10}	6	95	....	70	50
Linear sec alcohol E_9	6	95	....	81	52
ABS	6	....	18	....	....
OPE_{10}	3	90	....	54	47
Linear sec alcohol E_9	3	92	....	82	50

[a] Method of Crabb and Persinger (6).

[b] Based on foam volume of 20 ppm surfactant in feed (or in tap water): average, 18 ml for OPE_{10} and linear sec alcohol E_9. Effluent of blank unit (no surfactant) showed only a trace of foam (<1 ml.).

[c] Feed surface tension ranged from 40–44 dynes/cm. for OPE_{10} and linear sec alcohol E_9. Blank unit effluent surface tension ranged from 58–72 dynes/cm.

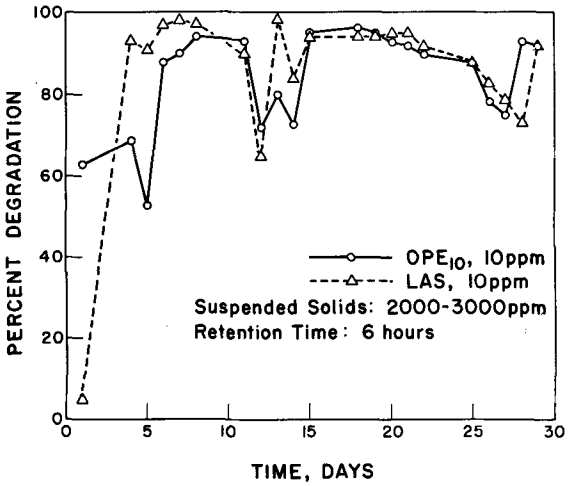

FIG. 2. Continuous activated sludge unit: biodegradation of an OPE_{10}-LAS mixture.

Duplicate units with fresh sludge were placed in operation with OPE_{10} which was randomly tagged with C^{14} in the poly(ethylene oxide) chain and with tritium in the benzene ring. The data obtained by cobalt thiocyanate analysis and by C^{14} loss (relative to the tritium) are presented in Figure 3. During the period of radioactivity observation, no loss in tritium counts was observed. Thus, loss of surfactant due to adsorption was negligible. The data in Figure 3 show that when a high degree of degradation of OPE_{10} is obtained by cobalt thiocyanate analysis, from 63% to 67% reduction in C^{14} also occurs. The loss in C^{14} is attributed to the conversion of the ethylene oxide chain to $C^{14}O_2$ and to a smaller extent to C^{14}-carbon assimilation by the microorganisms retained in the units. These results demonstrate extensive oxidative degradation.

The results presented in Figure 3 for a continuous activated sludge unit are confirmed by a similar experiment in a semicontinuous activated sludge unit. This unit was operated as before (4) with the exception that a 0.01-molar phosphate buffer was added to the feed so as to maintain the pH of the mixed liquor in the range from 7.1 to 8.0. The results are shown in Figure 4. By the 10th day of operation 98% degradation was occurring as indicated by cobalt thiocyanate (Crabb and Persinger) analysis. By the 15th day loss of carbon from the poly(ethylene oxide) chain amounted to 66% as indicated by C^{14} tracing.

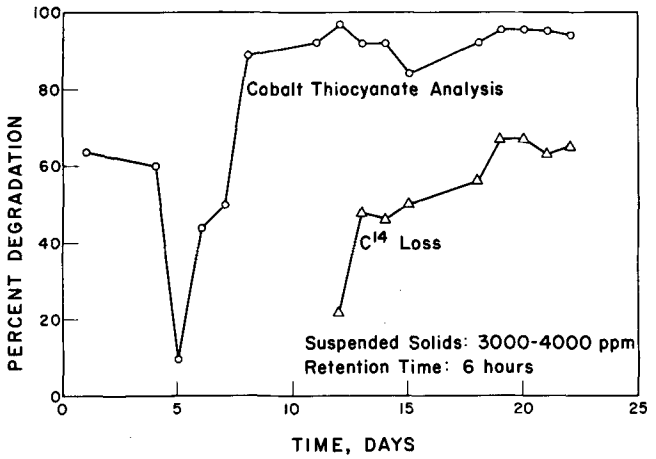

FIG. 3. Continuous activated sludge unit: biodegradation of OPE_{10} as determined by chemical analysis and C^{14} loss from the hydrophile.

