An antimicrobial polycationic sand filter for water disinfection
Annalisa Onnis-Hayden, Bryan B. Hsu, Alexander M. Klibanov and April Z. Gu

ABSTRACT
A new sand filtration water disinfection technology is developed which relies on the antimicrobial properties of hydrophobic polycations (N-hexylated polyethylenimine) covalently attached to the sand’s surface. The efficacy of the filter disinfection process was evaluated both with water spiked with E. coli and with real aqueous effluent from a wastewater treatment plant. For the former, over 7-log reduction in bacterial count was achieved. With real environmental wastewater secondary effluent samples, the E. coli concentration reduction declined to under 2 logs. This reduced inactivation efficiency compared to the model aqueous sample is likely due to the particulate or colloidal matter present that diminishes the contact between the immobilized polycation and the suspended bacteria. Preliminary sand washing methods were tested to assess potential ‘regeneration’ approaches. Potential advantages of the proposed approach over conventional disinfection in terms of eliminating harmful by-products and reducing energy consumption are discussed.

Key words | antimicrobial polymer, bactericidal, disinfection, sand filter

INTRODUCTION
Disinfection by chlorination as the last step of drinking water and wastewater treatments has played a critical role in protecting the U.S. water supply from waterborne infectious diseases. However, harmful disinfection byproducts (DBPs) produced during the chlorination process, such as carcinogenic trichloromethanes and chloroacetic acids, have raised concerns and motivated exploration of other disinfection agents (Cantwell et al. 2008). Such alternative disinfectants have included chloramines, ozone, UV, peracetic acid, bromine, and advanced oxidants (Freese & Nozaic 2004). Among these, the first three have been studied most extensively and proven effective for large-scale treatment.

Although these alternative disinfectants lower regulated chlorinated DBP levels, they can give rise to other toxic DBPs (Richardson 2005). Ozonation, for example, can lead to the formation of aldehydes, ketones and carboxylic acids, many of which have been found to be mutagenic or carcinogenic (Freese & Nozaic 2004). Although UV does not produce such residuals, it is energy-intensive and the effectiveness of UV disinfection varies with water quality (Moreno et al. 1997). Another troubling aspect is disinfection resistance phenomena. Different levels of resistance to both chlorination and UV have been observed with bacteria, viruses, and protozoa (Hijnen et al. 2006; LeChevallier et al. 1988). These issues motivate the exploration of novel effective disinfection methods that form no harmful DBPs.

Recently, a sterile-surface material – ‘antimicrobial polymer’ – has been developed and validated based on hydrophobic polycations, such as N-alkylated polyethylenimine (N-alkyl-PEI), either covalently attached or deposited (‘painted’) onto surfaces (Klibanov 2007; Lewis & Klibanov 2005). The resultant derivatized surfaces kill on contact both airborne and waterborne pathogenic bacteria (gram-positive and gram-negative), as well as fungi and influenza viruses (Klibanov 2007). The hypothesized antimicrobial mechanism involves the physical rupture of cellular membranes or viral envelopes by long-chained, moderately hydrophobic immobilized polycations (Milovic et al. 2005).

Polymeric disinfectants are ideal for applications in water treatment because they can inactivate, kill, or
remove target microorganisms by mere contact without releasing any reactive agents to the bulk phase to be disinfected (Kenawy et al. 2007); however, only a limited number of studies have explored this field of research. A water-insoluble matrix based on iodinated poly(methyl methacrylate-co-N-vinyl-2-pyrrolidone) has been synthesized for use in a portable water treatment device (Tyag & Singh 2000). In a recent study, N-halamine polymers in the form of highly cross-linked porous beads have been explored for use in drinking water disinfection (Chen et al. 2003). In this mentioned study, functionalization of methylated polystyrene by halogenated hydantoin and imidazolidinone derivatives was used in packed glass columns and the column filter biocidal efficacy tests showed effective reduction of *S. aureus* and *E. coli*. In both of these studies, however, the hydrophilic water-insoluble copolymer matrix or beads were used to immobilize a suitable antimicrobial agent, which can slowly release into the water, and therefore will need to be recharged, or discarded after use. In addition, the use of brominated beads is limited to disinfection of water systems because of the toxicity of emitted free bromine to the water.

