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Degradation of sodium polyglyoxylate, a non-persistent metal sequestrant, in laboratory ecosystems

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SUMMARY

Detergent builders such as zeolites, silicates and most organic polymers present concerns to some because of their environmental persistence. Sodium polyglyoxylate (SPG), on the other hand, is classified as readily biodegradable in a variety of aerobic and anaerobic biodegradation screening tests because it is extensively mineralized. Its persistence, however, is dependent on its rate of chemical hydrolysis to sodium glyoxylate, which is in turn controlled by parameters such as pH, temperature, metal ions and end capping group. The time for SPG's degradation ranges from a few hours at pH 5 to a few weeks at pH 9. Even though SPG is more persistent at alkaline pH values, it is rendered less bioavailable via precipitation/adsorption mechanisms. SPG's removal from and degradation in practically all ecosystems indicates that it will not have a significant impact on the environment with widespread use.

INTRODUCTION

The solubilization and control of metal ions are important in many commercial applications, including industrial water treatment, food processing and cleaning products. In cleaning applications, metal ion control agents are often referred to as builders or sequestrants. Detergent builders function primarily to bind or chelate metal ions, thereby softening water and permitting surfactant solutions to clean more effectively. Sodium tripolyphosphate (STP) is functionally the best all-around detergent builder. However, because of its potential to specifically stimulate algal growth in phosphate-limited environments, use of STP is being reduced around the world. Numerous non-phosphate replacements have appeared or are being developed. Inorganic materials such as zeolites and silicates and organic materials such as sodium citrate, nitrilotriacetate (NTA) and polymers are currently used, but all have some functional or ecotoxicological limitations.

Monsanto has evaluated numerous builder candidates over the past two decades for both functionality and biodegradability. We have concentrated on materials containing primarily carbon, hydrogen, oxygen and sodium, judging these to have the least serious impact on man and the environment. Furthermore, we feel that new organic

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builders must be acceptably biodegradable and/or treatable in conventional sewage treatment systems. Acceptably, in this context, means >60% mineralization in a standard biodegradation screening test and >80% removal in activated sludge biodegradation tests. In general, we have found that those materials which were acceptably biodegradable in an activated sludge screening test are functionally inferior to current detergent builders, and vice versa. Sodium polyglyoxylate (SPG) (**I**, below), was unique and of interest because it was effective as a detergent builder and was acceptably biodegradable in several screening tests [1].

Though the subject of this paper is environmental degradation or persistence of SPG, this aspect is but one of many which must be established in arriving at an environmental safety assessment of a material such as SPG. However, because such materials are highly water-soluble and do not bind heavy metals as strongly as chemicals such as NTA, EDTA and phosphonates, environmental concerns such as aquatic toxicity, bioconcentration and heavy metal mobilization are not major issues. The environmental safety assessment thus revolves around issues of persistence.

(I)

n = 50–100, X,Y = end-cap agents.

MATERIALS AND METHODS

Chemicals

All sodium polyglyoxylate (SPG) samples were synthesized at Monsanto. SPG containing a ^{14}C label in the methylene carbon had a specific activity of 2.22 μCi/mg and an average chain length of 75 monomer units. Sodium glyoxylate monohydrate (>99% purity, Fluka Chemical Co.) and glucose (> 99% purity, Difco) were used as reference chemicals. Commercial linear alkylbenzene sulfonate (LAS), average alkyl side chain length 11.8, and commercial Neodol 25-7 surfactant samples were also used as reference compounds and were obtained from Monsanto Co., St. Louis, MO and Shell Chemical Co., Houston, TX, respectively.

Procedures

Carbon dioxide evolution test

Measurement of ultimate biodegradation (carbon dioxide evolution) involved use of the shake flask procedure developed by Gledhill [2] and the medium proposed by Larson [4]. An inoculum was prepared by homogenizing activated sludge, allowing it to settle for 15 min, and adding 10 ml of the supernatant per liter of medium. Carbon dioxide was trapped as a precipitate in 10 ml of 0.15 N $Ba(OH)_2$ suspended in a tube above the medium and quantitated by titration to a pH 8.5 endpoint using 0.1 N HCl. For studies with radiolabelled compounds, barium hydroxide in the center well of the shake flask was replaced by 0.5 N KOH and radioactivity was quantitated by adding 5 ml to 15 ml Instagel (Packard) followed by liquid scintillation counting using a Beckman 6800 scintillation counter equipped with a DPM accessory for automatic quench correction. For studies involving pH variation, the normal buffer salts in the medium were replaced with 6.80 g/l KH_2PO_4 and adjusted to the desired pH with 0.1 N NaOH. All experimental flasks were set up in triplicate. Controls without test chemical were also included.