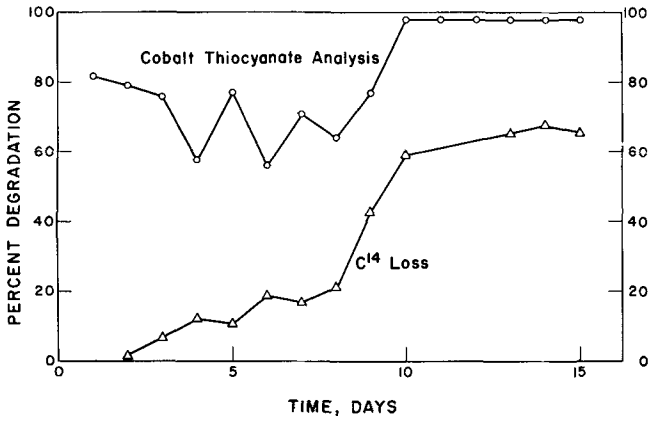

FIG. 4. Semicontinuous activated sludge unit: biodegradation of OPE_{10} as determined by chemical analysis and C^{14} loss from the hydrophile.

Again adsorption was ruled out as the basis for C^{14} loss by the tritium material balance.

Oxygen Consumption Experiments

In order to demonstrate that the loss of C^{14} was due to oxidative processes an oxygen consumption experiment was carried out as follows. Sludge from a bench-scale activated sludge unit (which was degrading OPE_{10} to greater than 95%) was centrifuged and washed twice with deionized water. The sludge was then suspended in the basal salt-buffer medium described above in the section on shake flask tests so as to give a bacterial solids content of approximately 10 ppm. OPE_{10} was added at 20 ppm and ammonium sulfate at 13 ppm. This solution was aerated by swirling in a large flask and was then poured into a screw-cap jar until no air space was left in the jar. A similar suspension was placed in a second jar without surfactant. The jars were kept at room temperature in the dark and oxygen concentration was measured periodically using the Beckman Oxygen Analyzer. Additional uninoculated, control jars were carried along as blanks—as a check, for instance, against oxygen dissolution during analysis. The results are given in Figure 5.

The difference between the oxygen consumption in the inoculated solution with surfactant and in the inoculated solution without surfactant was found to be 4.5 ppm at the end of 44 hr. At this point, the dissolved oxygen in the surfactant jar was depleted and no more oxygen consumption could, therefore, be observed. Note that the rate of oxygen utilization was greatest after an initial lag phase of 16 to 20 hr.

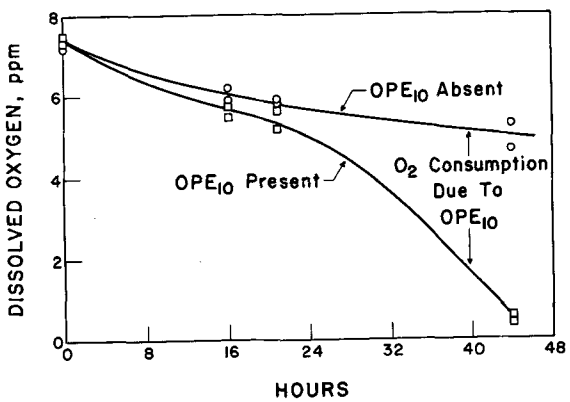

FIG. 5. Oxygen consumption by OPE_{10}-degrading microorganisms in an inorganic salts medium.