Herein, we explore the use of polymeric disinfectants for water disinfection by covalently attaching *N*,*N*-hexyl, methyl-PEI onto sand surface and using the resultant antimicrobial sand filter for water filtration and disinfection. There are several potential advantages of this disinfection method for water and advanced wastewater treatment or for portable water treatment devices. It eliminates DBPs since it involves no chemicals that react with organic precursors in water. Also, this disinfection approach requires neither added chemicals (as in chlorination) nor extensive energy (as with UV disinfection). Moreover, it can be combined into a single process with filtration, which is commonly used in water treatment as a final step to remove suspended particles and colloids before disinfection. The immobilized polycations do not seem to be subject to the existing mechanisms of microbial resistance (Milovic et al. 2005) and thus may be effective against microorganisms that have developed resistance to chlorine upon repeated long-term exposure. Finally, it has been previously shown that the antimicrobial property of these polycations can be easily regenerated by a simple washing step (Lin et al. 2005).

The overall objective of this study was to carry out a proof-of-concept investigation of the proposed antimicrobial polycationic sand filters for water disinfection. First, we developed a preparative protocol for attaching *N*,*N*-hexyl, methyl-PEI onto commercial sand surface and examined its robustness. We then evaluated the inactivation of indicator microorganisms in a lab-scale filter filled with polymer-coated sand. Different reactor operating conditions and water samples were evaluated to identify the parameters that affect the disinfection efficiency, including empty bed contact time (EBCT), characteristics of water matrix, and backwash/cleaning procedures.

**MATERIALS AND METHODS**

**Materials**

Silica sand with nominal diameter of 0.5 mm was obtained from Ricci Bros. Sand Co. Inc. (Port Norris, New Jersey). Branched PEI (average M<sub>w</sub> of 750 kDa, 50 wt% in water), organic solvents and chemical reagents used were obtained from Sigma-Aldrich Chemical Co. (Saint Louis, Missouri) and used without further purification except for *tert*-amyl alcohol, which was dried over 3A molecular sieves. *E. coli* (K12, MG1655) was kindly provided by Dr. Kim Lewis in the Biology Department at Northeastern University (Massachusetts, U.S.). Environmental wastewater secondary effluent was from the Deer Island wastewater treatment plant, Winthrop, Massachusetts.

**Polycation immobilization to sand**

This procedure was adopted from previous reports (Lin et al. 2002a; Lin et al. 2002b), and was modified to scale-up. For each batch, 90 g of sand was prepared by thoroughly washing in distilled water, sonicating in isopropanol for 5 min and drying at 80 °C. The cleaned sand was then silanized in 500 mL of 20% 3-aminopropyltriethoxysilane in anhydrous toluene with stirring at room temperature for 3 h, which was subsequently washed in toluene and methanol, and dried at 80 °C. This amino-sand was then stirred at room temperature with 500 mL of 5% *tert*-amyl alcohol in anhydrous chloroform for 5 h and washed with anhydrous chloroform and anhydrous methanol. PEI was then covalently crosslinked to the sand by stirring in a solution of 100 g PEI, 2.5 g potassium hydroxide, and 400 mL *tert*-amyl alcohol at 90 °C for 9 h. After washing with methanol, the sand was allowed to air dry. Alkylation of sand-immobilized PEI was achieved by stirring the sand in 50 mL 1-bromohexane, 2.5 g potassium hydroxide, and 450 mL *tert*-amyl alcohol overnight (~10 h) at 90 °C and then washing in methanol and air-drying. To increase the
extent of quaternionization, the sand was stirred with a solution of 100 mL iodomethane in 400 mL tert-amyl alcohol at 60 °C in a sealed container for 9 h, and then washed with methanol and air dried. The final yield of N,N-hexyl,methyl-PEI-treated sand was 79 g and the protocol was repeated several times to obtain a total of 250 g treated sand.

