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Removal and Inactivation of Bacteria During Alum Treatment of a Lake

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Flocculation and removal of bacteria were observed during two separate aluminum sulfate (alum) treatments for removal of phosphorus from a eutrophic recreational lake. In addition, die-off and release of bacteria from alum floc were studied in columns under laboratory conditions. Membrane filtration and spread plates were used to determine concentrations of indicator species and total cultivatable bacteria, respectively. During the alum treatment of the lake, 90% of the fecal coliform (FC) population and ca. 70% of the fecal streptococci population were removed from the water column within 72 h. Numbers of FC in the floc on the lake bottom exceeded 2,400/100 ml at 120 h compared with the pretreatment concentration of 30 FC/100 ml. Inactivation of FC in the floc proceeded at a rate of 200 FC/100 ml per 24 h. In a second alum application to the lake, 95% of the total culturable bacterial population was removed from the water column. In a laboratory column study of survival and release rates, over 90% of an *Escherichia coli* suspension was concentrated in a floc formed at the bottom. *E. coli* was not released from the floc. The numbers of and survival of *E. coli* in the floc suggest the probable concentration of other enteric organisms, including pathogens. Thus, the floc poses a potential human health risk if ingested by swimmers or if others use the lake as a potable water source.

Phosphate is a major limiting nutrient for nuisance algae and other plant growth in many recreational lakes. Since phosphate can be removed from the water column by aluminum sulfate (alum) (8, 18), this procedure has been used as a lake restoration technique to control nuisance algae and other plant growth (8, 9). Previous investigations of the use of alum in restorations have not addressed the removal, inactivation, and resurgence of bacterial communities in the water (8, 10). The literature is replete with references to the removal of bacteria and virus from wastewater and from water treated with high coagulant dosages >50 mg/liter. Malek et al. (11) presented a review of laboratory studies of the treatment of potable water with coagulant dosages <20 mg/liter.

Alum reacts with the natural hydroxide alkalinity of water to form an aluminum hydroxide complex (10, 16). The complex reacts with phosphorus and suspended particulates to form a relatively insoluble mass (floc) which settles due to many factors, including a reduction of electrical charge. Bacterial cells are colloidal particulates and can be aggregated by a variety of chemical additives, such as alum, and by the biopolymers (5, 16, 18). The removal of *Escherichia coli* and other indicators from the water column during lake renovation by alum concentrations <15 mg/liter suggests that enteric pathogens may also be concentrated in the alum floc (11). The concentration of bacteria and their survival in and potential release from the alum complex are of public health significance. In addition, removal of autochthonous planktonic bacteria may delay reestablishment of essential biogeochemical cycles.

Liberty Lake was selected for restoration by the Department of Ecology, state of Washington, and the Environmental Protection Agency in their Clean Lakes Program. Liberty is a recreation lake where use has been limited in recent years by nuisance algal blooms (9).

In this paper, we discuss the rates of removal of fecal coliforms (FC) and fecal streptococci (FS) from a natural lake as alum was added for removal of phosphorus as part of rehabilitation. The information presented represents a portion of the overall study of the effectiveness of alum as a restoration technique. For a discussion of the short- and long-term effects of the use of alum to reduce phosphate for control of algae and aquatic weeds, refer to Funk et al. (6–8). The fact that common indicators are removed from the water column and concentrated in the alum floc at the bottom of the lake is significant, since this indicates the potential for removal and concentration of pathogens also. Once the indicators are concentrated in the floc, it is important to know the kinetics of die-off and whether they are released into the overlying water. To answer these questions, laboratory experiments were performed on flocculated bacteria to ascertain rates of die-off and release. Knowledge of the potential for concentrating enteric pathogens in the floc is important since increased recreational use of the water is the major factor in lake restoration. Swimmers and other water users may contact and ingest the floc when it is resuspended in the water by wave action or swimmer activity after it has settled in the shallow beach areas. Of practical concern is the ingestion of the floc, which may contain essentially all of the bacteria (pathogens and nonpathogens alike) and viruses from the entire water body overlying the sediments.

