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Role of Lime Treatment in the Removal of Bacteria, Enteric Viruses, and Coliphages in a Wastewater Reclamation Plant

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Lime flocculation/sedimentation in the first process unit of a 4,500-m^3/day wastewater reclamation plant reduced numbers of microorganisms extensively when operated at pH 11.2. The efficiency was much less at lower pH values, and some bacteria even multiplied at pH 9.6. Data on reduction in the number of microorganisms in the lime treatment and subsequent units indicate that inactivation by hydroxide alkalinity plays an important role in the efficiency of lime treatment. Reductions in the numbers of enteric viruses were higher than those of coliphages, enterococci, and total plate and coliform bacteria, which indicate that lime treatment can be monitored by means of coliphage and conventional bacteriological tests. This paper illustrates the valuable role of high-pH lime treatment in reducing the load of pathogenic microorganisms on subsequent units, including ultimate disinfection processes, which is important in the multiple safety barrier concept of wastewater reclamation processes.

Lime treatment reduces the number of microorganisms by flocculation in sedimentation or flotation processes and, at the same time, the hydroxide alkalinity has an antimicrobial effect (9, 26, 29). In laboratory studies on the efficiency of lime treatment, the relative sensitivity of bacteria to hydroxide alkalinity differed extensively. Gram-negative bacteria such as *Escherichia coli, Salmonella typhi,* and *Shigella flexneri* proved highly sensitive, whereas gram-positive bacteria like *Streptococcus faecalis, Staphylococcus aureus,* and *Bacillus* species, and particularly their spores, as well as *Mycobacterium tuberculosis,* were much more resistant (9). These findings explained the selection for gram-positive bacteria, and especially sporeformers, during lime flocculation/flotation at pH 11.5 in the first process unit of an experimental plant for the advanced purification of secondary treated wastewater (9). The total bacterial plate count was reduced by more than 99% in this unit. Enteric viruses, which are a source of great concern in water reuse practices (6, 16, 17, 23, 24), may also be removed or inactivated by lime treatment (25). In laboratory experiments lime flocculation/sedimentation and hydroxide alkalinity at pH 11.5 reduced counts of poliovirus seeded into domestic wastewater by more than 99.99% (19), and exposure of poliovirus to pH 11.1 for 90 min resulted in 98.5% inactivation (2). However, additional work with more viruses under field operating conditions is needed for general conclusions on the behavior of viruses in lime treatment processes (25).

This paper deals with the effect of lime flocculation/sedimentation treatment on numbers of bacteria, enteric viruses, and bacteriophages of *E. coli* (coliphages) in a 4,500-m^3/day wastewater reclamation plant.

MATERIALS AND METHODS

Wastewater reclamation plant. The Stander water reclamation plant at Daspoort, Pretoria, which is being used for research on the reclamation of drinking water from wastewater, has been described (7, 10). The first process unit in this multiple safety barrier system is lime flocculation/sedimentation (Fig. 1). During this study the raw water intake consisted of effluent from an activated-sludge plant. Reductions in the numbers of microorganisms were studied during runs in which the lime [$Ca(OH)_2$] dosage was varied to obtain an average operational pH of 11.2, 10.5, 10.2, or 9.6. In addition to lime, a polyelectrolyte (0.5 mg/liter) and $FeCl_3$ (1.5 to 2.5 mg/liter as Fe) were also added. The retention period of the unit was about 50 min.

Bacterial counts. For total bacterial plate count, yeast extract agar pour-plate cultures were incubated at 37°C for 48 h (7). For total coliform count, membrane-filtered samples were incubated at 37°C for 24 h on a modified MacConkey medium (7); Sartorius SM11406 or Gelman GN-6 membranes (pore size, 0.45 μm) were used. For enterococci, membrane-filtered specimens were incubated at 44.5°C for 48 h on M-enterococcus agar (Difco) (7).

