

Bactericidal activity of Ag nanoparticle-impregnated fibreglass for water disinfection

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ABSTRACT

A new bactericidal system composed of fibreglass impregnated with silver (Ag) nanoparticles was developed and tested. Silver content, particle size and distribution were characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD). The antibacterial effectiveness was evaluated against *Escherichia coli* (*E. coli*, ATCC 29055). The minimum inhibitory loading was determined to be less than 1.8 wt% of silver nanoparticles per gram of fibreglass. In a 1 h immersion test, using a 0.1 mg fibreglass mat ml⁻¹, with 2.9 wt% loading of silver nanoparticles completely disinfected 100 ml of 10⁶ CFU ml⁻¹ of *E. coli*, dramatically outperforming activated carbon fibres impregnated with silver. Inactivation rate studies of 0.05 mg fibreglass mat ml⁻¹ (Ag 1.8 wt%) with 10¹² CFU *E. coli* displayed a 7 log reduction in 5 minutes. The activation and reuse of fibreglass (Ag 4.3 wt%) maintained its full effectiveness after two cycles of use and thermal regeneration at 350°C.

Key words | bactericidal, *E. coli*, silver nanoparticles, water disinfection

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INTRODUCTION

Silver (Ag) has long been utilized as a disinfectant for non-spore-forming bacteria and viruses (Thurman & Gerba 1988). Silver can serve as a disinfectant at concentrations 1,000 times lower than the level toxic to mammalian life (Environmental Protection Department 1996). The sulfhydryl-binding propensity of the silver ion disables proteins, inhibits enzyme activities and disrupts cell membranes (Thurman & Gerba 1988; Semikina & Skulacher 1990). Silver ions bind to DNA, RNA, enzymes and cellular proteins causing cell damage and death (Hambridge 2001).

Since the 1990s, several researchers have explored depositing silver onto carbon substrates to prevent bacterial growth during water purification (Oya & Yoshida 1993; Oya *et al.* 1993, 1994; Li *et al.* 1998; Wang *et al.* 1998). However, activated carbon filters alone are not effective against bacteria, viruses or protozoa. Carbon-based materials have excellent biocompatibility with bacteria and bacterial

growth is often enhanced in the presence of graphite or activated charcoal (Matsushashi *et al.* 1995). Bacteria, especially some Gram-positive, spore-forming species, tend to grow on carbon during use in water treatment causing secondary contamination (Li *et al.* 1998). Researchers have also examined the use of silver templated onto various inorganic substrates including zeolites and ceramics for water disinfection (Inoue *et al.* 2002). One drawback of this method is the possibility of biofilm formation on the supporting surface that may significantly reduce the bactericidal activity of the supported silver (Camper *et al.* 1986; Costerton *et al.* 1987; Brown & Gilbert 1993).

In this paper, a new kind of antibacterial fibre composed of fibreglass impregnated with silver nanoparticles is described. The antibacterial effectiveness in water was evaluated against *Escherichia coli* (*E. coli*) and compared with silver nanoparticles supported on carbon-based fibres

using United States Environmental Protection Agency's method number 10029 (US EPA 2002). The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. Performance in a dynamic filter cartridge form was evaluated along with the regeneration of the silver nanoparticles on the fibreglass system.

MATERIALS AND METHODS

The bacterial strain used in this work, *E. coli* (ATCC 29055), was cultured at $37 \pm 0.5^\circ\text{C}$ for 18 h in 25 ml of Luria Bertani broth prepared from 10.0 g Bacto™ Tryptone, 5.0 g Bacto™ yeast extract, 10.0 g NaCl, at pH 7.0 in 1 l of DI water. The bacteria were then centrifuged and resuspended with 0.01 M phosphate buffer ($0.690 \text{ g l}^{-1} \text{ NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, $0.71 \text{ g l}^{-1} \text{ Na}_2\text{HPO}_4$, pH 7.4) to obtain *E. coli* suspensions for static immersion and dynamic breakthrough tests. Cell concentrations were estimated by measuring the optical density at 600 nm. The viability of the *E. coli* was determined by the membrane filtration technique, US EPA method 10029, after a 24 h incubation period at $37 \pm 0.5^\circ\text{C}$ (US EPA 2002). Reasoner & Geldreich (1985) indicated that injured but still viable bacteria may not be recovered with some selective media used for *E. coli* enumeration. However, EPA method 10029-2002 indicates that m-ColiBlue24 broth enhances the growth rate of coliform bacteria and optimizes the recovery of stressed or injured organisms, while, special inhibitors in the medium efficiently minimize the growth of non-coliform bacteria (US EPA 2002).