River water studies

River water was collected from the Mississippi River above St. Louis, allowed to settle for 24 h, and the supernatant was transferred to shake flask carbon dioxide evolution flasks. SPG biodegradation was assessed at the higher concentration (20 mg/l) by measurement of CO_2 evolution and at the lower concentrations (44–790 μg/l) by radiolabelled carbon dioxide analysis as described above.

Soil studies

SPG soil exposure studies were conducted over

an 84-day period in Bartha-Pramer biometer flasks using procedures based on those of Laskowski et al. [5]. The soil, a clay loam (% organic C = 0.92, pH 7.4), was added in 50-g quantities to each flask, adjusted to 50% moisture-holding capacity and dosed with either SPG (1 or 45 μg/g) or glucose (1 μg/g). The side arm contained 25 ml of 0.5 N KOH for trapping CO_2. Flasks were connected to an oxygen source at slight positive pressure to replace oxygen consumed during the test. Evolved radiolabelled CO_2 was monitored by periodic analysis of the KOH via liquid scintillation counting.

Anaerobic sludge studies

Biodegradation screening in anaerobic sludge was based on the procedure of Shelton and Tiedje [8,9]. In this procedure, test chemical that was equivalent to 5 mg/l carbon was exposed in 160 ml sealed serum vials to 100 ml of a chemically defined medium containing 1 mg/l resazurin as an oxidation/reduction indicator and 10% (v/v) anaerobic sludge from a domestic sewage treatment plant. Total gas production (CO_2 + CH_4) was measured using a Unimeasure/80 pressure transducer equipped with a P-8 bellows (Unimeasure, Inc., Grants Pass, OR). Theoretical gas from 5 mg carbon is 10.5 ml at 35°C and atmospheric pressure. Connection to the serum bottles was made with a 20-gauge needle, 1/16-inch o.d. PTFE tubing and a three-way valve. A Digitac 2120 multimeter was used to measure the transducer signal.

Anaerobic sediment/water studies

SPG degradation under anaerobic conditions was carried out using a mixture of sediment and water from a local domestic sewage lagoon. A sediment/water slurry (0.4 g sediment/100 ml water, pH 7.4) was transferred under anaerobic conditions to 160-ml serum bottles. Bottles were sealed with butyl rubber septa and incubated at 20°C for a week before dosing with 1 mg/l radiolabelled SPG. Resazurin (1 mg/l) was used as an oxidation reduction indicator. Bottles were periodically sacrificed for methane, carbon dioxide and residue analysis. Methane was quantitated after addition of 1 ml 2.5 N KOH to the bottle via sparging, combustion of methane to CO_2 at 950 °C, trapping of evolved CO_2 in KOH and scintillation counting. After methane analysis, bottles were acidified with 1 ml conc. HCl, and sparged with CO_2-free air into a KOH trap. The KOH was analyzed via scintillation counting for radiolabelled CO_2.

Activated sludge studies

Procedures for measuring biodegradation in semi-continuous activated sludge systems were based on those developed by the Soap and Detergent Association [10] and the Organization For Economic Cooperation and Development [7]. The systems employed natural activated sludge (4000 mg/l) and raw sewage from a local domestic sewage treatment plant. Sewage buffered to pH 6.2, 7.0 and 7.8 was prepared by the addition of 50 mmol KH_2PO_4 and the appropriate amount of 1 N NaOH. The extent of removal was determined via dissolved organic carbon (DOC) analysis of effluent that had been centrifuged, acidified and sparged with CO_2-free air using a Beckman Model 915B Total Carbon Analyzer. For studies using ^{14}C-labelled SPG, 100 ml of the bufferred activated sludge was placed in a 250-ml biometer flask and supplemented with the desired concentration of ^{14}C-labelled SPG. The side well of the flask contained 5 ml of 0.5 N KOH to trap evolved radiolabelled CO_2. Flasks were agitated on a rotary shaker (100 rpm) in the dark at ambient (22°C) temperature. Periodically, the KOH was removed, added to 8.0 ml of Instagel and analyzed via scintillation counting as described above.

