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# LONG-TERM EVALUATION OF ALUMINUM HYDROXIDE-COATED SAND FOR REMOVAL OF BACTERIA FROM WASTEWATER

JIENAN CHEN[1], STEVE TRUESDAIL[2], FUHUA LU[3], GUOGEN ZHAN[1], CARTER BELVIN[1], BEN KOOPMAN[1]*, SAM FARRAH[3]Ⓜ and DINESH SHAH[2]

[1]Department of Environmental Engineering Sciences, University of Florida, Gainesville, FL 32611-6450, U.S.A., [2]Department of Chemical Engineering, University of Florida, Gainesville, FL 32611-6450, U.S.A. and [3]Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611-6450, U.S.A.

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**Abstract**—The effectiveness of sand coated with aluminum hydroxide by *in situ* precipitation was evaluated over a 4 month period during which the sand was exposed to wastewater. Biogrowth in one set of columns was prevented by chlorinating its wastewater influent, whereas a parallel set of columns received dechlorinated wastewater. Following an initial "conditioning effect" (i.e. an increase in bacterial removal capacity), bacterial removal capacity of coated sand gradually decreased to that of uncoated sand. Biogrowth on the sand accelerated this decline. The aluminum content of coated sands decreased by approximately 25% over the first two weeks, then remained relatively constant and well above that of uncoated sand. Similarly, zeta potential decreased over the first two weeks from above +20 to below −70 mV, which was still significantly more electropositive than that of uncoated sand. Zeta potential of coated sand without biogrowth subsequently remained approximately constant, while that of coated sand with biogrowth increased gradually. No apparent correlation was found between metal content or zeta potential and bacterial removal capacity. The results suggest that, absent of biogrowth, the effective lifetime of the aluminum hydroxide coated sand is approximately 4 months, whereas with biogrowth, the effective lifetime is reduced to approximately 3 months. This information is of importance for assessing the technological potential as well as economical implications of metallic hydroxide coating of filter media. © 1998 Elsevier Science Ltd. All rights reserved

*Key words*—biogrowth, surface modification, metallic hydroxides, *Escherichia coli*, filter media, sand

## INTRODUCTION

Removal of fine particles such as bacteria and viruses in filtration is difficult, since these biocolloids carry a negative surface charge in the pH range of natural waters (Loder and Liss, 1985; Marshall, 1976), and most filter media (e.g. sand, diatomaceous earth) are also negatively charged in this pH range. Coatings of metallic hydroxides, oxides, or peroxides on filter media enhance removal of bacteria, viruses and turbidity from water and wastewater (Ahammed and Chaudhuri, 1996; Farrah *et al.*, 1991; Gerba *et al.*, 1988; Mills *et al.*, 1994). The primary function of the coatings is to make the surface of the filter media more electropositive, thus facilitating colloidal attachment.

When coated filter media are placed in service, the metallic hydroxide surface will be subject to dissolution, attrition, change in surface area and roughness, and chemical or biological fouling, all of which can influence interaction of the surface with colloids. Thus far, the effects of these phenomena on media performance have been largely overlooked. Ahammed and Chaudhuri (1996) reported that coatings of iron hydroxide or iron and aluminum hydroxide on sand continued to effectively remove fecal coliforms from water after 50 days of intermittent operation. The effect of continuous exposure of coated media to water was not investigated, however. In water and wastewater treatment systems containing organic substrates, a large potential exists for biogrowth on filter media. Biogrowth in slow sand filters is essential for effective removal of microorganisms (Schuler *et al.*, 1991). Development of biofilm on glass, polycarbonate, and granular activated carbon surfaces enhanced removal of bacteria (Banks and Bryers, 1992) and bacterium-sized particles (Drury *et al.*, 1993; Rittmann and Wirtel, 1991; Sprouse and Rittmann, 1990). The effect of biogrowth on the performance of surface-modified filter media has not been reported.

This study is the first to examine the effect of long-term exposure to wastewater on the effectiveness of metallic hydroxide coating on filter media. The coating studied was aluminum hydroxide on

*Author to whom correspondence should be addressed.

sand. Wastewater was treated to allow biogrowth in one set of columns and to prevent biogrowth in a parallel set of columns. Performance of the coating was measured in terms of *E. coli* removal. The results indicated a progressive decline of removal capacity of the coating relative to that of the uncoated sand over a period of 118 days. Biogrowth on the filter media exacerbated this decline. These results provide important information on the stability and effective lifetime of the surface coating under wastewater conditions. This information is required to assess the technological potential, as well as economical implications, of metallic hydroxide coating of filter media.

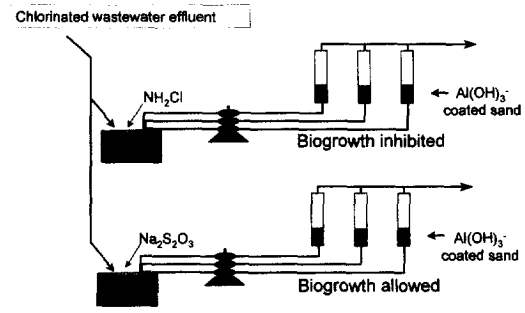


Fig. 1. Schematic diagram of column setup for exposing surface-modified sand to dechlorinated or chlorinated wastewater.