Since a portion of the autochthonous bacterial community is responsible for the biogeochemical cycles, total populations quantified on Henrici agar (17) indicate potential rates of reestablishment of the cycles. Removal and recovery of nonindicator populations will be discussed.

MATERIALS AND METHODS

Study area. Liberty Lake (Fig. 1) is a culturally eutrophic, shallow (8 m), soft-water lake, 288 ha in area with a mean residence time of 3 years (6). Weak stratification may occur for short periods during late summer. Recreational use is relatively heavy during summer with ca. 100,000 visitors per season (8).

Collection of water samples. After extensive pretreatment sampling, two sites were selected as representative of ambi-

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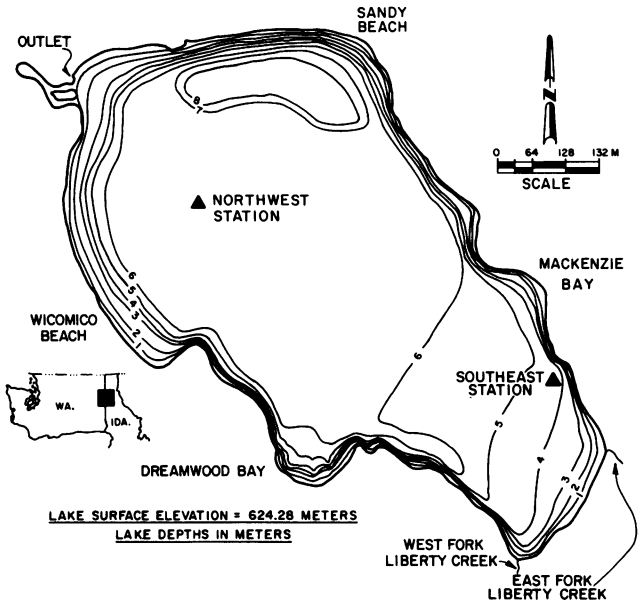

FIG. 1. Sampling stations at Liberty Lake, Wash.

ent lake conditions (Fig. 1). Water samples were collected from subsurface, middepth, and the lake bottom in sterile, 300-ml bottles from each site. Lake water was pumped to the surface from the middepth and bottom with a positive displacement pump fitted with Tygon tubing. Replicate sampling indicated no carry-over from different depths. The samples were transported to the laboratory in ice chests, and bacteriological analyses were initiated within 6 h.

Bacteriological methods. No attempt was made to separate the floc from the water in samples which contained floc. Therefore, numbers reported reflect the sample as withdrawn from the lake or experimental column. Enumeration of total bacteria (TB) and total FC bacteria (TC) was performed according to the *Standard Methods for the Examination of Water and Wastewater*, 15th ed. (2), by using the most probable number and the membrane filter techniques. FS were enumerated on m-Enterococcus agar (Difco Laboratories, Detroit, Mich.) after membrane filtration.

Henrici medium as modified by Stark and McCoy (17) was used for total plate counts. Water samples were diluted into sterile 0.1% peptone water (3) and distributed over the surface of the agar with a sterile glass rod. Dilutions were plated in duplicate and incubated at 21 ± 1°C for 7 days (D. J. Reasoner, M.S. thesis, Washington State University, Pullman, 1969). Plates with 30 to 300 colonies per plate were counted with the aid of a Quebec colony counter. Total numbers as well as numbers of pigmented (chromogenic) colonies were recorded.

Alum treatment. A pontoon barge system (6) was devised to mix and apply the granulated aluminum sulfate solution to the surface of the lake. Liberty Lake was first treated in the fall of 1980, when 80% of the lake was covered (Fig. 1) and again in the spring of 1981, when only the southern 30% was treated (Fig. 1). The alum concentration needed to bind the available phosphorus (while maintaining sufficient alkalinity) was 10 mg/liter as aluminum sulfate. The actual concentration in the lake was estimated to range from 6 to 15 mg/liter, depending on the degree of mixing.

