Anaerobic Biodegradability of Carboxymethyloxysuccinate, a Detergent Builder

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ABSTRACT AND SUMMARY

Controlled amounts of C-14 labeled trisodium carboxymethyloxysuccinate, an experimental detergent builder, were added to a laboratory anaerobic digester system. The daily dosages were increased incrementally from 4 to 20 ppm, dosages being held at each level for 24 days. Biodegradation commenced after 6 days, and once complete acclimation occurred, removals were rapid and complete at all dosage levels, yielding CO_2 and CH_4 as virtually the only end-products of decomposition. After 96 days of application, total removal was in excess of 95%. Further, this compound did not interfere with the normal digestion processes or display any inhibitory effects on the bacterial population.

INTRODUCTION

Trisodium carboxymethyloxysuccinate (CMOS) is a new sequestering agent that is currently being evaluated for use as a detergent builder (1). This compound is a synthetic ether-polycarboxylate that contains neither phosphorous nor nitrogen and has chelation properties similar to those of sodium citrate. Detergent formulations containing CMOS have proven to be both safe and effective performers. As a detergent ingredient, CMOS eventually becomes a component of wastewater, and, therefore, in addition to being interested in its chemical and performance characteristics, the detergent industry is also interested in the biodegradability and treatability of CMOS in conventional wastewater treatment processes or in systems that have potential effects on groundwater quality.

Past investigations have shown that CMOS is readily and completely biodegradable in aerobic environments (2-4). There is also evidence that CMOS degradation proceeds with relative ease under anaerobic conditions; however, due to the unavailability of the radioactive material at the time, the anaerobic study (2) could not demonstrate the extent of degradation of the CMOS molecule or the nature of its end-products. CMOS removals were monitored with a colorimetric assay which was designed to detect microgram quantities of the builder (5).

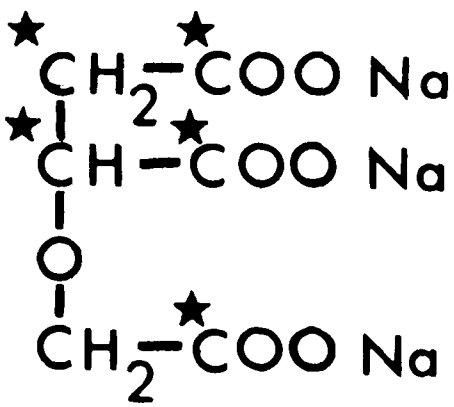

FIG. 1. C-14 labeled trisodium carboxymethyloxysuccinate.

The present research was initiated to re-evaluate the anaerobic biodegradability of CMOS and to determine conclusively whether CMOS is totally degraded or is only partially degraded to refractory end-products. Biodegradation studies were conducted in the laboratory with anaerobic sludge digesters and C-14 labeled CMOS. The performance characteristics and mode of operation of the digester units were patterned after those of a local sewage plant in Ridgewood, NJ.

This paper reports the ultimate fate of CMOS under strict anaerobic conditions and the effect that CMOS had on the anaerobic flora and on functioning of the treatment system.

MATERIAL AND METHODS

C-14 Labeled Trisodium Carboxymethyloxysuccinate ($C_6H_5O_7Na_3$)

The C-14 labeled compound was prepared as the anhydrous salt by Unilever Laboratories at Port Sunlight, England. Carboxyl labeled glycolic acid was reacted with a mixture of 1,4 and 2,3 labeled maleic acids. The product obtained was labeled at five carbon atoms (Fig. 1) with a uniform specific activity at each carbon atom. Specific activity of the compound was 0.45 μc per mg or ca. 1 x 10^6 dpm per mg. The abbreviation "CMOS" used in the present paper refers to the labeled trisodium salt, mol wt 258.

Sludges

The primary and anaerobic digester sludges were obtained from a sewage treatment plant in Ridgewood, NJ. The wastes treated at this installation are primarily of domestic origin. The digester sludge had a pH of 7.25 and contained 1.69% total solids and 67% total volatile solids. The primary sludge used as the feed mixture contained 4.34% total solids and 81% total volatile solids, and had a pH of 5.5. The primary sludge was stored at 4 C under 95% N_2-5% CO_2 mixture prior to use.