## MATERIALS AND METHODS

### *Surface modification of sand*

The fraction of 20 × 30 mesh Ottawa sand (Fisher Scientific, Springfield, NJ) passing a U.S. Standard No. 25 sieve was collected to obtain sand particles in the size range of approximately 600–700 μm diameter. The graded sand was coated with aluminum hydroxide to enhance adsorption of bacteria (Lukasik *et al.*, 1996) according to the following procedure. A 1000 g batch of sand was rinsed with deionized water until the supernatant became clear, air dried, then spread out to form a 3 cm thick layer in a plastic tray. A volume of 1000 ml of 1.0 M $AlCl_3 \cdot 6H_2O$ (Fisher Scientific) solution was added to the tray. After 30 min of contact, the excess solution was drained and the sand was air dried in the plastic tray for 24 h. The dried sand was transferred slowly into a 2.0 liter glass beaker containing 1000 ml of 3.0 M ammonium hydroxide and contacted for 10 min. The excess liquid was drained, then the sand was air dried in the plastic tray. The dried sand was rinsed vigorously with deionized water to remove loose precipitate, then air dried again in the plastic tray. Batches of coated sand were combined, mixed thoroughly, then stored at room temperature in sealed plastic bottles until use. A portion of the coated sand was not exposed to wastewater. Aliquots of this sand, which we term "positive control", were tested in parallel with all samples. Similarly, uncoated sand that was not exposed to wastewater was used as the "negative control".

### *Exposure of sand to wastewater in continuous flow columns*

Aluminum hydroxide-coated sand was exposed to wastewater in continuous flow-through columns that were operated for 118 days in an upflow mode using treated wastewater from the University of Florida Water Reclamation Facility. The typical wastewater composition before chlorination was: pH 7.2; alkalinity, 44 mg $l^{-1}$ as $CaCO_3$; turbidity, 0.7 NTU; $NO_3$-N, 1.2 mg $l^{-1}$; $NH_3$-N, 0.2 mg $l^{-1}$; total Kjeldahl nitrogen, 1.0 mg $l^{-1}$; orthophosphate-P, 1.2 mg $l^{-1}$; total phosphate-P, 1.8 mg $l^{-1}$; $Ca^{2+}$, 73 mg $l^{-1}$; $Mg^{2+}$, 8 mg $l^{-1}$; conductivity, 795 μmhos $cm^{-1}$. Sand was packed into the acrylic columns (3.2 cm I.D. × 1.5 m) to an initial depth of 0.75 m. A total of 6 columns was used in the experiment, three exposed to chlorinated wastewater and the other three to de-chlorinated wastewater (Fig. 1). Fresh chlorinated feed was obtained daily by dosing biologically treated wastewater with 10 mg $l^{-1}$ ammonia nitrogen, followed by titration with fresh sodium hypochlorite (Clorox Professional Products, Oakland, CA) to give a combined residual chlorine concentration of 2 mg $l^{-1}$. A combined residual was used to avoid rapid dissipation of the chlorine during storage of wastewater in the feed tank. Dechlorinated wastewater was obtained by dosing treated wastewater with sodium thiosulfate before it was pumped into the columns. The upflow rate in the columns was 57 ml $min^{-1}$, which gave a superficial velocity of 1.2 mm $s^{-1}$. The pH and temperature of the feedstocks were in the ranges of 6.6–7.0 and 22–25°C, respectively. Prior to the long-term experiment, a preliminary 45-day trial was carried out utilizing four small acrylic columns (1.5 cm I.D. × 1.0 m). The columns were packed to an initial depth of 0.5 m with $Al(OH)_3$-coated sand. Only dechlorinated wastewater was fed to the columns. The flow rate of each column was 20 ml $min^{-1}$, which gave a superficial velocity of 1.9 mm $s^{-1}$.

Rubber stoppers with tubing connectors were used to seal both ends of the columns. A fine nylon screen was used at the bottom of each column to prevent sand from entering the tubing. Columns and tubing were covered with black plastic to prevent growth of algae. The columns were backwashed for 15 min every 72 h at a superficial velocity of 17 mm $s^{-1}$, which was sufficient to completely fluidize the sand. Before each sampling, the columns were backwashed and then drained. Sand was emptied from each set of columns, combined, mixed, and sampled. The samples were rinsed with filter-sterilized wastewater to remove loosely attached biomass, then divided into subsamples. The subsamples were kept at 4°C for up to 24 h prior to analysis. Remaining sand was returned to the respective columns and feeding was resumed.

### *Preparation of test bacteria*

Batch cultures of *E. coli* C3000 (ATCC 15597) were grown overnight to early stationary phase in Nutrient Broth (Sigma Chemical, St. Louis, MO) at 35°C with shaking at 120 rev $min^{-1}$. A bacterial suspension of 50 ml from each batch was centrifuged at 3000 ***g*** for 10 min at 4°C to harvest the cells. The cells were resuspended in filter-sterilized MilliQ water to $A_{550} = 0.40$ and stained for 30 min with 4′,6-diamidino-2-phenyline-indole (DAPI) (Porter and Feig, 1980) at a final concentration of 25 μg $ml^{-1}$. Subsequently, the stained cells were harvested, washed in 2 × volume (100 ml) of artificial ground water (AGW) (McCaulou *et al.*, 1994) with vortexing, and reharvested by centrifugation at 7000 ***g*** for 10 min at 4°C. The cells were resuspended in AGW (pH 7.0) and kept at 4°C until use.