**Titration of quaternary ammonium groups**

To determine the amount of polymer covalently immobilized to sand, after covalent immobilization of N,N-hexylmethyl-PEI to the sand's surface, titration of the quaternary ammonium groups was performed using a fluorescein assay, as adopted from a previously described procedure (Tiller et al. 2001). 1.0 g of sand was shaken at room temperature with 5 mL of 1% fluorescein aqueous solution (Na salt) for 5 min. The sand was then thoroughly washed with 100 mL distilled water and then sonicated for 15 min in 4 mL of a 0.25% cetyltrimethylammonium chloride aqueous solution to displace the polycation-bound fluorescein into solution. This detergent solution was then decanted from the sand and 0.44 mL of 0.1 M phosphate buffer, pH 8.0, was added. After brief centrifugation to remove any fine sand particles, if present, the absorbance at 501 nm was measured. Using the previously determined molar extinction coefficient, $7.7 \times 10^4$ M$^{-1}$·cm$^{-1}$ (Tiller et al. 2001), the density of quaternary ammonium groups per gram of polycation-treated sand was determined to be 95 ± 32 nmol/g. In comparison, untreated sand gave a background density of 17 ± 1 nmol/g, which may be attributed to the heterogeneous elemental composition of the sand.

**Preliminary evaluation of antibacterial efficacy of the polycation-coated sand**

In addition to the titration of quaternary ammonium groups for evaluating the effectiveness of polymer coating onto sand surface, a pre-evaluation of the sand antibacterial efficacy was determined by an inactivation assay, in which 5.0 g of sand was shaken at 200 rpm with 10 mL of $10^7$ bacterial cells/100 mL for 2 h at 37 °C. Evaluation of the aliquots (100 µL) of the incubated bacterial suspensions diluted 10-fold in phosphate-buffered saline (PBS) were plated onto YDB-agar plates, in triplicate, and then incubated at 37 °C overnight. Comparison of the number of colony forming units (CFU) between bacterial suspensions incubated with untreated sand (351 ± 52 CFU) and polycation-treated sand (0 ± 0 CFU) demonstrated the latter had 100% bactericidal activity against waterborne *E. coli* under these assay conditions.

**Disinfection efficiency of the polycation-coated sand filter**

**Culture growth**

*E. coli* was grown in Luria-Bertani (LB) medium overnight at 37 °C to reach the stationary phase (~$3.5 \times 10^{10}$ CFU/100 mL). A known amount of the overnight culture was then spiked into either PBS (in 1 L of distilled water: 8.00 g of NaCl, 0.20 g of KCl, 1.44 g of Na$_2$HPO$_4$, 0.24 g of KH$_2$PO$_4$, with pH adjustment to 7.4) or other real environmental water samples to prepare influents for our disinfection filters (*E. coli* concentrations of $10^5$ to $10^9$ CFU/100 mL).

**Column testing set-up**

Two bench-scale sand filtration systems were set up in parallel, with one containing N,N-hexyl,methyl-PEI-sand and the other the same amount of clean sand as a control. Each filtration unit comprised a glass column (4.5 cm ID, 20 cm long) wet-packed by allowing sand grains to settle in deionized water upon agitation. The resultant column packing porosity was determined gravimetrically to be 0.40, and the total height of the packing in the column was approximately 10 cm (roughly 250 g of sand).

A typical experiment involved running 250 mL of the influent containing the bacteria through the column with a 1 cm water head maintained on top of the sand to allow even distribution of the influent. Effluent samples were collected after approximately two pore volumes (one pore volume is about 48 mL) of bacteria suspension were passed through the filter; about 150 mL of cumulated effluent was collected and used for further testing. Empty bed contact time ranging from 12 to 27 min was tested by adjusting the influent flow rates (7.8–14 mL/min).

**Inactivation efficacy assessment**

The influent and effluent samples from both the test and control filters were collected and quantified for *E. coli* contents using a membrane filter-plate counting method using LB agar medium or Coliscan medium (Microbiology Laboratories, Goshen, Indiana). Different sample dilutions were applied to ensure appropriate CFU after 18 to 24 h of
incubation at 37 °C; averages of triplicate samples were calculated for each sample. The bactericidal efficiencies and log reductions were then determined.