Enteric virus counts. Ten-liter samples were concentrated by means of ultrafiltration (Amicon model 2000, Amicon Corp., Lexington, Mass.), using 150-mm-diameter type XM50 membranes, followed by either evaluation of the 50% tissue culture infectious dose in roller tubes or inoculation of the 16-ml concentrate.

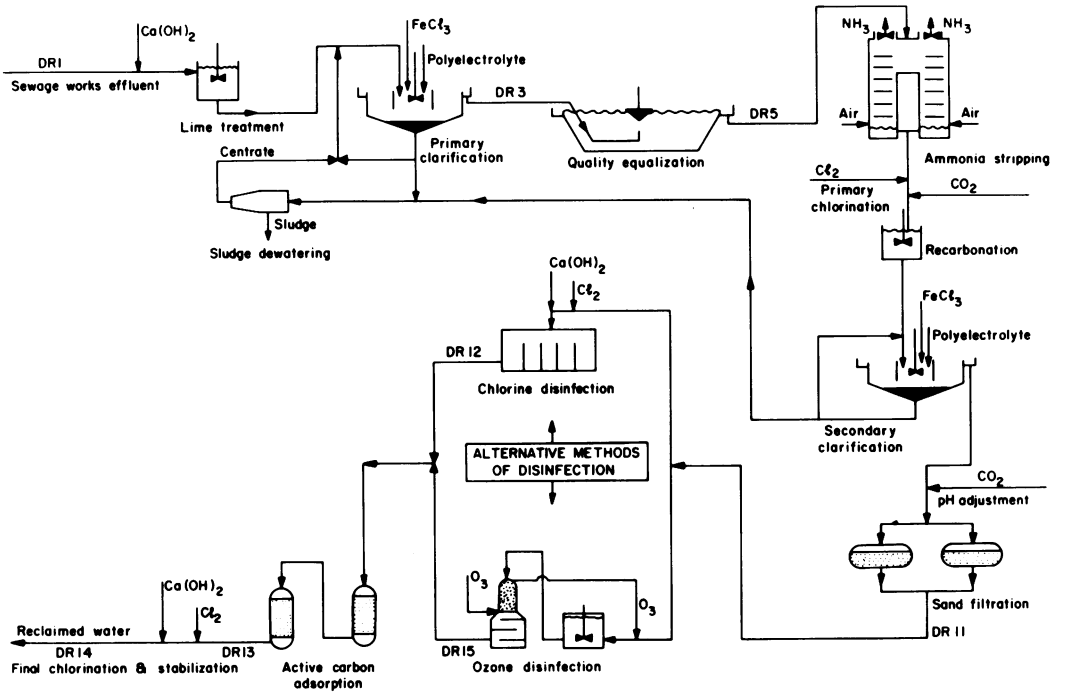

FIG. 1. *Flow diagram of the 4,500-m³/day Stander reclamation plant.*

from each 10-liter sample into a 1-liter Roux flask to obtain a qualitative positive or negative result. Primary vervet kidney tissue cultures were used in most tests. During the last 3 months of the study the BGM cell line was used in addition to, and occasionally instead of, primary cells (8).

Bacteriophage counts. Bacteriophages of *E. coli* B (coliphages) were assayed by means of a double-agar layer method (8). The bottom layer contained 11 g of agar (Difco), 13 g of tryptone (Difco), 8 g of sodium chloride, and 1.5 g of glucose per liter of distilled water. The top-layer medium contained 6 g of agar, 10 g of tryptone, 8 g of sodium chloride, and 3 g of glucose per liter. The bottom layer was poured into 85-mm-diameter plastic petri dishes and covered with a layer containing 2.5 ml of top-layer medium, 0.5 ml of the water sample to be tested, and 0.1 ml of an overnight nutrient broth (Difco) culture of *E. coli* B. Plaques were counted after incubation at 37°C for 16 h. Quantities of 10 ml were analyzed by plating 20 petri dishes with undiluted test samples. High counts were titrated by plating saline dilutions in triplicate (8).