Laboratory tests. Columns (27-cm inner diameter by 95 cm) were constructed from walled lucite (0.25 in. [0.64 cm]), which was drilled and fitted with septa at various depths to allow sampling at the water surface, just above the floc, and in the floc. Previous investigators (1, 6) reported that visible floc was not formed at an alum concentration of 15 mg/liter in small columns. Therefore, large columns were used to optimize floc formation. The columns were washed and rinsed with 70% ethanol and then with sterile distilled water to reduce contamination. Liberty Lake water was collected in sterile 18-liter bottles. Within 5 h of collection, the water was filtered with a Whatman no. 5 prefilter and a Millipore 0.45-μm filter. The columns were kept covered; however, because of the large size and the nature of the experiments, sterile conditions were not attempted.

E. coli ATCC 15224 was grown in tryptic soy broth (Difco) at 35°C. Culture age was determined by optical density with a spectrophotometer (Spectronic 20; Bausch & Lomb, Inc., Rochester, N.Y.). Cells, harvested after 24 h, were washed, resuspended in filter-sterilized lake water to prevent nutrient carry-over, and allowed to rest for 24 h at 14°C before being added to the columns.

An alum slurry was prepared from industrial-grade granular aluminum sulfate and applied across the surface with gentle mixing of the column to form a floc at the surface of the water, taking advantage of density removal by a settling floc (7). The effectiveness of alum rapidly deteriorates (1); therefore, solutions were fresh. Samples were withdrawn with syringes fitted with 1.5-in. (3.81-cm), 18-gauge needles and diluted in sterile 0.1% peptone (3). Replicate samples were taken at the same depth to determine whether the samples were consistent and representative.

Numbers of *E. coli* were determined by plating 0.1 ml of each dilution onto mFC agar (Difco) and incubating at 44.5 ± 0.2°C for 24 h. Cell injury (3) was determined by plating simultaneously on mFC and nutrient agar (Difco). Nutrient agar plates were incubated at 37°C for 48 h, giving injured cells ample opportunity to divide. The pH measurements were made with a Corning model 9 pH meter. Bicarbonate alkalinity (2) of the water was measured shortly after alum application to insure that adequate buffering capacity remained after alum addition.

Floc analysis. *E. coli* may be tightly bound to the floc and subsequently 1 CFU may represent several cells. Blending of samples containing the floc for short periods increased the number of *E. coli* detected on mFC agar. Samples containing floc were diluted and blended in a Waring model 700B blender for various lengths of time.

RESULTS AND DISCUSSION

October 1980 alum treatment of Liberty Lake. During the first alum treatment, FC and FS populations were monitored. The treatment covered the northern 80% of the lake. The south end was used as an untreated control station. Sampling began in August to obtain base-line information on numbers of bacteria.

Twenty-four hours after alum addition, FC decreased at the surface with a concomitant increase at the bottom (Fig. 2). Within 72 h of the alum application, 90% of the FC were removed from the overlying waters. Settling of coliforms was illustrated by the increase in concention at 24 h at the 3-m middepth, although the concentration decreased at the surface. Numbers of FC trapped in the alum floc at the bottom of the lake exceeded 2,400/100 ml at 120 h. After 120 h, FC numbers in the floc at the bottom decreased at the rate of 200 FC/100 ml per 24 h, reaching a concentration within the original confidence intervals at 600 h. Within 720 h of treatment, FC numbers returned to within their respective