For continuous flow activated sludge studies, the miniature 300-ml settler-type activated sludge units were used [12]. Units were equipped with traps for collecting radiolabelled CO_2 as described previously [3]. For primary treatability studies raw sewage was supplemented with 1.8 mg/l ^{14}C-labelled SPG and pumped to the unit to yield a mean hydraulic retention time of 7.0 h. The pH of the raw sewage was not controlled and averaged 8.1. SPG concentrations in the influent, effluent, centrifuged effluent and carbon dioxide traps were assayed by radiochemical analysis. For secondary

treatability studies, units were inoculated with fresh activated sludge from a local domestic sewage treatment plant and were fed refrigerated, settled raw sewage. Sewage was supplemented with 1.9 mg/l SPG and pumped to the unit to yield a mean hydraulic retention time of 7.6 h. The pH of the unit was maintained at 7.0 using a pH controller and 0.1 N HCl. Mean suspended solids concentration (MLSS) averaged 2550 mg/l, equivalent to a sludge age of approximately 7 days. SPG removal was determined as described above for the primary treatability study.

Hydrolysis studies

Hydrolysis studies were conducted under sterile conditions with 1 g SPG/l of the following buffers: pH 5.0–0.05 M $KHC_8H_4O_4$/KOH; pH 6.8–0.027 M KH_2PO_4 plus 0.039 M Na_2HPO_4; pH 9.0–0.05 M H_3BO_3/KOH. Experiments were performed at ambient temperature, 22°C, and at 40 and 60°C in a Precision Scientific incubator. Hydrolysis was monitored by measuring the concentration of the degradation product, sodium glyoxylate. Analysis involved use of a Waters Liquid Chromatograph equipped with a DuPont Zorbax NH_2 column (25 cm × 4.6 mm I.D.). The mobile phase was 0.04 M KH_2PO_4 in deionized water with a flow rate of 1 ml/min. Sodium glyoxylate was measured at 210 nm (0.04 AUFS).

Precipitation/adsorption studies

Abiotic removal studies in deionized, tap and well waters and activated sludge were conducted at ambient temperature (22°C) in 25-ml Corex centrifuge tubes with Teflon-lined screw caps. Twenty milliliters of the appropriate test solution were added to the centrifuge tube together with the desired concentration of ^{14}C-labelled SPG. Tubes were capped and placed on an orbital shaker (60 rpm). At each sampling point, tubes were centrifuged for 10 min at 15000 rpm on a DuPont Sorval RC-5B centrifuge with an SS-34 head. SPG remaining in solution was quantitated by scintillation counting (1 ml sample added to 15 ml Instagel). That material not remaining in the supernatant was assumed to be removed via precipitation in the three water systems and via a combination of precipitation and adsorption in the presence of activated sludge. Water hardness and suspended solids were determined by standard procedures [11].

RESULTS AND DISCUSSION

Mineralization of SPG in the CO_2 evolution test was extensive (Table 1). Polymers ranging in monomer content from 35 to 300 units (approximately 3500–30000 mol. wt.) averaged approximately 90% conversion to CO_2, similar to glucose. Lag times

Table 1

Ultimate biodegradation of SPG and reference compounds (pH 7.0)

	CO_2 evolution, day 35 (%, mean ± S.D.)	Rate constant, k ($days^{-1}$)	Half-life, $t_{\frac{1}{2}}$ (days)	Lag time (days)
SPG[a]				
n = 35	87 ± 8	0.100	16.4	9.5
100	88 ± 6	0.046	20.1	5.0
300	90 ± 7	0.108	14.7	8.3
Glucose[b]	87 ± 5	0.223	3.7	0.6
LAS[b]	63 ± 3	0.126	7.6	2.1
Neodol 25-7[b]	73 ± 8	0.147	6.0	1.3

[a] 50 mg/l.
[b] 20 mg/l.