RESULTS

The results presented in Tables 1 to 3 were obtained from 19 August 1976 to 7 October 1977. However, results for operation at pH 11.2 are typical of data recorded since the commissioning of the plant in 1970. The effect of lime treatment at different pH values on counts of microorganisms is illustrated in Table 1. Excellent reductions were recorded during an operational pH of 11.2. Reductions were much less at lower pH values. Enterococci and coliphages proved particularly resistant. The percent reduction values for enteric viruses were higher than those of the total plate, coliform, enterococci, and coliphage counts at all pH values. Numbers of enteric viruses in DR1 (raw water intake, activated-sludge effluent) were high enough for 50% tissue culture infectious dose titration of 10-liter concentrates. After lime treatment (DR3), regardless of the pH, enteric virus numbers were reduced to such an extent that they were only rarely detectable by qualitative Roux flask tests. A positive enteric virus result was obtained only once after lime treatment at pH 11.2. This was in one of eight 10-liter concentrates taken when biofilter humus tank effluent, which had considerably higher counts of all microorganisms than the activated-sludge effluent, was treated. Coliphage tests, on the other hand, yielded counts by direct titration in 5 of 22 (22.7%) samples of DR3 during lime treatment at pH 11.2. At lower pH values, 10 of 34 (29.4%) 10-liter concentrates of DR3 yielded positive results in qualitative enteric virus tests, whereas 36 of 39 (92.3%) samples yielded coliphage counts by direct titration. The number of coliphages in DR1 was about 60 times greater than that of enteric viruses.

TABLE 1. *Effect of lime treatment at different pH values on counts of microorganisms in the Stander plant*

Sample[b]	pH	Count/100 ml[a]				
		Total plate count	Total coliforms	Enterococci	Coliphages	Enteric viruses[c]
DR1	7.8	44×10^5	239×10^3	71×10^2	43×10^2	70
	7.5–8.4	$20–90 \times 10^5$	$85–390 \times 10^3$	$23–110 \times 10^2$	$9–120 \times 10^2$	10–400
	(27)	(28)	(27)	(27)	(27)	(17)
DR3	11.1	247×10^2	50	205	2	0/16
	10.9–11.3	$50–700 \times 10^2$	0–210	45–390	0–10	
	(27)	(27)	(27)	(21)	(22)	
Reduction (%)		99.44	99.98	97.11	99.95	100.00
DR1	7.7	29×10^5	110×10^3	21×10^2	40×10^2	65
	7.2–8.0	$10–110 \times 10^5$	$17–363 \times 10^3$	$10–53 \times 10^2$	$12–95 \times 10^2$	15–159
	(25)	(25)	(25)	(25)	(25)	(25)
DR3	10.5	118×10^3	632	184	168	7/25
	10.1–10.9	$15–300 \times 10^3$	30–1,700	39–425	0–710	
	(25)	(25)	(24)	(16)	(24)	
Reduction (%)		95.93	99.43	91.24	95.80	>99.98
DR1	7.9	28×10^5	124×10^3	25×10^2	31×10^2	60
	7.8–8.0	$3–55 \times 10^5$	$17–186 \times 10^3$	$14–37 \times 10^2$	$11–50 \times 10^2$	10–150
	(7)	(7)	(7)	(7)	(7)	(7)
DR3	10.2	50×10^4	83×10^2	727	687	0/7
	10.1–10.3	$25–96 \times 10^4$	$15–150 \times 10^2$	120–1,600	110–2,100	
	(7)	(7)	(7)	(6)	(7)	
Reduction (%)		82.14	93.27	70.92	77.84	100
DR1	7.8	39×10^5	162×10^3	33×10^2	35×10^2	58
	7.7–8.1	$19–65 \times 10^5$	$50–390 \times 10^3$	$4–54 \times 10^2$	$14–60 \times 10^2$	10–140
	(8)	(7)	(7)	(8)	(8)	(8)
DR3	9.6	23×10^5	61×10^3	1,044	1,505	3/8
	9.5–9.6	$11–39 \times 10^5$	$22–96 \times 10^3$	550–1,900	630–2,960	
	(8)	(8)	(8)	(8)	(8)	
Reduction (%)		41.03	62.35	68.36	57.00	>99.98

[a] Average count and range for number of samples given in parentheses.