Laboratory Anaerobic Digester System

Two glass aspirator bottles, of one-liter capacity, were modified to serve as anaerobic digesters. One unit was employed as the control, while the other was used for the CMOS degradation studies. To initiate the operation, both units were filled to 800 ml with anaerobic digester sludge, providing a head space of ca. 200 ml. The digesters were then maintained in a 35 C water bath with gas lines leading to an outside collection apparatus. This arrangement is illustrated in Figure 2.

Operation and Maintenance of Digesters

The digesters were fed daily by first mixing the sludge thoroughly and then withdrawing the appropriate sample volume (25 ml). This volume was replaced with an equal volume of the primary sludge adjusted to the desired volatile solids concentration. This feed mixture was introduced rapidly into the digester via the feed input tube with the aid of a 50 ml hypodermic syringe without needle. The only mixing that occurred in the digester was at the time of sampling and feeding. At the above hydraulic loading rate, the digester detention time was ca. 32 days. The organic

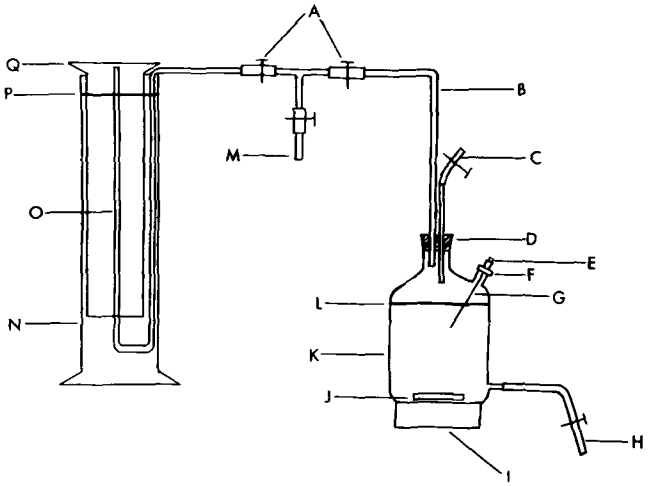

FIG. 2. Laboratory anaerobic digester system. (A) Clamps, (B) Digester gas line, (C) Feed input, (D) Rubber stopper, (E) Stopper in hypodermic needle, (F) Rubber septum, (G) 14G X 4 in. hypodermic needle, (H) Sludge effluent tube, (I) Water immersible stirrer, air operated, (J) Magnetic stirring bar, (K) One-liter aspirator bottle, (L) Sludge level, (M) Digester gas outlet, (N) Two-liter graduate, (O) Digester gas line, (P) 2% H_2SO_4 solution level, (Q) One-liter graduate.

loading rate was initiated at 0.0016 lb volatile solids per day per digester and was gradually increased to the desired or final operating level of 0.00190 lb over a period of 18 days. After the digesters were operating at desired efficiency (14 days), the specified concentration of CMOS in 1 ml of distilled water was injected daily into the side arm of the unit immediately following the addition of primary sludge. This procedure was used to insure complete delivery of the CMOS dose. The initial daily dose was a quantity (3.2 mg) which produced a concentration of 4 μg per ml or 4 ppm of CMOS. The dosage was increased to the next higher level every 24 days to yield 8, 12, 20 ppm concentrations. The actual amount of CMOS added daily was ca. 3% higher than the indicated concentration to take into account the quantity removed from the digester during the sampling.

CMOS Analysis

After the digester sludge was thoroughly mixed, a sample volume, (25 ml), was withdrawn and acidified with concentrated HCl to a pH value of ca. 4.0. The acidified sludge was placed into a 60 C water bath, and dry nitrogen gas was vigorously bubbled into the mixture for 20 min. This procedure was employed to facilitate the total removal of $^{14}CO_2$ which existed prior to acidification as either $H^{14}CO_3^-$, $^{14}CO_3^=$, or $^{14}CO_2$. Following the nitrogen treatment, an aliquot of the acidified sludge was centrifuged at 20,000 x g for 20 min. The supernatant solution was collected, diluted back to the original aliquot volume with distilled water washings from the precipitate, and subsequently filtered through a 0.22 μ Millipore membrane. Both the clear filtrate and acidified sludge were analyzed for C-14 in the Intertechnique SL 30 Liquid Scintillation Counter. The difference between these two C-14 values was interpreted as the amount of CMOS (or its equivalent) adsorbed onto sludge solids and converted into biomass. The radioactivity contained in the acidified sludge was considered totally as ppm of CMOS and/or its equivalent remaining from the dosage level.