Silver nanoparticles were deposited on non-woven fibreglass mats (diameter ranging from 6.5 to 13 μm) with 7 wt% polyvinyl alcohol (PVA) binder (Craneglas® 230, from Crane & Co., Inc.) by impregnating 300–400 mg of mat with 100 ml of 0.5 M aqueous silver nitrate (AgNO_3 , Sigma Aldrich) solution overnight. The impregnated fibreglass mats were then washed with deionized (DI) water until neutral and dried at 120°C for 1 h. The fibreglass mats were then heated in nitrogen at $20^\circ\text{C min}^{-1}$ to 300°C and then held at 300°C for 0.5 h to decompose the PVA and reduce Ag(I) to Ag(O) (Longenberger & Mills 1995). The silver content of the mats was determined by acid digestion followed by analysis with inductively coupled plasma mass

spectrometry (ICP/MS). These samples are designated as FG(Ag). Variations of fibreglass impregnated with lower concentrations of AgNO_3 produced fibres with lower silver content (see below).

Commercial activated carbon fibres (ACF), from American Kynol, Inc. (ACC-5092-10B, -15, -20) were also impregnated with silver nanoparticles by the methods of Le Pape *et al.* and Wang *et al.* (Wang *et al.* 1998; Le Pape *et al.* 2002). The fibres were prepared by an overnight impregnation with 0.5 M aqueous silver nitrate, followed by a DI water rinse and heat treatment at 400°C for 0.5 h in nitrogen. The fibres are designated as ACF-10(Ag), ACF-15(Ag) and ACF-20(Ag).

Silver impregnated phenolic based ACFs were prepared by treating 300–400 mg of phenolic resin impregnated fibreglass mat with 100 ml of 0.5 M aqueous silver nitrate solution overnight. The impregnated mats were then washed with DI water until neutral and dried at 120°C for 1 h. The fibres were then carbonized at 600°C in nitrogen for 1 h and then activated at 800°C in steam-saturated carbon dioxide for 0.5 h. These fibres are designated as ACF(ph-Ag). Phenolic based ACFs with phenolic resin and silver precursor were impregnated and processed in the same manner and are designated as ACF(ph-1-Ag).

For immersion tests, $0.1 \text{ mg mat ml}^{-1}$ silver impregnated fibreglass mats were added to 100 ml of bacterial suspension with 10^6 CFU ml^{-1} and sampled intermittently. The sample solutions were serially diluted and transferred to a membrane filter and processed according to the membrane filtration method 10029 for *E. coli* (US EPA 2002). Colonies of *E. coli* were counted in each Petri dish after incubation at $37 \pm 0.5^\circ\text{C}$ for 24 h. When plating our samples we did not use a silver neutralizer as previous studies indicated that using a thionneutralizer has no benefit when the exposure time of the silver to the bacteria exceeds 10–20 minutes (Tilton & Rosenberg 1978; Landeen *et al.* 1989).

Antibacterial filters were fabricated from silver impregnated fibreglass mats, prepared as described above, rolled tightly around a glass rod and then wrapped with parafilm. Connectors were put on both ends of the cylindrical tube to direct the water flow, and silicone rubber was used to seal the filters as described in Figure 1. Each filter was degassed under vacuum to remove trapped gases and

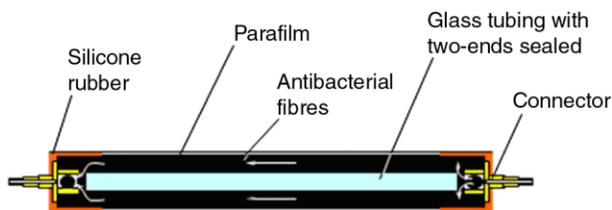


Figure 1 | Schematic structure of antibacterial filters.

prevent air pockets from interrupting the water flow through the filter.