### *Batch and column removal tests*

Batch removal tests were carried out using samples of $Al(OH)_3$-coated sand from the columns fed with wastewater. Parallel tests were also run with $Al(OH)_3$-coated (positive control) and uncoated (negative control) sands that were not exposed to wastewater. Initial bacterial concentrations were adjusted to $10^5$–$10^6$ cells $ml^{-1}$. The test bacteria were contacted for 2 h with the respective sands

in 50 ml plastic centrifuge tubes affixed to a 70 cm diameter wheel that was rotated vertically at 30 rev $min^{-1}$ at room temperature. Each test tube contained 5 g of wet sand (rinsed with AGW) and 20 ml of bacterial suspension. Blanks containing bacterial suspension but no sand were run along with the other tubes to obtain initial counts. After mixing, sand in the tubes was allowed to settle for 10 min at room temperature, then supernatants were sampled.

Parallel filtration tests were carried out on days 1 and 118 in four acrylic columns (1 m length × 1.5 cm I.D.). The columns were packed with sands from the same samples and controls used in the corresponding batch removal tests. Glass wool was used at either end of the columns to prevent loss of media. The porosity of the packed media was 40%. Columns were run in an upflow mode at a flow rate of 20 ml $min^{-1}$. The packed columns were initially rinsed with 20 pore volumes of AGW (pH 7.0). Influents to the four columns were drawn from a tank containing bacterial suspension adjusted to the same cell concentration ($10^5$–$10^6$ cells $ml^{-1}$) using bacteria from the same preparations used for the corresponding batch removal tests. The influent tank was continuously mixed with a paddle stirrer. Composite effluent samples were collected at the 71st through 75th pore volumes to obtain steady-state data (Küçükçolak *et al.*, in press). The influent lines were sampled while pore volumes 71–75 were fed to the columns. The batch and column removal tests were run in triplicate. Samples were stored at 4°C until enumeration.

*Bacterial enumeration*

Direct counts of bacterial samples were obtained within 48 h (preliminary experiments showed that cell losses were less than 5% after this length of storage) using epifluorescence microscopy (APHA *et al.*, 1992; Sherr *et al.*, 1993). The samples were not re-stained, hence only pre-stained test cells were counted. This prevented interference from indigenous cells (background contamination or sloughed biomass). A Leitz Microlab microscope, equipped with appropriate filter blocks for desired light excitation and emission, was used for visualization and counting. Samples were vortexed vigorously to mix well immediately before filtration. A 1.0 ml aliquot of sample was filtered through a sterile 0.20 μm pore size, black, nucleation track, Nuclepore polycarbonate filter (Costar Scientific, Cambridge, MA), with a 0.45 μm pore size (GN-6) membrane filter (Gelman Sciences, Ann Arbor, MI) placed underneath to evenly distribute the vacuum. Filters were pre-wetted with filter-sterilized MilliQ water and a vacuum of 600 mm Hg applied. 3 ml of filter-sterilized MilliQ water was added to the filter funnel immediately before applying vacuum to help disperse bacteria on the filter. Ten microscopic fields were counted per filter. Sample concentrations typically gave 25–50 cells per field, hence the total number of cells counted per sample exceeded the minimum range recommended by Kepner and Pratt (1994).

*Protein assay*

The Lowry method (Lowry *et al.*, 1951), as modified (Bensadoun and Weinstein, 1976; Peterson, 1977), was employed, based on the procedure described in the Sigma Protein Assay Kit (Sigma) to estimate protein accumulation due to biogrowth on sand exposed to wastewater. 20 g of wet sand was extracted in 8.0 ml of 0.1 M NaOH in sterile 50 ml plastic centrifuge tubes. The tubes were vortexed vigorously for 30 s and let stand at room temperature for 5 min. These steps were repeated, then the tubes were placed in −40°C freezer for at least 30 min. After freezing, the tubes were thawed in a 50°C water bath for 10 min, followed by vortexing for 30 s. The supernatants were transferred to 13 × 100 mm glass test tubes and allowed to settle for 30 min. The supernatants were then distributed in 2.0 ml aliquots to triplicate 15 ml plastic centrifuge tubes. Protein standards (bovine serum albumin from Sigma) were made in the concentration range of 25–400 μg $ml^{-1}$. Filter-sterilized MilliQ water (2.0 ml) was used in a separate centrifuge tube as a blank. Supernatant samples, standards and blank were run together through the same steps according to instructions provided in the Sigma kit. Absorbance was measured at 750 nm after allowing color to develop at room temperature for 30 min.