Table 2

Effect of pH on SPG and glucose biodegradation

	pH	CO_2 evolution, day 55 (%, mean ± S.D.)	Rate constant, k ($days^{-1}$)	Half-life, $t_{1/2}$ (days)	Lag time (days)
SPG[a]					
n = 100	6.2	86 ± 6	0.131	5.4	0.1
	7.0	69 ± 0	0.037	18	0.1
	7.8	25 ± 3	0.007	106	0.3
Glucose[b]	6.2	99 ± 14	0.532	2.3	1.1
	7.0	98 ± 20	0.330	3.2	1.1
	7.8	100 ± 34	0.082	9	1.5

[a] 50 mg/l.

[b] 20 mg/l.

averaging about 1 week and degradation half-lives averaging about 17 days were also noted. Based on these results, SPG, as well as glucose, LAS and Neodol 25-7, would be classified as readily biodegradable. However, it was subsequently found that this apparent ease of biodegradation was dependent on pH (Table 2, Fig. 1). The rate of biodegradation was found to decrease as the pH was increased, and at pH 7.8 only 25% conversion to CO_2 was noted. In this study, little if any lag time was noted and mineralization at pH 7.0 was not as extensive as in Table 1. This may be related to the higher-strength buffers used in the latter study. The extent of glucose biodegradation was not affected by pH; however, the rates of degradation decreased at higher pH values.

The pH dependence observed together with the absence of a lag phase suggested chemical degradation to be a major step in SPG biodegradation. Consequently, a detailed set of hydrolysis studies in sterile demineralized water was initiated and confirmed the chemical degradation of SPG to sodium glyoxylate as a function of pH. Sodium glyoxylate did not further degrade in this system. Rates of hydrolysis were found to increase at lower pH and higher temperature values (Fig. 2). Metal ions had variable effects on the rate of hydrolysis (Table 3). Calcium and magnesium significantly increased the half-life, especially at neutral and alkaline pH values. The three transition metal ions did not have as great an effect on hydrolysis at pH 5.0 as they did at higher pH values. Copper significantly decreased

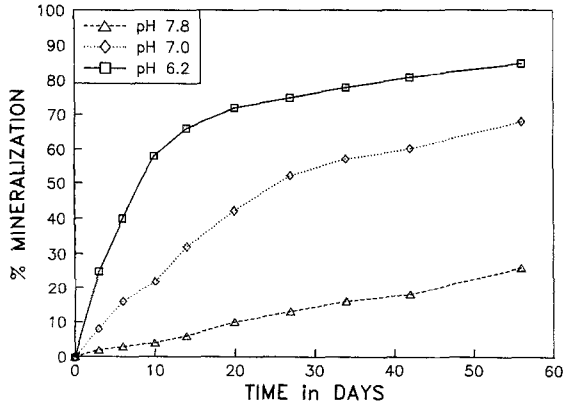

Fig. 1. SPG biodegradation as a function of pH.

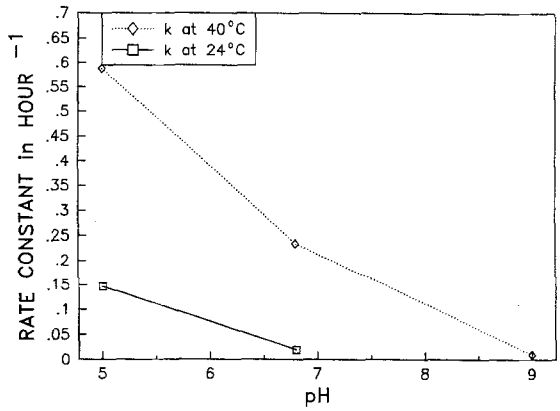

Fig. 2. SPG hydrolysis at different temperatures.

Table 3

Effect of pH, temperature and metal ions on the rate of SPG hydrolysis

Metal ion	Temp. (°C)	pH 5.0		pH 6.8		pH 9.0	
		k	$t_{1/2}$ (h)	k	$t_{1/2}$ (h)	k	$t_{1/2}$ (h)
None	22	0.147	4.7	0.019	36.8	–[a]	–[a]
None	40	0.588	1.2	0.234	3.0	0.01	71.5
None	60	2.030	0.34	0.859	0.81	0.018	38
Mg^{2+} [b]	40	0.500	1.2	0.037	19	0.001	552
Ca^{2+} [b]	40	0.110	6.5	0.001	720[d]	ND[e]	ND[e]
Cu^{2+} [c]	40	0.190	3.6	0.610	1.1	0.190	3.6
Zn^{2+} [c]	40	0.530	1.3	0.063	11	0.017	42
Fe^{3+} [c]	40	0.220	3.1	0.120	5.8	0.0035	200

[a] Not determined, rate too slow.
[b] Metal ion/SPG = 1:3.
[c] Metal ion/SPG = 0.1:3.
[d] Calcium phosphate precipitated.
[e] Not determined.

the hydrolysis half life at higher pH 9.0, while Fe^{3+} increased hydrolytic stability. Zn^{2+} decreased the half-life at pH 9.0 and increased it slightly at pH 6.8. Despite these results it is anticipated that transition metal ions will not have a great effect on SPG in natural waters because their concentrations are normally quite low [6].