Bacterial breakthrough tests were conducted by adjusting the 25 ml *E. coli* stock suspension in 0.01 M phosphate buffer to give a bacterial suspension of 10^6 CFU ml⁻¹. The suspension was then pumped at a flow rate of 20 ml min⁻¹ through a small filter with 5.0 wt% silver impregnated fibreglass (7.1 ml bed volume) fabricated as described in Figure 1. Duplicate samples were collected intermittently, serially diluted, and the bacteria enumerated using the US EPA membrane filtration method 10029.

The spent antibacterial mats were regenerated by heating at 350°C in air for 1 h. The oxidized fibres were subsequently reduced by heating in a nitrogen environment at 350°C for 30 min. It is well established that silver oxide begins to lose oxygen when heated above 190°C (Remy 1956).

RESULTS AND DISCUSSION

The silver nanoparticles deposited on the surface of the fibreglass were characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD). Figure 2 clearly displays well-formed silver nanoparticles deposited

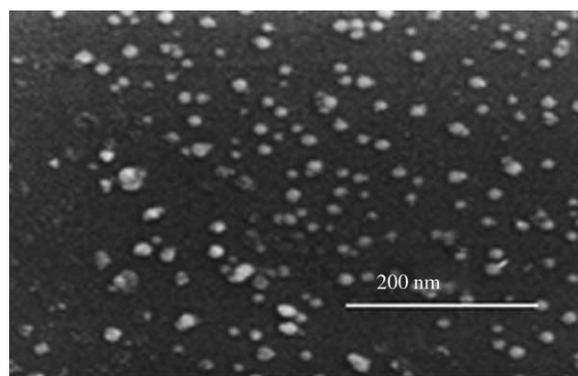


Figure 2 | SEM image of silver nanoparticles on fibreglass mat; surface morphology at 200,000 × magnification.

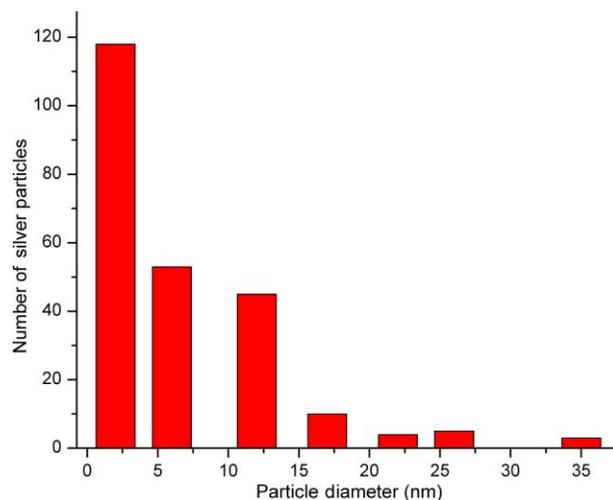


Figure 3 | Particle size distribution of silver-impregnated fibreglass.

on a glass fibre. Image analysis of the SEM image using Scion Image[®] showed that the silver particles were in the range of 17 ± 7 nm in diameter, and 97.7% of the particles were less than 30 nm in diameter as shown in Figure 3. XRD clearly indicates the formation of silver nanocrystals with characteristic peaks corresponding to Ag(O) 110, 200, 220, 311 reflections as shown in Figure 4. The peak width at half height verified the silver crystals are nanostructures with an average size of 20 nm.

The silver impregnated ACF were characterized for their surface area (with and without silver), silver content and bactericidal properties. The ACFs take up more silver during the impregnation process and display a higher BET (Brunauer-Emmett-Teller) surface area in ultra high purity