*Scanning electron microscopy (SEM)*

SEM was carried out in parallel with protein assay (i.e. at 1, 15, 62, 97, and 118 days) to visualize biogrowth. Sand samples were fixed in fresh 2.5% glutaraldehyde in phosphate buffered saline (PBS, pH 7.2) for 15–20 min at room temperature, washed in PBS, 2 times, 5 min each, and then subjected to post-fixation at room temperature in 4% osmium tetroxide ($OSO_4$) in PBS for 10 min or 4% $OSO_4$ in 0.1 M cacodylate buffer for 5 min. The fixed samples were rinsed twice with deionized water for 5 min each time, then dehydrated successively in a graded ethanol series (50%, 75%, 95%, 100%) for 5 min in each step, followed by two 5 min changes in hexamethyldisilizane (HMDS). Subsequently, the samples were air-dried on filter paper placed in petri dish, mounted on aluminum support using colloidal graphite with isopropanol as adhesive, then oven-dried at 60°C, and finally sputter-coated with gold on an IB-2 ion coater (Eiko) according to manufacturer's recommendations. The gold-coated samples were viewed on a Hitachi S-4000 Field Emission SEM at 6 kV. Images were saved digitally using a Advanced Database Systems ImageSlave System. Final prints were printed on a Kodak 8600 PS Dye Sublimation printer.

*Metal content analysis*

A quantity of 10.0 g sand was digested in 25.0 ml of *aqua regia* (1:2:2::HCl:$HNO_3$:$H_2O$) for 20 min. The mixture was heated to near the boiling point. The digestate was diluted to 1000 ml, then filtered through a 0.2 μm pore size filter. The filtered digestate was then used for metal content analysis by ICP.

*Granular surface potential*

Surface charge is a key variable affecting bacterial interactions with granular media. Zeta potential, which approximates surface charge, was determined for each sample using a streaming potential apparatus that was constructed at the University of Florida. The apparatus consisted of a flow-through cell that was packed with the granular media to be analyzed and a tank to supply electrolyte ($1.0 \times 10^{-4}$ M KCl) to the flow-through cell. A water manometer was used to monitor the pressure drop across the cell and a Keithly 610c electrometer was used to measure the electrical potential between two silver chloride electrodes which preceded and followed the granular sample. The sample cell was made from a clear polycarbonate pipe with a $\frac{1}{4}$ in. inner diameter and a length of 20 cm (8 in.). The electrodes consisted of 99% pure 18 gauge silver wire and 40 mesh silver gauze (Newark Wire Cloth) which was cut to match the 1.9 cm ($\frac{3}{4}$ in.) circular diameter of the sample pipe. The wire and mesh were spot welded together so that the silver wire extended perpendicular from the center of the circular mesh piece. The mesh portion of the electrode was then anodized in 0.1 M HCl for 1 h using a copper cathode and a 5 mA current. The finished electrode was then allowed to stabilize overnight in distilled water. Electrodes were regenerated every two to three weeks to ensure proper performance.

The sample cell was filled completely with sand. Uniform packing was obtained via tap and fill method-

ology. The sample cell was then flushed with carbon dioxide and then the KCl electrolyte solution to remove any potential air pockets. The actual physical operation of the streaming potential device allowed for a $1.0 \times 10^{-4}$ M KCl salt solution to flow through the granular sample under investigation. Pressure was used as the controlled variable in these measurements and was allowed to range over the entire laminar flow regime. Electrodes at the edges of the sample monitored the voltage, $\Delta E$, generated as the charged ions in the KCl solution pass the charged sample surface under investigation. This voltage varied as a function of pressure drop, $\Delta P$. The slope of the measured voltage and pressure values was then used along with the measured solution conductivity, $K$, to calculate the corresponding zeta potential, $\zeta$, with the Smoluchowski relation (Overbeek, 1952):

$$\zeta = \frac{4\pi\mu K}{\varepsilon\varepsilon_0}\frac{\Delta E}{\Delta P} \quad (1)$$

where $\varepsilon$ is the dielectric constant of the medium, $\varepsilon_0$ is the dielectric constant of a vacuum, and $\mu$ is the viscosity of water.

Due to the weak electrolytic nature of the solution used in these measurements, the zeta potential is assumed to be very close to the surface potential and is the value used in the DLVO calculations.

The above mentioned apparatus typically provides a reproducibility within an error around 10% of the measured zeta potential. This error value was based upon the 95% confidence interval for the solution conductivity and the pressure–voltage slope variables used in the Smoluchowski equation. Standard deviations between samples were generally determined to be less than 3%.

## RESULTS AND DISCUSSION

### *Biogrowth development*

The protein content of $Al(OH)_3$-coated sand exposed to chlorinated wastewater remained at background levels throughout the 118 days of the experiment (Fig. 2). Similarly, no bacteria were found on this sand using SEM (data not shown). In contrast, both protein assay and SEM indicated time-dependent biogrowth on the sand that was exposed to dechlorinated wastewater. The protein content was significantly above background levels ($P < 0.05$, $t$-test) after 15 days. It increased exponentially through day 97, after which the rate of increase declined (Fig. 2). This time-dependent biogrowth could be described by a logistic curve. The protein content after 118 days was approximately one-half of what we previously obtained for filter sand samples from the Kanapaha Water Reclamation Facility in Gainesville, Florida, which has for over 25 years received clarified effluent from an activated sludge process without upstream chlorination.