**Sand washing**

Before and after each test, 400 mL of sterile PBS was run through the filter to clean it and remove any residual bacteria. When a decrease in efficiency was observed, a more rigorous cleaning procedure was performed prior to further testing using 70% (v/v) ethanol (EtOH), followed by distilled water. The former was performed by leaving the ethanol in static contact with the sand for 10 min, then allowing it to flow through the sand with similar rates as in the tests, followed by 10 pore volumes of deionized water (about 400 mL).

**RESULTS AND DISCUSSION**

The efficacy of N,N-hexyl,methyl-PEI-immobilized sand in batch filtration mode was first evaluated herein using PBS spiked with *E. coli*. The results depicted in Figure 1 indicate a greater than 5-log reduction and essentially 100% removal of *E. coli* for the first three test runs conducted on different dates with initial bacterial concentration of 10^5 CFU/100 mL. When the influent *E. coli* concentration was increased to 10^9 CFU/100 mL, a bacterial count reduction of over 7 logs was achieved in the initial test (Figure 1, Test 4A); however, subsequent tests of the filter conducted on the same day showed decreased reduction (Figure 1, Tests 4B and 4C). To address this lowered performance, we performed a longer PBS cleaning of the sand by passing PBS through the filter for 15–20 min and then conducting the test with initial *E. coli* concentration at 10^6 CFU/100mL. This treatment essentially recovered the original high disinfection efficiency – 100% bacterial removal and an over 6-log reduction.

To evaluate the impact of wastewater matrix on the antimicrobial performance of N,N-hexyl,methyl-PEI-sand, we conducted similar tests with both raw secondary effluent from a local wastewater treatment plant (Deer Island) and autoclaved effluent spiked with *E. coli*. Figure 2 shows that, with raw wastewater effluent samples, only a limited reduction (1.2 to 2 logs) of *E. coli* was achieved at the initial concentration of 10^3 CFU/100 mL. Thus the disinfection efficiency with real wastewater is much lower than that with a PBS model system at the same initial concentration (2.9–5.5 log kill, see Figure 1). These results suggest that the wastewater matrix, likely organic particulate or colloidal particulate matter, reduces the contact efficiency between the immobilized polycation and the suspended bacteria. However, it should be noted that we employed the worst-case scenario with the secondary wastewater effluent here. The proposed disinfection method would be most suitable for water treatment or for advanced tertiary wastewater treatment for water reuse, and not for wastewater secondary effluent as above. Moreover, for practical applications, a dual-sand filter in series could be implemented with the first stage filter removing the particulate/colloids, followed by a polymer-coated sand filter for disinfection. The upper stage of the filter containing untreated sand, with the aid of coagulants, will act as a regular filter for removing colloidal and some organic matters that may potentially

![Figure 1](imageurl) | Inactivation of *E. coli* by N,N-hexyl,methyl-PEI covalently immobilized on the surface of sand filtration in PBS buffer. Influent: influent to sand filters; BDL: below detection limit. PBS: phosphate-buffered saline; EtOH: 70% (v/v) ethanol solution; the numbers in parentheses represent the corresponding EBCT in minutes.
compete with microorganisms for active sites on the sand surface. More testing will be needed to determine the optimal washing/backwashing duration and operating conditions for this dual filter system, and it will be part of our future study.

As shown in Figure 1, three consecutive tests conducted on the same day (Tests 4A, 4B, and 4C) with the same initial E. coli concentration showed decreased antibacterial performance over time, likely due to bacterial debris contaminating the surface and shielding suspended live bacteria from contact with the coated surface. After thorough cleaning with PBS, the original microbicidal efficacy was essentially recovered. However, further continuous tests of the filter resulted in reduced performance again. We then explored the cleaning using ethanol, and the antibacterial efficiency was re-established, as seen in the following tests (tests 7 and 8 in Figure 1).