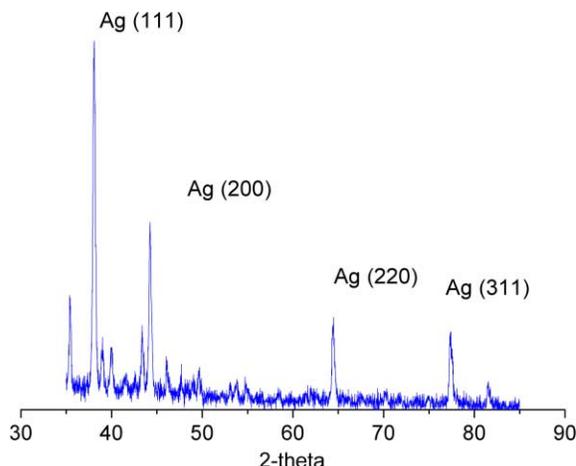


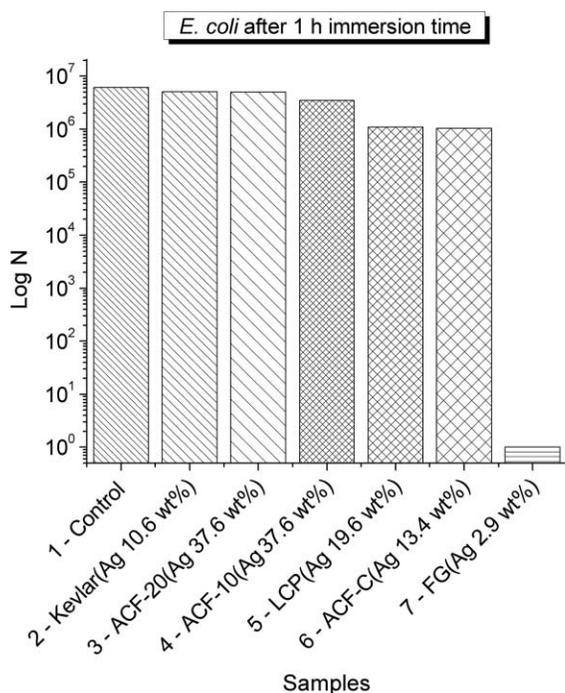
Figure 4 | XRD confirmation of Ag nanoparticles on fibreglass.

Table 1 | Silver adsorption by different fibre substrates

Sample name	Fibre substrates	Substrate surface area ($\text{m}^2 \text{g}^{-1}$)	Silver content (wt %)	Surface area of silver-impregnated fibre ($\text{m}^2 \text{g}^{-1}$)
ACF-20(Ag)	ACF 20	1,890	40.2	1,606
ACF-20(Ag)	ACF 15	1,500	14.3	1,208
ACF-20(Ag)	ACF 10	1,000	5.9	1,072
FG(Ag)	Fibreglass	100	1.1	101

N_2 gas, as determined with an Autosorb-1 MP (Quantachrome Corporation), than the silver impregnated fibreglass mats as shown in Table 1.

Immersion tests against *E. coli* indicated the inactivation efficiency without filtration effects for the silver nanoparticle-impregnated fibreglass mats. Equal loadings of $0.1 \text{ mg mat ml}^{-1}$ of each set of fibres listed in Table 1 were placed in a 100 ml bacterial suspension of *E. coli*, 10^6 CFU ml^{-1} . After 1 h immersion time, the CFU in the suspension was assessed. The bacterial suspension was serially diluted and filtered through a $0.45 \mu\text{m}$ membrane filter. The filter was then transferred to a 50-mm Petri dish containing an absorbent pad saturated with m-coliBlue24

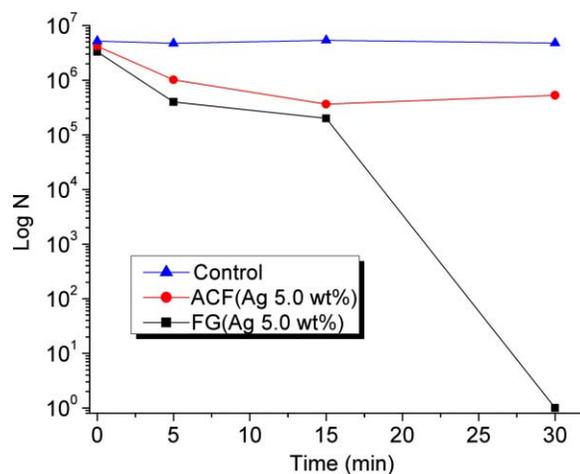
**Figure 5** | Comparison of bactericidal activity for silver-impregnated fibres; 1 h immersion test, Ag fibre loading = $0.1 \text{ mg mat ml}^{-1}$.

Broth and then incubated at $37 \pm 0.5^\circ\text{C}$ for 24 h. Blue colonies under fluorescence indicated the presence of *E. coli*. Figure 5 displays the CFU of *E. coli* after 1 h contact time with the silver impregnated fibres.