[b] DR1, Raw water intake, activated-sludge effluent; DR3, lime treatment effluent (see Fig. 1).

[c] DR1: 50% tissue culture infectious doses/100 ml; DR3: number of positive results/number of 10-liter samples tested.

The quality equalization unit (Fig. 1) consisted of a pond with surface aeration and a mean residence time of about 10 h (28). The main purpose of this unit was to facilitate the downstream control of breakpoint chlorination as well as carbon dioxide and alkali dosing (28). Additional advantages were ammonia desorption and a reduction in calcium carbonate supersaturation, and Table 2 shows that, generally, counts of microorganisms were also reduced. The pH during quality equalization depended on lime dosage in the lime treatment unit. Reductions were recorded for numbers of coliforms, enterococci, coliphages, and enteric viruses, but the total plate count usually tended to increase. The advantage of high-pH lime treatment for reduction in the numbers of microorganisms was still evident after quality equalization. During lime treatment at pH 9.6, the total plate count was 40 times higher after quality equalization than during treatment at pH 11.2, whereas the coliform and coliphage counts were 3,500 and 5,275 times higher, respectively. Nine 10-liter samples taken after quality equalization (DR5) during lime treatment at pH 11.2 all yielded negative results for enteric virus tests, whereas

TABLE 2. *Effect of quality equalization on counts of microorganisms in the Stander plant*

Sample[b]	pH	Count/100 ml[a]				Enteric viruses (no. of positives/10-liter test)
		Total plate count	Total coliforms	Enterococci	Coliphages	
DR3	11.1	247×10^2	50	205	2	0/16
DR5	10.7 10.4–11.1 (27)	480×10^2 $60–1,060 \times 10^2$ (27)	12 0–90 (26)	90 34–190 (21)	0.7 0–10 (22)	0/9
Reduction (%)		−94.3[c]	76.0	56.1	65.0	
DR3	10.5	118×10^3	632	184	168	7/25
DR5	10.0 9.8–10.3 (25)	148×10^3 $18–550 \times 10^3$ (25)	388 50–1,050 (25)	153 10–290 (16)	136 0–750 (25)	7/25
Reduction (%)		−25.4[c]	38.6	16.9	19.1	
DR3	10.2	50×10^4	83×10^2	727	687	0/7
DR5	9.8 9.7–9.8 (7)	58×10^4 $18–99 \times 10^4$ (7)	56×10^2 $9–162 \times 10^2$ (7)	388 120–810 (6)	410 50–880 (7)	0/7
Reduction (%)		−16.0[c]	32.5	46.6	40.3	
DR3	9.6	23×10^5	61×10^3	1,044	1,505	3/8
DR5	9.4 9.2–9.5 (8)	19×10^5 $13–24 \times 10^5$ (8)	42×10^3 $24–88 \times 10^3$ (8)	855 480–1,800 (8)	1,055 220–1,850 (8)	0/8
Reduction (%)		17.4	31.2	18.1	29.9	

[a] Average count and range for number of samples given in parentheses; range and number of samples for DR3 appear in Table 1.

[b] DR3, Lime-treated effluent; DR5, quality equalization effluent (see Fig. 1).

[c] Percent increase.

4 of 22 (18.2%) samples yielded coliphage counts by direct titration. At lower pH values positive enteric virus results were obtained for 10 of 40 (25.0%) 10-liter concentrates, and coliphage counts were obtained for 37 of 40 (92.5%) samples.