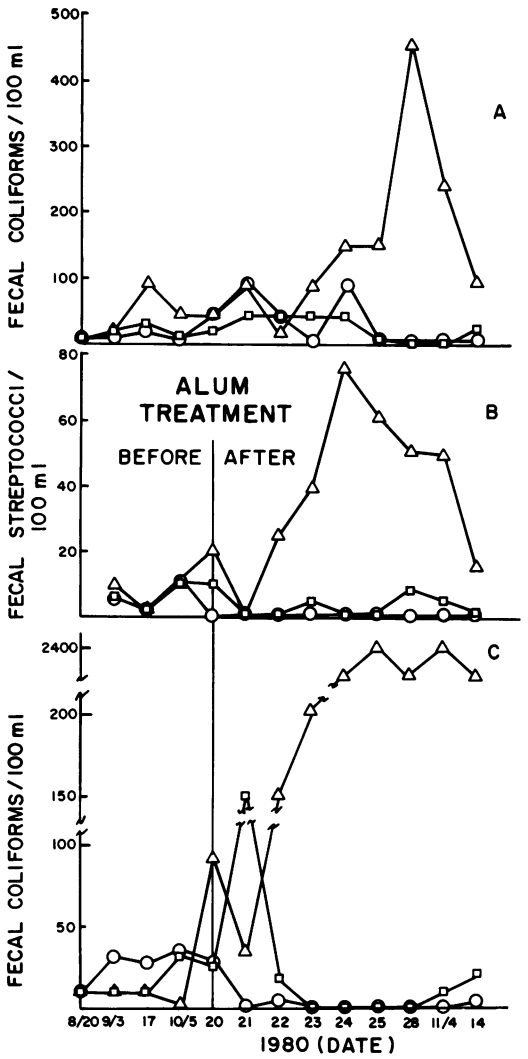

FIG. 2. Change in number of bacteria before and after alum addition to the north end during 1980. A, Southeast station, untreated area; B, northwest station; C, northwest station. Symbols: ○, surface; □, middepth (3 m at northwest and 2 m at southeast); △, bottom (6 m at northwest and 4 m at southeast).

confidence intervals at all three sampling depths, thus suggesting a possible time limit during which recreational use of the lake could be considered. FC addition to the lake surface from runoff and other extraneous sources (6) may have been sufficient for concentrations to increase at the surface almost 1 month after treatment (Fig. 2).

However, the decline in FC concentration in the alum floc at the lake bottom suggests accelerated die-off. The decline indicates that FC were not released from the floc to the overlying waters, since numbers at 3 m remained low (Fig. 2). The results of the laboratory column studies presented below confirm that the FC were not released. The observations on 28 October after treatment in the northwest section of the lake (Fig. 2A) demonstrate that the floc containing FC was redistributed from the northwest section to the untreated southeast section. As soon as quiescent weather conditions returned, the concentration of FC returned to the pattern that was observed throughout the rest of the study. Water transparency, aluminum, solids, and phosphorus analyses confirmed that the floc was resuspended (8).

The concentrations of FS at the northwest station were generally below the accepted limits (2) but consistent, giving narrow confidence intervals. Concentrations at the surface and middepth exhibited no significant difference (Fig. 2). FS numbers increased significantly at the bottom within 72 h after the alum treatment (Fig. 2). Thus, not only were FC removed with alum but also FS were, the former to a greater extent. The difference in flocculation is apparently due to the different polymers and charge densities associated with streptococci (gram-positive cocci) and coliforms (gram-negative rods) (12).

A statistical analysis of the data gathered before and after the alum treatments of 1980 and 1981 is presented in Table 1. The t statistics calculated for FC from the northwest station were significant at $P = 0.2$ for all depths, surface and bottom. Hence, there was a difference in the FC density before and after the alum treatment in that section of the lake (Fig. 2). At the surface, FC density decreased after the alum treatment as compared with before. At the 3-m depth, the FC density also decreased but not enough to be statistically significant. At the bottom, FC densities were greatly increased due to their entrapment in the floc. FS densities followed a pattern similar to that of the FC.

At the southeast station, the only calculated t value that was statistically significant was for the bottom FC data. The difference in FC densities in the bottom samples before and after indicates that drift occurred in the 1980 treatment of the northern section of the lake. The lack of impact on the densities of FS was probably the result of two factors. First, the floc was already formed when it moved into the southeast station; second, FS were not held in the floc as strongly as FC.