With this value and knowledge of the theoretical or total amount of CMOS added to the digester, one can readily calculate total removal and removal per day of CMOS. The C-14 activity present in the whole sludge (unacidified) was determined by withdrawing a 10 ml volume from the

TABLE I

Performance of Anaerobic Digesters

		Control digester				CMOS[a] digester			
		Gas composition		Gas production		Gas composition		Gas production	
Day	Organic loading lb VS[b] per day	CO_2 (%)	CH_4 (%)	Liter per day	Cu ft per lb VS destroyed	CO_2 (%)	CH_4 (%)	Liter per day	Cu ft per lb VS destroyed
2	.0016	27.5	72.5	.155	6.9	26.4	73.6	.140	6.2
6	.0016	28.0	72.0	.200	8.9	27.3	72.6	.180	8.0
10	.0017	29.5	70.5	.230	8.2	27.5	72.5	.230	9.2
14	.0017	29.9	70.1	.290	11.6	30.7	69.3	.275	11.3
18	.0019	32.9	67.1	.425	15.9	30.5	69.5	.410	15.3
20	.0019	32.5	67.5	.410	13.9	31.5	68.5	.395	14.1
30	.0019	31.4	68.6	.385	13.7	32.5	67.5	.390	13.9
50	.0019	28.5	71.5	.400	14.9	31.0	69.0	.420	15.6
75	.0019	29.5	70.5	.405	13.3	28.8	71.2	.415	14.8
90	.0019	28.2	71.8	.415	14.8	31.0	69.0	.430	14.5
105	.0019	27.8	72.2	.345	12.3	28.7	71.3	.360	12.8
110	.0019	29.3	71.7	.355	12.1	28.9	71.1	.350	12.5

[a]Trisodium carboxymethyloxysuccinate.
[b]Volatile solids.

TABLE II

Operational Characteristics of Anaerobic Digesters

	Control digester					CMOS[a] digester				
Day	Bacteria x 10^6/ml	Volatile suspended solids (%)	Alkalinity as $CaCO_3$ (mg/l)	Volatile acids as acetic (mg/l)	pH	Bacteria x 10^6/ml	Volatile suspended solids (%)	Alkalinity as $CaCO_3$ (mg/l)	Volatile acids as acetic (mg/l)	pH
2	0.4	67.0	3220	190	7.20	1	67.0	3225	194	7.20
6	0.6	68.1			7.20	0.9	67.6			7.20
10	10		3110	190	7.15	25		3200	186	7.20
14	16	72.8			7.25	40	72.6			7.30
20	3	70.5	3370	240	7.40	14	72.1	3440	280	7.30
30	15	72.5	3420		7.35	20	73.6	3580		7.30
50	12		3285	270	7.30	8		3371	292	7.35
75	6	73.8	3370		7.20	10	71.0	3340		7.25
95	10		3340	226	7.20	16		3490	240	7.30
110	9	68.0		280	7.20	6	69.2		265	7.15

[a]Trisodium carboxymethyloxysuccinate.

digester side arm with a hypodermic syringe. This sample was then immediately injected into a 50 ml serum vial (sealed with rubber septum) containing 25 ml of 4N NaOH. The mixture was thoroughly agitated to insure complete dissolution of the $^{14}CO_2$ before an aliquot was removed for liquid scintillation counting. The C-14 contained in this fraction represents CMOS or its end products and the $^{14}CO_2$ which did not evolve but remained in solution. The amount of radioactivity in the whole sludge was of value in determining the steady state condition of the system.

The scintillation solution used for these analyses consisted of 0.6 g 1,4-bis-2 (4 methyl-5-phenyloxazolyl)-benzene, 7.0 g of 2,5-diphenyloxazole, and 100 g of naphthalene in a mixture of toluene (700 ml) and methyl cellosolve (300 ml). Thixotropic gel powder (Packard Instrument Co, Downers Grove, IL) was added (3-5%) to the counting vials containing the sludge samples. This method increased the counting efficiency by suspending the insoluble particles.