The hydrolytic stability of SPG was also found to be influenced by the end-capping group (X in **I**, above). Uncapped SPG quickly hydrolyzed in water to sodium glyoxylate. Different end-capping groups rendered varying degrees of hydrolytic stability to the molecule which translated into varying degrees of biodegradability (Table 4). Material capped with ethyl and other alkylvinyl ether was less hydrolytically stable and consequently yielded the highest mineralization rates in the carbon dioxide evolution test. Because ethylvinyl ether end-capped material was sufficiently stable during detergent formulation and storage and sufficiently unstable in the environment, it was selected as the preferred builder.

Results from a variety of biodegradation tests in different aerobic and anaerobic ecosystems also demonstrated the effect of pH on the persistence of SPG. Tables 5 and 6 briefly summarize the results of several experiments. In Mississippi River water (pH 8.3) at 50 mg/l, SPG underwent little mineralization. In activated sludge at the same concentration, removal (as judged by DOC disappearance) was extensive at all pH values and ranged from 88 to 95%. Removal in activated sludge systems apparently occurred by a combination of hydrolysis, biodegradation, precipitation and adsorption. Rapid removal via precipitation was confirmed by studies of SPG in the presence of hard water (Table 7). At 1 and 10 mg/l, 40–60% of the SPG precipitated within 1 h. In the presence of activated sludge an additional 20% of the SPG was apparently removed by adsorption to suspended solids. In acti-

Table 4

Biodegradation as a function of end-capping group

X	% CO_2 evolution[a]
Ethylvinyl ether	66–94
Alkylvinyl ether (other than ethyl)	44–67
Ethylene oxide	3–37
Methoxy	14
Carboxymethyl	16
Ethoxyethyl	33
Methoxymethyl	5

[a] pH 7.0, 28 days.

Table 5

Biodegradation of SPG and reference compounds in several screening test systems

Conc. (ppm)	% $^{14}CO_2$ evolution							
	Mississippi River water[a]			Activated sludge — SPG[b] at pH:			Soil[c]	
	SPG	Glucose	Glyoxylate	6.2	7.0	7.8	SPG	Glucose
0.044	–	–	–	53	48	48	–	–
0.390	–	–	–	50	37	29	–	–
1.0	–	–	–	–	–	–	21	55
20.0	–	93[a]	87[a]	–	–	–	–	–
45.0	–	–	–	–	–	–	21	57
50.0	4[a]	–	–	(88)[d]	(95)[d]	(95)[d]	–	–

[a] pH 8.3, 28 days, conc. in mg/l, % CO_2 evolution (no ^{14}C label).
[b] 10 days, conc. in mg/l.
[c] pH 7.5, 84 days, conc. in μg/g.
[d] % DOC removal, 24 h.

vated sludge at a concentration closer to that anticipated in nature (0.39 mg/l, Table 5), pH was found to affect mineralization of SPG. However, at a lower concentration (44 μg/l ppb), mineralization was equivalent at all pH values tested. In soil (Table 5) and anaerobic systems (Table 6) (pH 7.4–7.6), mineralization ranged from 21 to 53%, approximately one-third to one-half the extent of glucose and glyoxylate biodegradation.

Thus, results of screening studies in a variety of natural systems indicated that SPG would not be persistent in nature, and that rates of degradation would be dependent on factors such as pH, concentration and type of ecosystem. Based on SPG's rapid hydrolysis to sodium glyoxylate in sterile water, SPG's stability at alkaline pH values in Mississippi River water and its stability when end-capped with groups other than alkylvinyl ether, it is con-

Table 6

SPG and glyoxylate mineralization in anaerobic sludge and anaerobic sediment/water systems

Day	Anaerobic sludge[a] (% gas production)				Anaerobic sediment/water[b] (% $^{14}CO_2$ + $^{14}CH_4$)
	SPG, avg. monomer content:			Glyoxylate	SPG
	35	100	300		
7	23	13	11	13	5
14	39	20	20	50	11
21	47	31	24	64	ND[c]
28	53	36	33	98	20
56	68	40	44	104	30
84	–	–	–	–	39

[a] Conc. 20 mg/l (5 mg C/l), pH 7.6.
[b] Conc. 1 mg/l, pH 7.4.
[c] Not determined.