SEM examination of sand exposed to dechlorinated wastewater indicated that bacteria on the sand surface were scarce initially, but increased in number with exposure time (Fig. 3). Surface coverage remained low even as protein content approached a stable level. Early biogrowth consisted of single, mostly rod-shaped bacterial cells (Fig. 3(b)). As the length of exposure increased, biogrowth composition became more diverse, with an increasing presence of filamentous microbes and polymeric network-like structures (Fig. 3(c, d)).

### *Metal content and zeta potential of test sands*

Coating the sand with $Al(OH)_3$ increased the Al content from approximately 0.05 to 0.4 mg Al $g^{-1}$ dry sand. The Al content of the coated sands decreased by approximately one-fourth over the first two weeks of exposure to wastewater, then remained approximately constant but well above (approximately 6-fold) the level of uncoated sand (negative control) throughout the rest of the experiment (Fig. 4). This initial loss of coating may be attributed to a combination of attrition and leaching of loosely attached precipitates. Biogrowth does not appear to affect Al content, since there was no significant differences ($P < 0.05$, paired $t$-test) in Al content between the sand exposed to dechlorinated wastewater vs. the sand exposed to chlorinated wastewater.

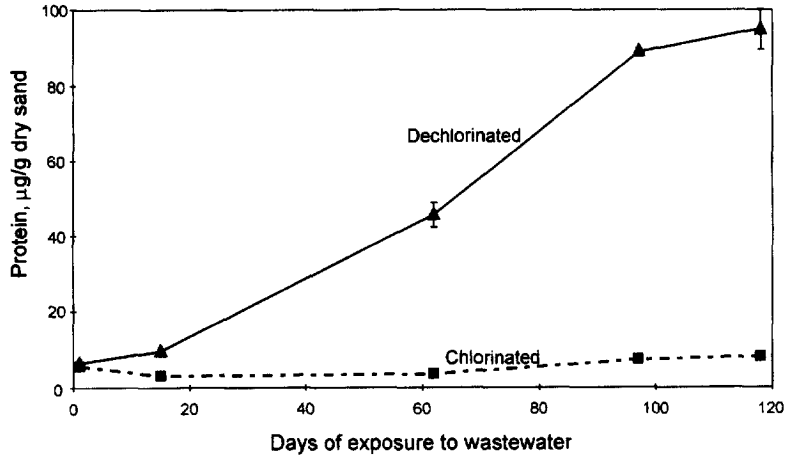


Fig. 2. Development of biogrowth on aluminum hydroxide-coated sand exposed to dechlorinated or chlorinated wastewater as measured by protein content. Error bars represent ±1.0 SD.