Gas Analysis

The total volume of gas evolved per day was determined by the liquid displacement method (Fig. 2). The gas collector system consisted of a two-liter graduate which contained an inverted one-liter graduate and 2% H_2SO_4 solution. The evolved gas entered the gas collector through a glass tube and displaced the H_2SO_4 solution from the one-liter graduate. This displaced volume, which represents the evolved gas, was determined directly from the one-liter graduate markings after the graduate was lifted slightly to alleviate gas compression.

The digester gas was analyzed daily by several methods for CO_2 and CH_4 during the initial 15 days of operation. The amount of CO_2 present in the evolved gas was determined by bubbling a known volume of gas through a solution of 4N NaOH contained in a separate gas collector system. The volume of digester gas absorbed was considered CO_2 and the nonabsorbed CH_4. Carbon dioxide was also determined from the Na_2CO_3, formed as a result of the procedure, by titration (6) and by combustion with the carbon analyzer (Beckman Instrument Co., Cedar Grove, NJ). Carbon dioxide and CH_4 were measured directly by gas liquid chromatography (7). Since all procedures gave similar results, the digester gas was analyzed routinely for its CO_2 and CH_4 composition by the NaOH absorption method.

The absorption procedure was also used for determining the amount of radioactivity in both gases. The 4N NaOH solution was analyzed for $^{14}CO_2$ (as Na_2CO_3) by liquid scintillation. The nonabsorbed gas or CH_4 was collected into metal cylinders and analyzed for $^{14}CH_4$ by Teledyne Isotopes, Westwood, NJ.

Growth Medium

Preliminary experiments revealed that the addition of digester liquor to a culture medium shortened incubation time and increased growth. To obtain this supplement, anaerobic digester sludge was centrifuged at 20,000 x g for 30 min. The clear supernatant fluid was collected and sterilized by Millipore filtration. The sterile solution was stored under 95% N_2-5% CO_2 atmosphere at 4 C prior to use. The plate medium employed for the enumeration of anaerobes consisted of brain heart infusion (BHI) agar containing 0.2% sodium thioglycolate, 0.1% cysteine HCl, .0001% resazurin, and 10% v/v of the supplement. Upon completion, these plates were immediately stored in desiccators under 95% N_2-5% CO_2 atmosphere until ready for use. The digester samples were serially diluted with a solution composed of 0.075M phosphate buffer (pH 7.2), 2% peptone, 0.5% sodium thioglycolate, and 0.2% cysteine HCl. This solution (9 ml) was introduced into loosely

capped, 16 x 150 mm screw top test tubes, and then autoclaved at 121 C for 15 min. Immediately after the sterlization period, while the tubes were still hot, the caps were screwed tightly into place, and the tubes were allowed to come to room temperature before usage. These dilution tubes and the BHI-supplement agar plates were freshly prepared at the required time and used within a 24 hr period.

Enumeration of Anaerobic Bacteria

One ml sludge samples were withdrawn from each digester, via the 14 gauge x 4 in. hypodermic needle contained in the side arm, by means of a sterile 2 ml syringe. After the serial dilutions were made, each sample was quickly plated onto the BHI-supplement agar plates. All dilutions and plating procedures were accomplished in a glove box which was continuously flushed with 95% N_2-5% CO_2 gas. The inoculated plates were incubated in desiccators at 35 C for 3-5 days under an atmosphere similar to that present in the digesters; 69% CH_4-30% CO_2-1% H_2 (gas mixture was prepared by Matheson Gas Products). Prior to incubation, the desiccators were thoroughly evacuated and refilled several times to a pressure slightly less than normal with the digester gas mixture.

Organisms from the control digester were also cultured under the same conditions on a series of BHI-supplement plates containing, in addition, various concentrations of CMOS, ranging from 10 to 500 ppm.

Total Alkalinity

Alkalinity of the digester sludge was determined by the method described in "Standard Methods for the Examination of Water and Wastewater" (8). The total alkalinity values were reported as mg per liter of $CaCO_3$.

Volatile Acids

The concentration of volatile acids present in the digester sludge was measured according to the method described by Montgomery et al. (9). The acidity values were reported as mg per liter of acetic acid.