BOD (biochemical oxygen demand) tests were also made using seed from the OPE_{10}-degrading activated sludge units and employing the standard dilution BOD method (3). BOD values were obtained with three surfactant concentrations and on three separate occasions. With the standard five-day BOD test, the BOD values ranged from 18% to 26% of the theoretical COD. Extension of the BOD test to seven days resulted in BOD values of from 23% to 29% of the theoretical COD. BOD tests performed with unacclimated seed from trickle filter effluents gave five-day BOD values of 0% to 15% of the theoretical COD.

All of the results of the oxygen consumption tests just presented indicate that OPE_{10} can be oxidized significantly by microorganisms and that these microorganisms are able to utilize OPE_{10} as a major, if not sole, source of carbon and energy. The use of an acclimated seed is necessary to demonstrate a more rapid rate of oxidation. In connection with this, a bacterial culture cannot be considered truly acclimated unless some evidence for degradation can be presented. The increase in the seventh-day results above the fifth-day BOD test results with acclimated seed suggest that the maximum BOD was not realized.

The extent of oxidation on the basis of the C^{14} loss occurring in continuous and semicontinuous activated sludge units indicates a minimum of a ⅔ loss of the poly(ethylene oxide) chain. This conversion corresponds to at least a 40% loss in the chemical oxygen demand of the surfactant. It is particularly noteworthy that such extensive oxidation is occurring where alternate food sources are available for the microorganisms.

Model Septic Tank-Percolation Field System

The model septic tank was a stoppered, one-gallon glass jar with an inlet and outlet tube which could be sealed off so that the contents would remain anaerobic. The percolation field was a 3-in. diameter column of sand, with 0.5% peat moss added, of 2-ft height with a water table at the bottom. The septic tank was operated at an average retention time of 67 hr. A 48- to 96-hr retention would be normally expected in a household unit (5). The percolation field had a low percolation time of 40 to 50 sec. This column was fed three times a day so that the hydraulic loading was 2.5 gallons per day per square foot of surface area, which would be typical of a properly designed percolation field. The septic tank was initially charged with mixed liquor from an activated sewage plant and fed daily a solution which contained the feed nutrients at the concentration suggested in West German law (7). The surfactant concentration in the feed solution was 30 ppm. The pH of the effluents from both the septic tank and the percolation field remained between 7 and 8 throughout the course of the experiment. The septic tank and percolation field were kept at room temperature, 22 to 28C. The OPE_{10} employed was again the tritium- and C^{14}-tagged material. A several-month period was allowed for development of a bacterial culture in the percolation field. The results obtained during the final test period with OPE_{10} and LAS are presented in Figures 6, 7 and 8.

LAS undergoes only a minor degree of degradation in the anaerobic septic tank; no loss in foaming characteristics and 20% loss of methylene blue response. After passage through the aerobic percolation field, however, LAS does suffer appreciable degradation as shown by an overall loss of 84% of response to methylene blue test. An 86% loss of foam was also observed. These results for LAS are in substantial agreement with those reported by others (14,18). OPE_{10} showed an appreciable degree of degradation in the septic tank; a 58% loss of response to cobalt thiocyanate and a 63% loss of foam. The loss of C^{14} was only 7%. Additional degradation is observed in the percolation field to produce an overall loss of most of the cobalt thiocyanate response (93%) and most of the foam (84%). An average total loss of 46% of the C^{14} was obtained with a maximum loss of 60% to 65% at the end of the test period. Since no loss of tritium was observed during this period, the C^{14} loss can be interpreted as degradation of the ethylene oxide chain and conversion to CO_2 and/or bacterial protoplasm. Independent experiments have shown that loss of phenyl-H^3 from OPE_{10} by exchange with water is negligible.