The silver impregnated fibreglass reduced the CFU of the *E. coli* by six orders of magnitude from the test water. The activated carbon-based materials loaded with silver displayed significantly lower effectiveness in inactivating *E. coli* under the same conditions as the silver impregnated fibreglass. The higher inactivation efficiency of the silver impregnated fibreglass may be due to the inorganic nature of the system since bacteria have been found to easily adhere to and metabolize activated carbon materials (Gottschalk 1986; Wang et al. 1998).

In a head-to-head comparison between 5.0 wt% silver impregnated ACF and fibreglass, the fibreglass system outperformed the ACF system as seen in Figure 6. An equivalent amount of ACF(ph-1-Ag 5.0 wt%) and FG(Ag 5.0 wt%), $1.8 \text{ mg mat ml}^{-1}$ were placed into a separate 4 ml suspension of 10^6 CFU ml^{-1} *E. coli*. Duplicate samples were withdrawn intermittently, serially diluted, plated and incubated overnight at $37 \pm 0.5^\circ\text{C}$ and counted.

The bactericidal activity of the FG(Ag) mats vs. time are shown in Figure 7. Each set of fibres was placed into a separate *E. coli* suspension of 10^6 CFU ml^{-1} at a loading of $0.05 \text{ mg mat ml}^{-1}$. Duplicate samples were withdrawn intermittently, serially diluted, plated and then incubated overnight at $37 \pm 0.5^\circ\text{C}$ and counted.

**Figure 6** | Antibacterial activity of ACF(ph-1-Ag) and FG(Ag); 10^6 CFU ml^{-1} *E. coli* suspension, material loading per sample = $1.8 \text{ mg mat ml}^{-1}$.

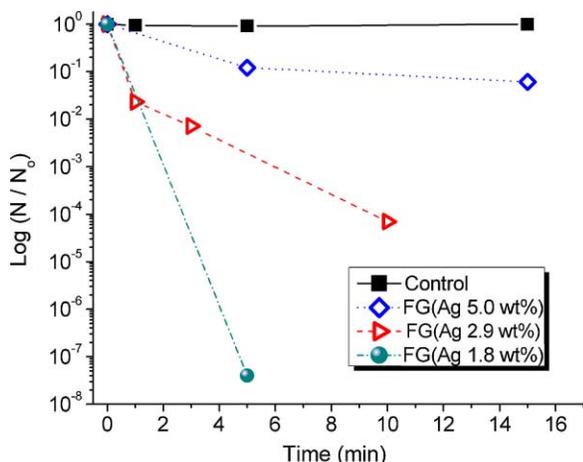


Figure 7 | Bactericidal effect of 0.05 mg mat ml⁻¹ FG(Ag) at varying silver content in a 100 ml suspension of *E. coli*.

The Ag impregnated fibreglass system displayed rapid bactericidal activity against *E. coli*. At a silver content of 1.8 wt%, 0.05 mg ml⁻¹ FG(Ag) reduced the presence of *E. coli* in a 100 ml suspension of 10¹² CFU ml⁻¹ by 7 log in 5 min. At the identical material loading, FG(Ag 1.2 wt%) reduced the presence of *Aeromonas hydrophila* in a 100 ml suspension of 10¹² CFU ml⁻¹ by 6 logs within 5 min (Xiao 2005). In previous work Xiao reported that the bactericidal efficiency increased with increasing silver content from 0.5 up to 1.2 wt% on the fibreglass (Xiao 2005). However, the data presented in Figure 7 indicates a decrease in disinfection efficiency with increasing silver content above 1.8 wt%. This result is most likely due to the increased Ag particle size at higher silver loadings and a lower surface area to volume ratio for the larger Ag crystallites, which results in lower contact efficiency with the bacteria. We have recently shown that as the Ag loading increases, the particle size increases (Nangmenyi et al. 2009).