Sludge Solids

Samples (20-25 ml) of primary and digester sludges were placed into tared crucibles, evaporated to dryness at 105 C, cooled, and subsequently weighed to determine the percent total solids. Total volatile solids or organic solids content was then determined by ashing the above dried sample for 30 min at 575 C and measuring the weight lost. The amount of suspended solids was obtained by filtering a known volume of sludge through a Selas filter crucible (fine porosity) and drying the material retained on the filter to a constant weight at 105 C. This dried residue was ignited as previously described to determine the suspended volatile solids. The calculation of percent volatile solids destroyed was determined as follows:

$$D = VS_a \times VS_d \times 100/VS_a - (VS_a \times VS_d).$$

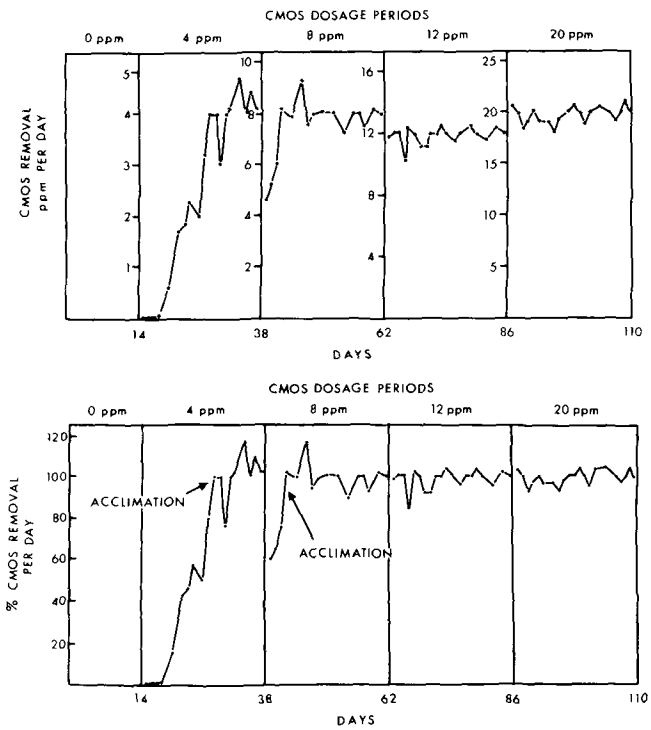

FIG. 3. Trisodium carboxymethyloxysuccinate concentrations and removals during the entire treatment process.

D is the percentage of volatile solids destroyed, VS_a is the amount of volatile solids added to digester, and VS_d is the volatile solids concentration in digester sludge.

The above procedures are those employed at the sewage treatment plant in Ridgewood, NJ.

RESULTS AND DISCUSSION

The digester gas from both units generally contained a CH_4 content of 67-73% and a CO_2 concentration of 27-32%. These values are typical of a well operated digester fed domestic sludge (10). The efficiency of digestion was initially lower than normal, based on 10-20 cu ft of gas produced per lb of volatile solids destroyed (10), but reached a normal level after 14 days of operation (Table I). The total alkalinity and acidity remained within normal limits of 2500-4000 mg per liter (10-12) and 100-300 mg per liter (10-14), respectively (Table II); thus, there was no need to add $NaHCO_3$ or change the final organic loading rate of 0.0019 lb of volatile solids per day. Under these conditions, a pH range of 7.2-7.4 was maintained, which in turn reflected normal digestion (10,15). The relatively constant bacterial numbers and volatile suspended solids contents (Table II) were also indicative of normal operation (15-17). Any significant decrease in these values would have resulted in lowered efficiency. According to the prescribed method, total bacterial counts ranged from 4 x 10^5 to 40 x

TABLE III

Summary of Trisodium Carboxymethyloxysuccinate (CMOS) Removal in Anaerobic Digester System

CMOS dosage level (ppm)	Acclimation[a] period for each dosage level (days)	Mean percent removal following acclimation	Total removal of CMOS after 96 days of application (%)
4	14	104.0	
8	4	98.6	
12	0	97.9	
20	0	97.6	
End of feeding	–	--	95.6

[a]Refers to complete acclimation or ca. 100% removal of the CMOS dosage level.