The effect of ammonia stripping, recarbonation, secondary clarification, and pH adjustment (Fig. 1) on counts of microorganisms was not investigated in this study. Table 3 shows that the plate count and numbers of coliforms and coliphages were 2.3, 61.0, and 254.5 times higher, respectively, after sand filtration (DR11) during lime treatment at pH 9.6 than at pH 11.2. During lime treatment at pH values below 11.2, 6 of 40 (15.0%) 10-liter concentrates of DR11 yielded positive results in qualitative enteric virus tests, whereas coliphages were detected by direct titration in 32 of 40 (80.0%) samples.

During disinfection by either breakpoint chlorination or ozonation, followed by active carbon treatment and final chlorination (Fig. 1), counts of microorganisms were consistently reduced to less than the following limits: total plate count, 100/1 ml; total coliforms, 0/100 ml; enteric viruses, 0/10 liters; and coliphages, 0/10 ml. Neither enteric viruses nor coliphages were detected after disinfection by either breakpoint chlorination or ozonation. The pH at which lime treatment was carried out had no effect on numbers of bacteria after disinfection.

DISCUSSION

Although the lime flocculation/sedimentation unit of the Stander plant reduced numbers of bacteria, enteric viruses, and coliphages extensively at pH 11.2, the efficiency was much less at lower pH levels. This indicates that inactivation by hydroxide alkalinity plays an important role in the reduction in numbers. The percent

TABLE 3. *Effect of sand filtration on numbers of microorganisms in the Stander plant*

Sample[b]	pH	Count/100 ml[a]				Enteric viruses (no. of positives/10-liter test)
		Total plate count	Total coliforms	Enterococci	Coliphages	
DR5	10.7	5×10^4	12	90	0.2	0/9
DR11	7.6 6.8–8.4 (45)	63×10^4 $5–310 \times 10^4$ (46)	200 0–970 (43)	2 0–5 (21)	2 0–10 (38)	1/20
Reduction (%)		−1,160.0[c]	−1,566.7[c]	97.8	−900.0[c]	
DR5	10.0	148×10^3	388	—[d]	136	7/25
DR11	7.2 6.8–8.3 (24)	300×10^3 $20–1,000 \times 10^3$ (25)	648 0–1,700 (25)	—	135 0–1,100 (25)	6/25
Reduction (%)		−102.7[c]	−67.0[c]		0.7	
DR5	9.8	58×10^4	56×10^2	—	410	0/7
DR11	7.3 7.0–7.5 (7)	66×10^4 $10–120 \times 10^4$ (7)	40×10^2 $21–72 \times 10^2$ (7)	—	196 0–830 (7)	0/7
Reduction (%)		−13.8[c]	28.6		52.2	
DR5	9.4	19×10^5	42×10^3	—	1,055	0/8
DR11	7.3 7.2–7.3 (8)	15×10^5 $6–44 \times 10^5$ (8)	12×10^3 $2–20 \times 10^3$ (8)	—	509 0–1,120 (8)	0/8
Reduction (%)		21.1	71.4		51.8	

[a] Average count and range for number of samples given in parentheses; range and number of samples for DR5 appear in Table 2.

[b] DR5, Quality equalization effluent; DR11, sand filtration effluent (see Fig. 1).

[c] Percent increase.

[d] —, Not done.

reduction values for the total plate and coliform counts indicate that some bacteria may even multiply during lime treatment at pH 9.6. The reduction values for these counts were generally higher than those for enterococci and coliphages at all pH levels except 9.6, at which they were lower. In addition, the reduction value for enterococci, which are probably not inactivated at pH 9.6 (9), indicates that physical removal by flocculation/sedimentation should account for higher reductions in the total plate and coliform counts than those recorded at pH 9.6. Increases in the total plate count were also recorded during quality equalization at pH values as high as 10.7 (Table 2). The advantage of high-pH lime treatment was evident from counts even after sand filtration (Table 3). The differences in counts of total bacteria and coliforms during lime treatment at different pH values would have been much higher if their numbers had not increased between quality equalization and sand filtration. Bacterial growth in the pressure type of sand filters used in the Stander plant is not unusual. The limited differences in numbers of bacteria after sand filtration following lime treatment at different pH values indicate that, at all pH values within this range, the filters reached a saturated microbial growth capacity. The apparent reduction in numbers of bacteria after sand filtration during lime treatment at low pH values may be due to removal of bacteria by secondary clarification, followed by regrowth on the sand filters to saturation numbers, which were lower than the counts in the quality equalization effluent.