Due to extensive mixing caused by severe weather conditions, some flocculation occurred at the southeast station (Fig. 1). Although not as intensive as at the north end, FC were effectively flocculated by drifting alum at the south end, reducing the value of the station as an effective control. As with the alum-treated section of the lake, FC concentrations significantly increased at the bottom and simultaneously decreased in the overlying waters at the southeast station.

FS numbers were generally low, yet occasionally increased

TABLE 1. The t statistics applied to indicator populations before and after alum applications in Liberty Lake

Indicator group	Sample depth	SE[a] (1980)	NW[a] (1980)	Table $t_{0.2}$	SE[a] (1981)	Table $t_{0.2}$
FC	Top	0.61	3.85	1.397	ND[b]	ND
	Mid	0.81	1.42	1.397	ND	ND
	Bottom	2.65	2.92	1.397	ND	ND
	All depths	1.84	1.96	1.313	ND	ND
FS	Top	0.17	1.97	1.440	ND	ND
	Mid	0.64	1.27	1.440	ND	ND
	Bottom	1.12	2.37	1.400	ND	ND
	All depths	0.51	1.58	1.321	ND	ND
TC	Top	ND	ND	ND	2.54	1.886
	Mid	ND	ND	ND	4.03	1.886
	Bottom	ND	ND	ND	2.49	1.886
	All depths	ND	ND	ND	0.57	1.372

[a] Sampling station: SE, southeast; NW, northwest.
[b] ND, Not determined.

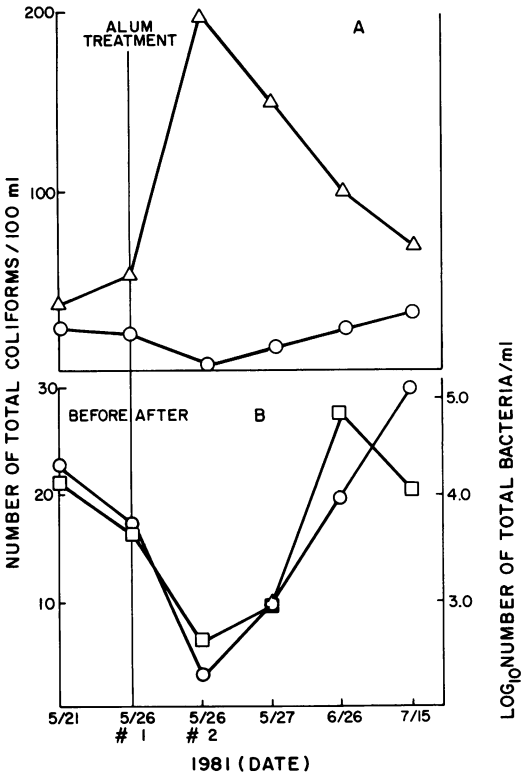

FIG. 3. Change in number of bacteria before and after alum addition to the south end during 1981. 5/26 #1 is at 0900 h, and 5/26 #2 is at 1700 h. A, TC at the southeast station (treated): at the surface (○) with a mean concentration of 20 ± 1.4 TC/100 ml (standard error) and at the bottom (△) with a mean concentration of 38 ± 3.1 TC/100 ml before treatment. B, TC (○) and TB (□) as determined on m-Henrici medium at the southeast station at the surface during 1981, TC with a mean concentration of 20 ± 1.4 TC/100 ml and TB with a mean concentration of 10,400 ± 2,420 TB/100 ml before treatment.

10-fold at the southeast station. This was attributed to the influx of as many as 1,000 waterfowl each night to that section of the lake (T. Noyes, M.S. thesis, Washington State University, Pullman, 1981). Because the FS concentrations were erratic, the confidence intervals were large, making comparison of the before and after treatments difficult.