In this study, we attempted two alternative cleaning methods including PBS and ethanol washing. PBS is commonly used in biological applications as a multipurpose sterile and benign medium because of its physiological pH and osmotic balance with the cell cytoplasm. It was shown in the previous study that transiently attached bacterial cells on the slides coated by the same polymer could be easily washed off using PBS (Milovic et al. 2005). We also tried ethanol, because the hypothesized mechanism of the hydrophobic polycations, bactericidal activity involves disruption of the bacterial membrane by electrostatic and hydrophobic interaction with the polymer coating. Ethanol at 70% (v/v) is known to be very effective in dehydrating proteins, and therefore this property can be used to render the attached dead bacteria insoluble and remove them simply by running distilled water after the treatment with ethanol. To displace the biological debris into solution, it has been shown that common hand-soap is effective and can refresh bactericidal activity of the polymer (Lin et al. 2005). While PBS was shown to be somewhat effective and the ethanol seemed to be more effective, these two cleaning procedures showed that it is possible to refresh the bactericidal activity of the polymer, assuming an appropriate washing solution is chosen. Further research is needed to find the most cost-effective, as well as most sustainable, back-washing and regeneration alternative.

The treatment of the regeneration wastewater needs to be considered as well. The wastewater generated from back-washing will comprise particulate matter (dead bacteria debris, and particulates initially present in the water), as well as the agent used for the washing/regeneration process. Depending on the agent used for the washing procedure, the generated wastewater could be either sent back to the main treatment train or sent into the sewage system. For the specific case of ethanol, there are no health based standards or guidelines for its presence in drinking water or reuse water. For wastewater treatment, addition of an external carbon source such as ethanol is commonly practised to enhance denitrification. It is most likely that the influent...
water at the head of the treatment plant will provide the necessary dilution of the filter wastewater, so that no specific treatment will be needed. Moreover, compared to regular backwash wastewater produced in sand filter, the backwash wastewater of this specific filter does not contain different composition except a higher concentration of cell debris.

Figure 3 shows the relationship found between the log reduction and the EBCT. Within 12 to 27 min of EBCT, the increasing of contact time did not seem to enhance bacterial removal as expected. This implies that the inactivation process is likely limited by the unencumbered immobilized N,N-hexyl,methyl-PEI available to the microbes. The results indicated that the cleaning protocol and matrix of the sample play important roles; thus, providing more contact area (e.g. increased filter column depth, or greater available surface area) will be crucial in the application of this technology. The EBCT for effective inactivation was within the range of typical sand filter operation, suggesting the inactivation rate and kinetics are sufficient for real practical application. Previous inactivation kinetics studies with glass surface coated with this same antimicrobial polymer showed that, with initial E. coli concentration up to $2 \times 10^7$ cells, 6-log reduction can be achieved within half an hour when the polymer-coated slide is submerged in cell suspension (Milovic et al. 2005). This rate is comparable to the inactivation rate observed with our sand filter and the difference is due to the different hydraulic and contact conditions. In addition, real water effluents containing interfering organics and other compounds can compete with the bacteria for active sites on the surface, therefore reducing the disinfection capacity of the system. Thus, further studies will be needed to determine the rate, kinetics and capacity of the disinfection filter with various process configurations (e.g. dual sand media, increased column depth).

In closing, in this work we explored a sand filtration water disinfection technology which relies on antimicrobial properties of the immobilized hydrophobic polycation N,N-hexyl,methyl-PEI. The polycation was successfully immobilized onto the surface of sand of the type commonly used for water treatment. The efficacy of the sand-immobilized polycation in inactivating an indicator water quality microorganism was evaluated with a bench scale filter packed with the polymer-treated sand. Promising results were obtained with clean water matrix; however, alternative and more efficient ‘regeneration’ methods will be needed in the future studies in order to apply this technology in practical systems. In addition, inactivation efficiency towards other indicator pathogenic microorganisms such as protozoa, Cryptosporidium and Giardia should be evaluated. The advantage of this technology for these applications resides in the fact that it does not require any chemicals, or energy (e.g. electricity) consumption, and it can be easily built in any small water filters. Finally, the application of this modified sand filter is not limited to drinking water treatment and tertiary wastewater treatment for reuse, but it has the potential to be used to create a portable device that can provide drinkable water treatment under adverse conditions (e.g. rural areas in developing countries, or for military personnel in war zones).

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