Although the last three process units of the Stander plant, namely, disinfection by either breakpoint chlorination or ozonation followed by active carbon adsorption and final chlorination (Fig. 1), consistently reduced numbers of

bacteria to minimal levels and enteric viruses as well as coliphages to undetectable levels, the present findings illustrate the value of high-pH lime treatment in the multiple-barrier concept (31) of reclamation processes. The excellent reduction in numbers of even highly resistant organisms at pH 11.2 shows that high-pH lime treatment greatly reduced the load of pathogenic microorganisms, particularly enteric viruses, which proved more sensitive than coliphages and many bacteria, on subsequent units and ultimate disinfection. Bacteria that multiply in certain process units such as quality equalization and sand filtration are members of the natural microflora of water, which have negligible health importance (20, 30). One advantage of these bacteria is that they facilitate the microbiological monitoring of the efficiency of subsequent process units.

The present findings on the behavior of bacteria, enteric viruses, and coliphages in lime treatment under field conditions support evidence (8, 10) that water reclaimed from wastewater by multiple-barrier processes such as the Stander plant is microbiologically safe for human consumption provided it conforms to the following limits: total plate count, 100/1 ml; total coliforms, 0/100 ml; enteric viruses, 0/10 liters; and coliphages, 0/10 ml. This conclusion is in opposition to the view that drinking water can be regarded as safe only if the absence of enteric viruses in 379 to 3,785 liters (100 to 1,000 U.S. gallons) has been proved (1, 6, 16, 23). This view is largely based on the unjustified assumption that viruses are generally more resistant than bacteria to water purification processes, since laboratory experiments showed that certain enteric viruses are more resistant than *E. coli* to processes such as chlorination (12, 17, 18, 21, 24). Table 1 shows that enterococci and many bacteria included in the total plate and even coliform counts are more resistant to lime treatment than enteric viruses. The behavior of *E. coli* in lime treatment could not be used to represent that of bacteria in general, since laboratory experiments showed that it is one of the most sensitive organisms to hydroxide alkalinity and even other members of the coliform group, such as the capsulated *Klebsiella pneumoniae,* are much more resistant (9). Similarly, both field studies on the Stander plant (8) as well as laboratory experiments (4, 5) proved that many bacteria, especially gram-positive organisms and bacterial spores, are more resistant to chlorine and ozone than any enteric viruses tested so far; *E. coli* again proved one of the most sensitive bacteria. The value of coliphages as an indicator of the removal of enteric viruses in wastewater reclamation processes (8) is supported by the finding that they are more resistant to lime treatment (Table 1) and that their numbers in wastewater generally exceed those of enteric viruses by far (Table 1; 11, 14). The indicator value of coliphages is probably increased by coliform bacteria, which multiply in certain units and liberate additional phages (Table 3), and the host bacteria may also protect phages against disinfectants prior to release. Furthermore, field studies as well as laboratory experiments showed that, generally, coliphages are at least as resistant as enteric viruses to many water purification processes including chlorination (3, 13–15, 27). These properties of coliphages explain their detection by direct titration of samples even after sand filtration (Table 3), where only a few of the 10-liter concentrates yielded positive results in qualitative Roux flask tests for enteric viruses.

This study illustrates the important contribution that lime treatment can make to wastewater reclamation. It also supports views (7, 10, 22) that the technology to reclaim microbiologically safe drinking water from wastewater is available and that routine monitoring of multiple safety barrier systems (31) such as the Stander plant is possible by means of practical methods within the capabilities of many microbiological laboratories.

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