May 1981 alum treatment of Liberty Lake. In May 1981, the southern 30% of Liberty Lake was treated with 10 mg of aluminum sulfate per liter (mean) to remove available phosphorus released during a dredging operation. During the spring, the FC and FS counts were below the acceptable limits for reporting (2) and thus are of limited statistical value. Enumeration of TC and TB proved to be sufficient for a statistical comparison of the effects of alum on bacteria.

Eight hours after treatment, the concentration of TC at the southeast station increased 500% at the bottom and decreased at the surface (Fig. 3). TB reduction paralleled TC reduction at the southeast station surface (Fig. 3). The TB concentration at the surface decreased by more than 1.3 log units within a few hours of treatment; this was a 95% reduction compared with a 60% reduction in TC. TC concentrations rebounded to their average value and stabilized within 720 h, whereas TB concentrations continued to increase for several months. Increased lake productivity (8) may account for the TB proliferation.

In addition to TB, chromogenic bacterial populations were monitored since they represent ca. 50% of the countable bacteria in lake waters (13–15). The numbers of chromogens are presented as a percentage of the population established before the application of alum. The chromogens responded acutely to alum. Within 8 h of treatment, the percentage of chromogens in both the surface and bottom samples decreased by 99 and 50%, respectively (Fig. 4). At 25 h after treatment, the chromogens in surface samples returned to normal distribution percentages, whereas in bottom samples, the percentage of chromogens increased 100%, comprising a greater portion of the TB at the bottom for several months. The rise in percentage of chromogens is in part a seasonal variation (Reasoner, M.S. thesis). In addition, much of the organic debris was removed after alum addition, decreasing turbidity and increasing light penetration (7). Those organisms with greater adaptability and faster generation times will reappear first. Another possibility is that if bacteria are released from the floc, chromogens may exist in greater percentages, but we have no data to support this contention.

For the spring 1981 treatment of the southern end of the lake, the TC densities before and after treatment were statistically different at all depths (Table 1). Thus, the alum application affected the TC densities by reducing their density in the surface and mid-water columns and increasing their density in the bottom column. This was due to the trapping of TC by the alum floc.

Laboratory study. Since bacteria remained viable in the floc at the lake bottom, a laboratory investigation was conducted to determine whether *E. coli* was released or remained entrapped in the floc. Large lucite columns and direct plating techniques most closely approximated lake conditions. Mechanical treatment of the floc samples was performed to determine the accuracy of numbers of bacteria obtained by direct plating techniques.

Experiments were conducted with a column with a 27-cm inner diameter. A sampling port was located 3.5 cm from the bottom of the column and immediately above the floc to ascertain release of bacteria from the floc once it had settled to the bottom. Flocculation of *E. coli* was observed in Liberty Lake water with 15 mg of alum per liter. Under these conditions, flocculation of *E. coli* increased dramatically

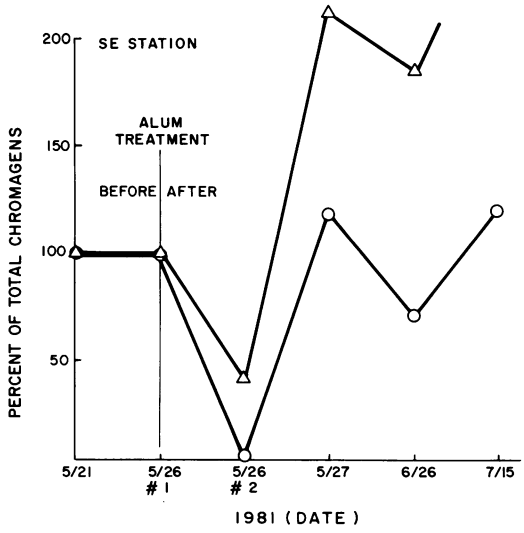

FIG. 4. Change in percentage of chromogens as determined on m-Henrici agar. 5/26 #1 is at 0900 h, and 5/26 #2 is at 1700 h. Surface (○) with a mean percentage of 100 ± 20 (standard error) and bottom (△) with a mean percentage of 100 ± 21 before treatment.