TABLE IV

Biodegradation of C-14 Labeled Trisodium Carboxymethyloxysuccinate (CMOS) to $^{14}CO_2$ and $^{14}CH_4$ in Anaerobic Digester System

Day	CMOS dosage level (ppm)	^{14}CMOS added (dpm x 10^6)	^{14}C in evolved CO_2 (dpm x 10^6)	^{14}C in evolved CH_4 (dpm x 10^6)	Ratio of $^{14}CO_2$:$^{14}CH_4$	Volume of digester gas evolved per day (liter)	Percent of ^{14}CMOS degraded to $^{14}CO_2$ and $^{14}CH_4$
71	12	9.6	4.9	4.5	1.08	.425	97.9
74	12	9.6	4.8	4.6	1.04	.420	97.9
93	20	16.0	8.2	7.6	1.08	.415	98.7
94	20	16.0	8.2	7.5	1.09	.415	98.1

10^6 per ml for both digesters (Table II). These counts compare closely to those previously reported (18-20). The digesters functioned normally throughout the entire study, and since the physical and chemical operational characteristics of the digester receiving CMOS were essentially the same as those of the control unit, it was concluded that neither CMOS nor its by-products had any effect on the digestion processes or on the microflora.

The plate study employing the BHI-supplement agar with CMOS (10-500 ppm) provided additional evidence of the builder's nontoxic properties. The cell counts were similar at each CMOS concentration, averaging 8 x 10^6 bacteria per ml. The major contribution of this experiment is that CMOS levels far in excess of those one would expect to occur in wastewater appear to be innocuous to sewage anaerobes.

Figure 3 shows both the absolute and percent removals of CMOS through the entire treatment process. The time at which the sludge appeared to become completely acclimated (100% removal) at each dosage level is indicated in the lower graph of Figure 3. CMOS degradation was initially detected after 6 days at a rate of ca. 0.6 ppm per day. However, an additional 8 days or a total of 14 days was required before 100% removal (degradation of 4 ppm per day) occurred. When the dosage level was increased from 4 to 8 ppm per day, the acclimation period was considerably shorter, requiring only 4 days. There did not appear to be any acclimation period for the higher dosage levels (Table III). It was also observed, especially in the earlier days of the study, that the removal rate occasionally exceeded 100%, or that removal in absolute values was greater than the dosage level. This greater rate of removal was attributed to the availability of surplus CMOS which accumulated during the pre-acclimation period.

As illustrated in Figure 3, a period of acclimation was needed before maximum CMOS removals occurred, both in the initial exposure and on subsequent increases in dosage level. This acclimation behavior may be due to both the gradual establishment of CMOS catabolic pathways in the existing biological community and to a population shift favoring the organisms which have the capability of metabolizing CMOS.

Also of significance was not only that maximum CMOS removals reached 100% at each dosage level but that this removal rate remained relatively constant from day to day. Supplementary kinetic studies, in which the percent removal was determined at 4-8 hr intervals over a 96 hr period, revealed that the rate of CMOS removal was indeed a constant function. These additional experiments showed that acclimated sludge (at each dosage level) adjusted its degradation rate or required 21-26 hr to completely remove the added CMOS. This indicates that the microbial community has a tendency to develop a degradation capacity proportional to the rate of CMOS application but does not develop an excess capacity or show a preference for CMOS over the more plentiful nutrients present in sludge. Further, the rate of CMOS removal by fully acclimated sludge appears to be a zero-order reaction. In terms of enzyme kinetics, the enzymes catalyzing the rate-limiting degradation step seem to be saturated with CMOS at all dosage levels.

The study also revealed that there was no appreciable adsorption of CMOS. Less than 1.9% of the added CMOS was found in the sludge solids. This small amount was assumed to be primarily the CMOS assimilated into biomass. Ancillary experiments showed the digester sludge adsorbed a maximum of 1.0 to 1.6 ppm of CMOS.

To confirm the nature and extent of CMOS removal by anaerobes, the digester gas was analyzed for C-14 during a time in which the system displayed a steady-state condition and apparent degradation rates of 12 and 20 ppm per day.

As shown in Table IV, the results were similar in all instances; ca. 98% of the added C-14 labeled CMOS was accounted for in the digester gas as $^{14}CH_4$ and $^{14}CO_2$. In addition, these radioactive gases were always produced in relatively equal amounts. The conversion of CMOS to CH_4 and CO_2 also implies that both groups of bacteria, acid-producers and methanogenics, are involved in the biodegradation of CMOS.