Table 7

SPG precipitation and adsorption

Solution	% SPG removal from solution[a] at time (h):							
	0		1		2		3	
Deionized water	0	(0)	2	(5)	1	(6)	3	(10)
Tap water[b]	0	(0)	62	(38)	61	(50)	62	(54)
Well water[c]	0	(0)	54	(56)	62	(59)	63	(60)
Activated sludge[d]	ND	(0)	ND	(74)	ND	(78)	ND	(80)

[a] SPG conc. tested = 10.6 mg/l (1.06 mg/l).
[b] pH 9.1, 98 ppm hardness.
[c] pH 8.3, 214 ppm hardness.
[d] pH 6.7, 220 ppm hardness, 2920 ppm suspended solids.

cluded that abiotic degradation is the primary initial mechanism for SPG's decomposition.

Since the primary use of SPG will be in detergent formulations, it will enter the environment mainly as a component of sewage. Thus, its treatability in a conventional activated sludge sewage treatment plant was of interest and was assessed in laboratory-scale continuous flow activated sludge units simulating both primary and secondary treatment. Fig. 3 summarizes the treatability of SPG, as measured by radiochemical analysis, in both primary and secondary systems. Upsets in pH control on days 15 and 40 caused by pump failure were reflected in activated sludge performance. Removal averaged 62% for primary and 69% for secondary treatment, respectively. Overall SPG removal through both systems was 88%. Approximately 10% of the label in secondary treatment and 3% of the label in primary treatment was evolved as carbon dioxide. The remainder of that not in the effluent was removed either by precipitation with or adsorption to the primary or activated sludge. No attempt was made to characterize that material in the effluent. However, because the primary degradation product of SPG, sodium glyoxylate, is known to be readily biodegradable, the material leaving the treatment plant with effluent or sludge was most likely a mixture of intact polymer and ^{14}C-labelled cells.

Had the previous studies been conducted in series instead of separately, as would be expected in actual practice, overall removal might have been expected to be about 90%. Based on the results of this study, of the 2 mg/l entering the treatment plant, about 0.2 mg/l would be in the secondary effluent, about 0.5 mg/l would be mineralized and 1.3 mg/l would end up with waste sludge. Based on results of our anaerobic screening studies, it would be expected that substantial mineralization to carbon dioxide and methane would occur in anaerobic digestors where longer detention times favor hydrolysis of SPG.

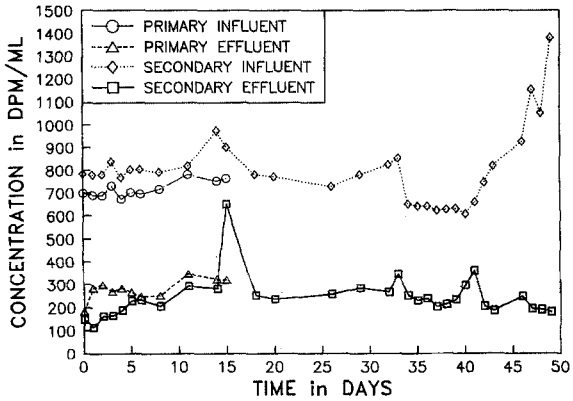

Fig. 3. SPG aerobic treatability.

In summary, and in contrast to other polymers and non-phosphate inorganic detergent builders in use today, SPG has been shown to be non-persistent in a variety of ecosystems under both aerobic and anaerobic conditions. SPG's persistence is dependent on its rate of hydrolysis to sodium glyoxylate, which in turn is controlled by such parameters as pH, temperature, concentration and metal ions. Even though SPG's stability is increased at alkaline pH values, its rapid removal from aqueous systems via precipitation/adsorption mechanisms renders it less bioavailable. For these reasons, SPG is not anticipated to present a significant impact on the environment.

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