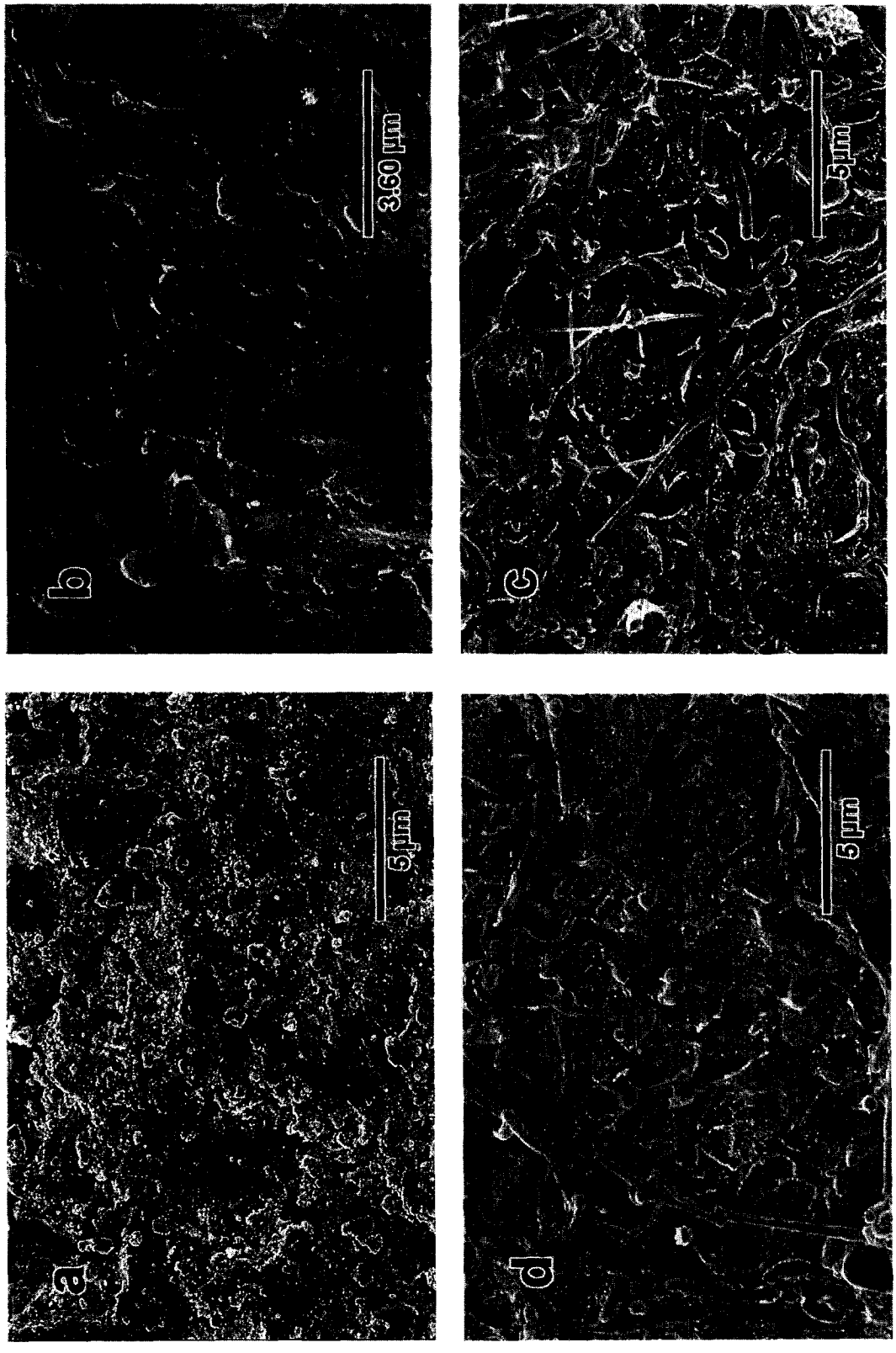


Fig. 3. Development of biogrowth on aluminum hydroxide-coated sand exposed to dechlorinated wastewater as observed by SEM: (a) 1; (b) 62; (c) 97; (d) 118 days.

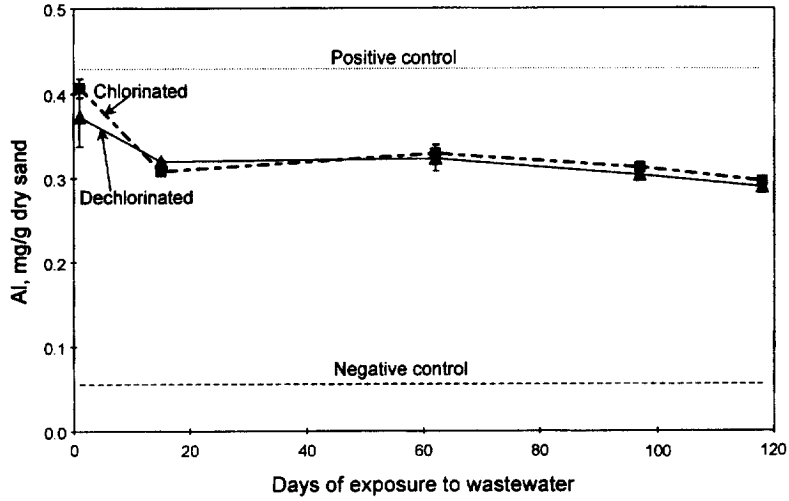


Fig. 4. Metal content of aluminum hydroxide-coated sand exposed to dechlorinated or chlorinated wastewater. Positive control = aluminum hydroxide-coated sand that was not exposed to wastewater; negative control = uncoated sand that was not exposed to wastewater. Error bars represent ±1.0 SD.

The zeta potential of $Al(OH)_3$-coated sand, before exposure to wastewater, was markedly higher than that of uncoated sand (above +20 mV vs. below −90 mV) (Fig. 5). Zeta potential of the coated sands decreased to the range of −50 to −65 mV after one day of exposure to wastewater and further decreased to below −70 mV over the next two weeks. This was still significantly ($P < 0.05$) above the negative control. After this initial drop, zeta potential of coated sand exposed to chlorinated wastewater remained approximately constant, whereas zeta potential of coated sand exposed to dechlorinated wastewater gradually became less negative. The trend in zeta potential of coated sand exposed to chlorinated wastewater was similar to the trend in metal content. The increase in the positive nature of the zeta potential of coated sand exposed to dechlorinated wastewater paralleled the development of biogrowth. Previously, Rao *et al.* (1993) observed that the zeta potential of bacteria-coated apatite minerals was intermediate between that of the minerals alone and of the bacteria alone. We can thus attribute the increased zeta potential of sand with biogrowth to an "averaging" of the zeta potential of bacteria (typically from −10 to −60 mV) (Achouak *et al.*, 1994; Nicholov *et al.*, 1993; Vitaya and Toda, 1991) and that of the coated sand exposed to wastewater (around −80 mV, as seen in Fig. 5).