The present research also confirms the findings of the field investigations (flooded percolation fields and oxidation ponds) of Klein and Jenkins (2) in that CMOS degradation is rapid and complete in anaerobic environments.

The over-all data from this investigation indicate that after 96 days of treatment, CMOS removal by sedimentation was negligible, and that all CMOS removals were accomplished mainly by the digestion process. Of the total amount of CMOS added (1056 ppm), over 95% was totally degraded (Table III), and, as previously mentioned, less than 2% accumulated in the biomass. Following acclimation, CMOS removals were rapid and essentially complete, averaging over 97%, (Table III), yielding CO_2 and CH_4 as virtually the only end products of decomposition. In addition, there were no identifiable effects on the digestion system or on the microflora, even though both were exposed to excessive levels of CMOS. The 20 ppm level employed is considered to be 2-3 times higher than any anticipated concentration of CMOS that would occur in a typical municipal wastewater, even if CMOS were incorporated into the builder system of all laundry detergents. Furthermore, due to the builder's high solubility and low adsorption properties, very little of the CMOS present in wastewater is expected to be found in the primary sludge. Although it is difficult to predict quantitatively the behavior of a full scale system on the basis of laboratory data, the results indicate that the anaerobic digestion system of a municipal treatment plant should be capable of completely removing CMOS from the primary sludge.

Of greater importance is that this study conclusively demonstrates that CMOS will degrade readily under strict anaerobic conditions in the presence of naturally occurring biota. In addition, once the appropriate biota are established, CMOS will be completely removed at the rate it is applied. From the present evidence, there is little possibility of appreciable amounts of CMOS accumulating in anaerobic environments.

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REFERENCES

1. Lamberti, V., M.D. Konort, and I. Weil, Lever Brothers Co., U.S. Patent 3,692,685 (1972).
2. Klein, S.A., and D. Jenkins, J.W.P.C.F. 46:2107 (1974).
3. Peterson, D., and J. Llaneza, Arch. Biochem. Biophys. 162:135 (1974).
4. Schwartz, A.M., and A.E. Davis, "The Development of Phosphate-Free Heavy Duty Detergents," Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC, Report No. EPA-600/2-74-003, March, 1974.
5. Viccaro, J.P., and E.L. Ambye, JAOCS 50:213 (1973).
6. Litwack, G., "Experimental Biochemistry," John Wiley and Sons, New York, NY, p. 218.
7. Van Huyssteen, J.J., Water Res. 1:237 (1967).
8. "Standard Methods for the Examination of Water and Wastewater," Twelfth edition, American Public Health Association.
9. Montgomery, H.A., J.F. Dymock, and N.S. Thom, Analyst (London) 87:949 (1962).
10. "Anaerobic Biological Treatment Process," Advances in Chemistry Series 105, Edited by R.F. Gould, American Chemical Society, Washington, DC, 1971, p. 132.
11. Copeland, W.R., Sewage Ind. Wastes 27:224 (1955).
12. Simpson, J.R., "Waste Treatment," Edited by P.C. Isaac, Pergamon Press, New York, 1960, p. 31.
13. Backmeyer, D.P., Wat. Sewage Wks. J. 102:369 (1955).
14. Sawyer, C.N., "Chemistry for Sanitary Engineers," McGraw-Hill, New York, 1960.
15. Agardy, F.J., R.D. Cole, and E.D. Pearson, San. Eng. Res. Lab. Report No. 63-2, University of California, Berkeley, CA, 1963.
16. Hattingh, W.H.J., J.P. Kotzé, P.G. Theil, D.F. Toerien, and M.L. Siebert, Water Res. 1:255 (1967).
17. Kotzé, J.P., P.G. Thiel, D.F. Toerien, W.H.J. Hattingh, and M.L. Siebert, Water Res. 2:195 (1968).
18. Burbank, N.C., J.T. Cookson, J. Goeppner, and D. Brooman, Int. J. Air Wat. Pollut. 10:327 (1966).
19. Toerien, D.F., M.L. Siebert, and W.H.J. Hattingh, Water Res. 1:497 (1967).
20. McCarty, P.L., J.S. Jeris, R.E. McKinney, K. Reed, and C.A. Vath, "Microbiology of Anaerobic Digestion," Report No. R62-29, Sedgwick Laboratories of Sanitary Science, Mass. Instit of Technol., Cambridge, MA, 1962.

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