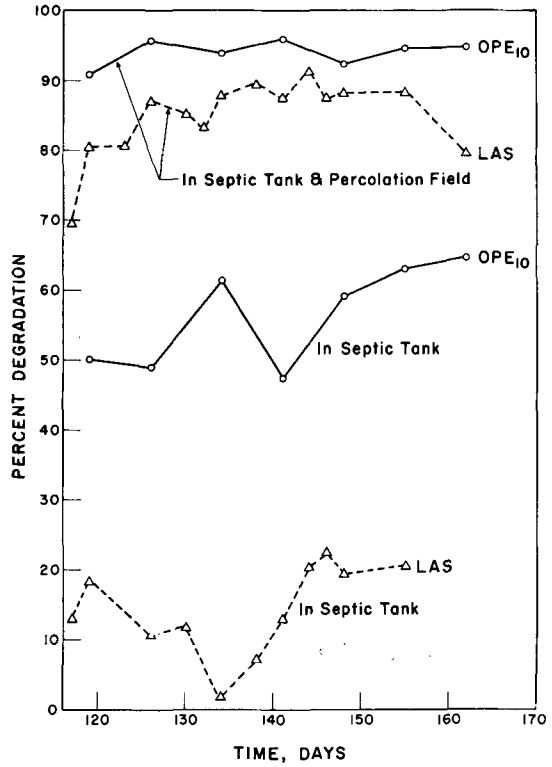

FIG. 6. Septic tank-percolation field: comparison of OPE_{10} and LAS biodegradation by chemical analysis.

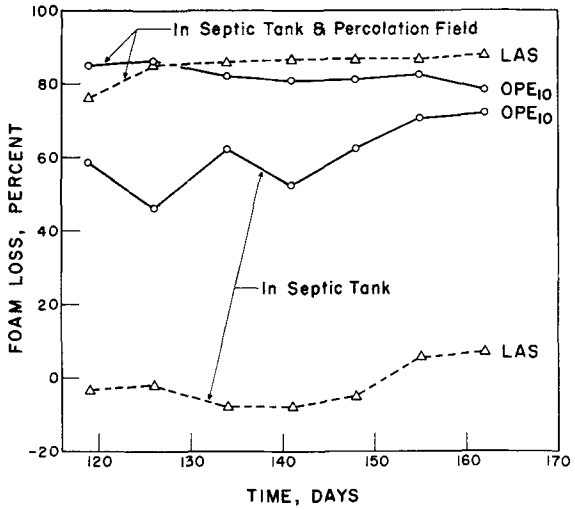

FIG. 7. Septic tank-percolation field: loss of foam due to OPE_{10} and LAS.

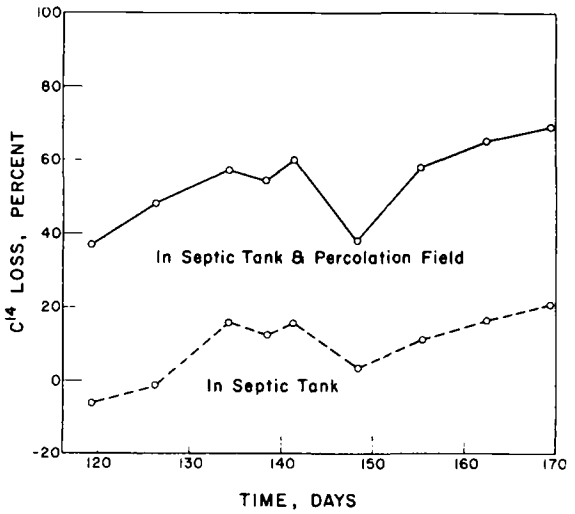

FIG. 8. Septic tank-percolation field: loss of radioactivity from OPE_{10}, C^{14}-tagged in the hydrophile.

The OPE_{10}, LAS and blank (no surfactant) septic tank-percolation field units produced a 60% reduction in COD of the feed. The 84% removal of LAS by methylene blue and the 93% removal of OPE_{10} by cobalt thiocyanate compared quite favorably to the COD loss. The 60% to 65% loss of C^{14}-tagged ethylene oxide observed at the end of the test period, which corresponds to at least a 40% loss of COD of the OPE_{10}, approaches the degree of oxidation of the feed nutrients.

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