*Removal of E. coli*

Coated sand (positive control) was significantly better than uncoated sand (negative control) in the removal of *E. coli* (approximately 45–60% vs. 10–

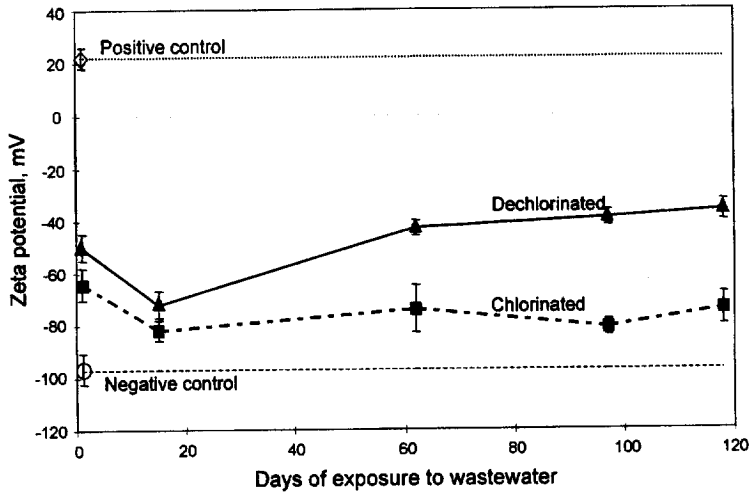


Fig. 5. Zeta potential of aluminum hydroxide-coated sand exposed to dechlorinated or chlorinated wastewater. Positive control = aluminum hydroxide-coated sand that was not exposed to wastewater; negative control = uncoated sand that was not exposed to wastewater. Error bars represent ±1.0 SD.

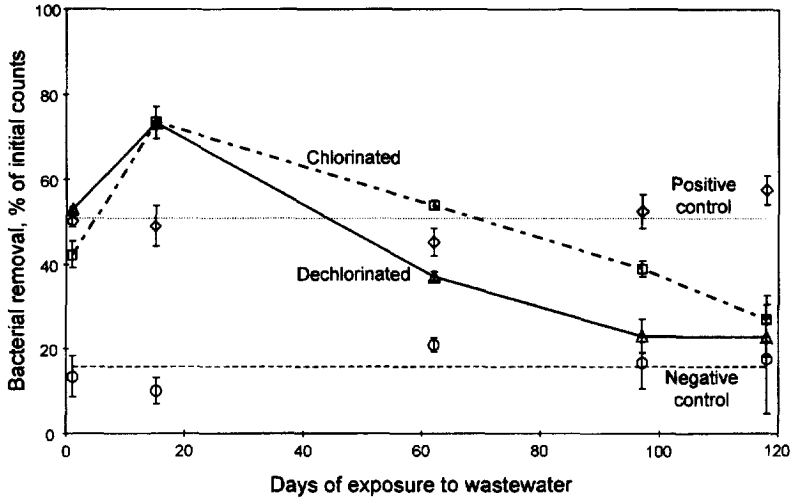


Fig. 6. Batch removal of *E. coli* by aluminum hydroxide-coated sand exposed to dechlorinated or chlorinated wastewater. Positive control = aluminum hydroxide-coated sand that was not exposed to wastewater; negative control = uncoated sand that was not exposed to wastewater. Error bars represent ±1.0 SD.

20%) (Fig. 6). Performance of coated sand was strongly influenced by exposure to wastewater. A significant enhancement relative to the positive control ($P < 0.05$) was evident after two weeks. Subsequently, the effect of exposure to wastewater was negative, i.e. removal efficiencies for the exposed sands decreased with time (Fig. 6). Such negative effect was intensified by biogrowth accumulation (as indicated by protein content and SEM) on sand exposed to dechlorinated wastewater. Enhancement of bacterial removal ("conditioning effect") upon short-term exposure of granular media to natural waters was observed by Schneider and Marshall (1994). Our preliminary work (Fig. 7) gives trends in bacterial removal and biogrowth (as measured by protein) that are consistent with results of our long-term experiments.

Overall trends seen in the batch tests were in good agreement with the column results shown in Fig. 8. After 1 day of exposure to wastewater, all the coated sands removed approximately the same amount of bacteria (50–55%), significantly exceeding the removal by the uncoated sand (14%). By day 118, removal by the coated sands exposed to wastewater had decreased to a level near that achieved by the uncoated sand (negative control). In fact, the exposed sands performed equivalently to the uncoated sand at this time, whereas the coated sand that was not exposed to wastewater maintained a removal of approximately 55%.

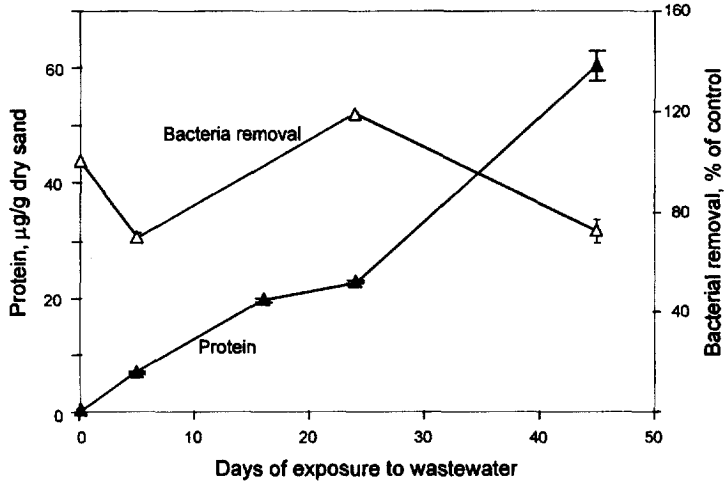


Fig. 7. Correlation between biogrowth (as measured by protein content) and batch removal of *E. coli* by aluminum hydroxide-coated sand exposed to dechlorinated wastewater. The right axis gives the removal percentages of the test sand divided by the respective removal percentages of the positive control. Error bars represent ±1.0 SD.