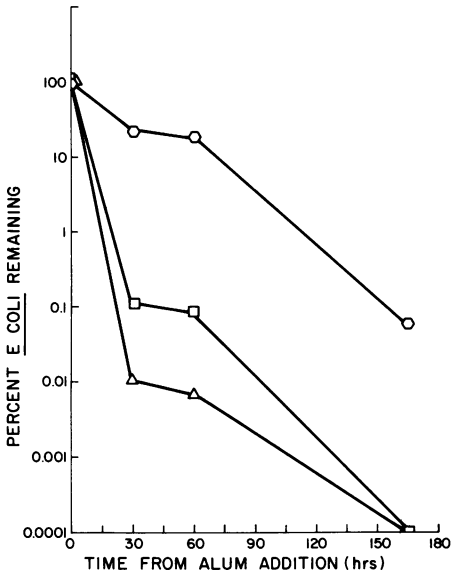

FIG. 5. Percent *E. coli* remaining after alum addition (15 mg/liter) to a column with a 26.8-cm inner diameter with filter-sterilized Liberty Lake water. Initial *E. coli* concentration is 3×10^7 cells per 100 ml. Symbols: △, surface; □, 91 cm; and ○, 94.5 cm (bottom).

(Fig. 5). At 30 h after flocculation with alum, 99.99% of the original population of *E. coli* was removed from the surface. The concentration of *E. coli* in the floc remained elevated over 1,000% above the concentration directly above the floc (3.5 cm) and over 10,000% above the concentration at the surface. The concentration at the surface of the column decreased from 15×10^7/100 ml to 15×10^2/100 ml in 30 h.

The 3.5-cm depth is directly above the floc; subsequently, any release of *E. coli* from the floc would increase the concentration at that depth in conjunction with a concomitant decrease at the bottom. Aside from the initial decline, the concentrations of *E. coli* in the floc and directly above it remained proportional to each other for the duration of the experiment. Thus, release of *E. coli* from the floc was not observed.

Dislodgment of *E. coli* from the floc was investigated by blending. After 2 s of blending, the numbers of *E. coli* increased, suggesting that flocculation decreased, possibly due to removal of pili and a decrease in the number of reactive sites (4, 5). The concentration of *E. coli* then decreased with increasing blending times up to 10 s, when the concentration stabilized. The decrease in concentration was not due to increased cell injury caused by shear since the percentage of injury displayed no significant change for up to 18 s of blending. The percentage of injury does increase with longer blending times. According to McGregor and Finn (12), aggregated cells can be dislodged with a blender. The resultant flocs are smaller and more compact (12).

In summary, during both alum treatments of Liberty Lake, bacteria were effectively flocculated and removed from the overlying waters and concentrated in the alum floc at the bottom of the lake. Allochthonous bacteria, as represented by the indicators, and autochthonous bacteria flocculated and were removed from the water column at different rates. The TB population, which included autochtohonous bacteria, recovered faster than indicator bacteria. Results from flocculation of *E. coli* with alum in columns showed that *E. coli* was not released from the floc but remained entrapped and was inactivated at a faster rate than the unflocculated cells.

Based on the results of this study, consideration should be given to a posttreatment restriction on the recreational use of the lake during the time when the user might contact the floc. When alum is used in lake restoration, application is done when soluble phosphate is near the seasonal high concentration(s). Application during the nonpeak recreation season should be considered to avoid any potential health risk that might be associated with the alum floc. For example, in some lakes in the northwest it would be advantageous to treat a lake during the fall to allow a long die-off period for the microorganisms in the floc.

Since the die-off of pathogens is not always the same as indicator populations, the rates of removal, inactivation, and release of pathogenic bacteria and virus from alum floc should be investigated further.

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