If the loss of efficiency of the FG(Ag) mats over time with use is due to the formation of a biofilm, then simply heating the sample to kill the biofilm followed by rinsing with clean water would be expected to regenerate the filter. To examine this possibility, 9 mg of FG(Ag) mats were used to treat 90 ml of 10⁶ CFU ml⁻¹ *E. coli* suspensions. After each bactericidal run, the fibres were then exposed to high concentrations of *E. coli* to completely 'exhaust' their bactericidal properties. The mats were then regenerated by heating in air at 350°C for 1 h, followed by heating under

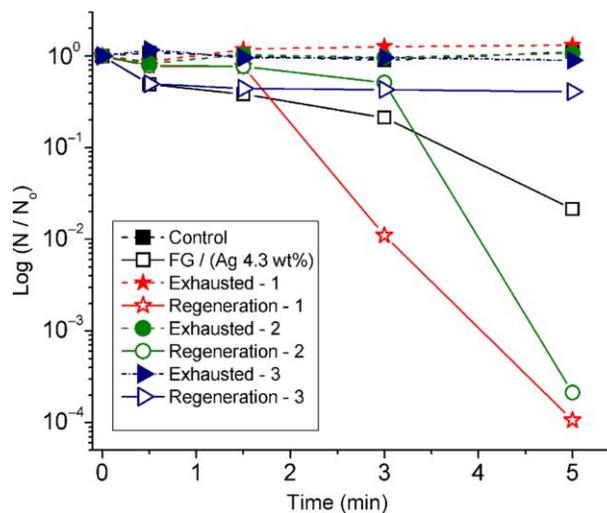


Figure 8 | Bactericidal activity of regenerated FG/Ag (4.3 wt%).

nitrogen at 350°C for an additional 0.5 h. As can be seen in Figure 8, the FG(Ag 4.3 wt%) mat maintained full activity after two regeneration cycles with a drop off after the third regeneration treatment. An SEM of the FG(Ag) mat after three regeneration cycles clearly shows the morphology of the silver has been maintained with a particle size range of 20–30 nm and the silver content after three regenerations (4.3 wt%) was within the experimental error of the initial silver loading (Figure 9).

Filters fabricated from silver impregnated fibreglass displayed dynamic disinfection capacity. In breakthrough tests conducted with 10⁶ CFU ml⁻¹ *E. coli* stock solution, no *E. coli* were detected up to 421 bed volumes of effluent at

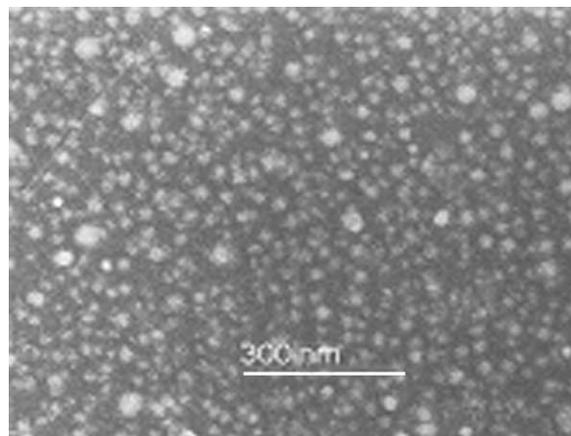


Figure 9 | SEM image of silver nanoparticles on fibreglass mat; surface morphology at 200,000 × magnification after three regenerations.

a flow rate of 20 ml min⁻¹. The average filter volume was 7.1 ml and the total amount of contaminated water processed was 3 l. In preliminary field experiments the FG(Ag) was found to be highly effective in inactivating waterborne pathogens found in natural waters in South Africa. FG(Ag) loaded with 2.9 wt% Ag was effective in eliminating *Salmonella*, *Shigella* and fecal coliform found in the raw groundwater supply in rural communities of South Africa. Further field trials are currently in progress.

CONCLUSIONS

Fibreglass mats impregnated with silver nanoparticles exhibit strong bactericidal activity against *E. coli* (ATCC 29055) under both static and dynamic testing, and exhibit superior performance over silver-impregnated activated carbon fibres. These silver-impregnated fibreglass mats can be readily regenerated by thermal treatment. Further research on the synthesis and properties of silver nanoparticle-impregnated fibreglass systems is warranted. A better understanding of the factors affecting the silver crystallite size and the mechanical properties of the material at varying silver loadings as well as the interface between the fibreglass and silver would enable the development of improved systems.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Crane & Co., Inc., for providing free samples of the Craneglas 230™ product used in this research. We would also like to acknowledge Dr James Imlay for the use of his laboratory for some of the microbiological testing. This work was partially supported by The WaterCAMPWS, a Science and Technology Center of Advanced Materials for the Purification of Water with Systems under the National Science Foundation agreement number CTS-0120978.

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First received 10 August 2007; accepted in revised form 23 January 2009. Available online July 2009