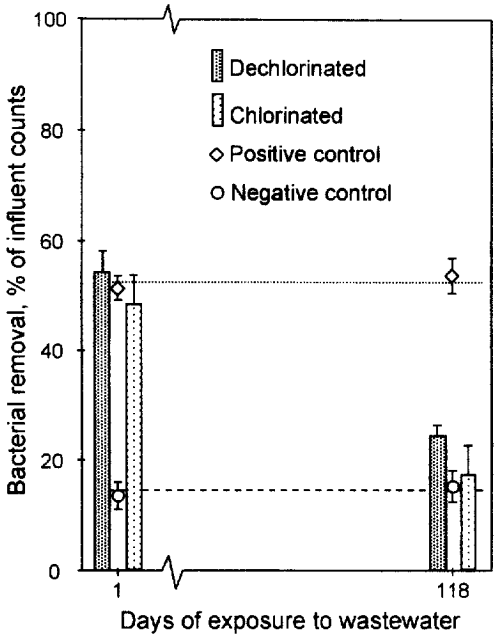


Fig. 8. Column removal of *E. coli* by aluminum hydroxide-coated sand exposed to dechlorinated or chlorinated wastewater. Positive control = aluminum hydroxide-coated sand that was not exposed to wastewater; negative control = uncoated sand that was not exposed to wastewater. Error bars represent ±1.0 SD.

After the initial conditioning period, sand with biogrowth had a significantly lower bacterial removal capacity than sand without biogrowth ($p < 0.05$) (Fig. 6). Removal efficiency for sand with biogrowth declined to the level of the negative control sooner than sand without biogrowth (97 vs. 118 days). Others have reported improved removal of bacteria (Banks and Bryers, 1992) and bacterium-sized particles (Drury *et al.*, 1993; Rittmann and Wirtel, 1991; Sprouse and Rittmann, 1990) by biofilm-coated media. The contradictory results of our study may be attributable to differences in the substratum (sand in our study vs glass, polycarbonate, or granular activated carbon), particles removed (bacteria vs latex beads or organic solids), and fluid medium (treated wastewater effluent vs. synthetic wastewater or artificial medium). The phenomenon of enhanced bacterial removal following initial exposure of sand to wastewater may be effected by a "conditioning film" of adsorbed ions and other inorganic and organic colloids on the media surface (Schneider and Marshall, 1994).

Changes in zeta potential do not appear to explain the decreased removal capacity of the sand in the presence of biogrowth, as opposite trends were observed in zeta potential (Fig. 5) and bacterial removal (Fig. 6) of sand with biogrowth. Normally, increased positive character of zeta potential would be expected to enhance bacterial removal (Van Loosdrecht *et al.*, 1990). Reduced removal capacity also does not seem to be consistent with the expected increase in hydrophobicity of sand surface due to biogrowth. For example, coating quartz surface with polyethyleneoxide (hydrophobic) enhanced removal of hydrophobic bacteria, but had a marginal (positive) effect on the removal of hydrophilic bacteria (McCaulou *et al.*, 1994). The negative effect of biogrowth may be interpreted as blockage of attachment sites by the bacterial cells and associated exopolymers making up the biogrowth. For example, protein films on inanimate substrata have been found to generally inhibit bacterial attachment (Fletcher, 1976).

Since Al content and zeta potential did not change significantly after day 14, reduced removal capacity in the absence of biogrowth must be due to factors other than the loss of coating or decrease in surface charge. Loss of fine structures (on the order of 0.1 μm) of coated sand surface was observed by SEM (6000×) after 14 days of exposure to wastewater. This would suggest that the concomitant loss of surface area and surface roughness played a role in the aging of $Al(OH)_3$ coated sand as a consequence of prolonged exposure to wastewater. Another possible mechanism for the loss of coating effectiveness is the saturation of attachment sites as a consequence of adsorption of macromolecules present in the wastewater.

## CONCLUSIONS

This is the first study to examine the aging phenomena of metallic hydroxide-coating on filter media. The effective lifetime of the aluminum hydroxide coating was 4 months without biogrowth and 3 months with biogrowth under conditions employed in this study. About a quarter of the Al content was lost within the first two weeks of exposure, but no significant loss was observed thereafter. Loss of coating effectiveness as a consequence of prolonged exposure to wastewater was due neither to loss of coating quantity nor to change in zeta potential. SEM observation of coated sand surface suggests that loss of surface microstructure plays a role in the aging effect of prolonged exposure to wastewater. Biogrowth adversely affected the ability of coated sand to remove bacteria. Even without biogrowth, the coating progressively lost its enhancing effect when exposed to wastewater. Short-term (hours to days) exposure to wastewater has a reproducible "conditioning" (positive) effect on bacterial removal.

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