Contract No:

This document was prepared in conjunction with work accomplished under Contract No. 89303321CEM000080 with the U.S. Department of Energy (DOE) Office of Environmental Management (EM).

Disclaimer:

Enhanced Filter Material for Pathogen Removal

Project Start and End Dates
Project Start Date: December 1, 2020
Project End Date: June 1, 2021

Project Highlight
It was determined that spherical silver (Ag) nanoparticles either bound to 316 stainless-steel filter material or as unbound nanomaterials in deionized water had anti-microbial activity on *Escherichia coli* K-12 (*E. coli)* cultures when aerosolized or waterborne. This effect however was attenuated when in the presence of the high-salt growth media. Nanoparticles are known to agglomerate in high salt solutions and this may have limited their ability to cross the cell membrane of the microorganisms and cause fatal damage.

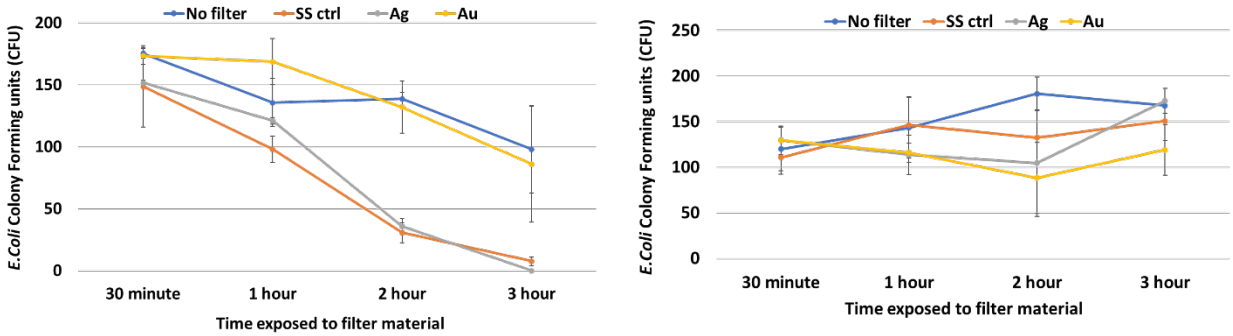

Figure 4. (Left) Colony Forming Units (CFUs) of waterborne *E. coli* in deionized water exposed to filter materials containing bound silver (Ag) or gold (Au) nanoparticles compared to 316 stainless steel (SS) filter control and no filter control (n=3). **(Right)** CFUs of waterborne *E. coli* in growth media exposed to filter materials containing bound silver (Ag) or gold (Au) nanoparticles compared to 316 stainless steel (SS) filter control and no filter (n=3).

Project Team
Principal Investigator: Wendy Kuhne
Team Members: Candace Langan
External Collaborators: None

Abstract
In this project we evaluated two spherical nanoparticles, gold (Au) and silver (Au), embedded separately on 316 stainless steel (SS) filter materials to remove potentially pathogenic microorganisms from airborne and waterborne environments. *Escherichia coli* K-12 (*E. coli)* was chosen for airborne experiments where an aerosol stream was generated for 75 sec using deionized water (DI) in a nebulizer (fan speed set to ~11.2 L per min, sonication at 50%). *E. coli* were collected on agar plates 1" from the nebulizer with and without filter material present. Treatment conditions were SS alone, SS + Ag, and SS + Au, and no filter conducted in triplicate. Ag filter material showed a 90% reduction in colony forming units (CFUs) in the aerosolized stream using DI water. Waterborne experiments, using liquid *E. coli* cultures in DI water exposed to SS, SS + Ag, and SS + Au filters resulted in a statistically significant reduction of CFUs compared to the no filter material control. When evaluated using a high salt growth media as a delivery source

instead of DI water, the reduction in viable colonies was not present. Comparison of the SS filter alone vs the SS + Ag show similar levels of CFU reduction (~70-79%) in the aerosolized stream of DI water.

Objectives

- Determine suitable viral pathogen surrogates relevant and ensure that adequate analytical tools exist.
- Create and functionalize nanoparticles (different geometries and compositions; native or coated with surrogate-specific receptors) on filter materials, such as polypropylene fibers and/or stainless-steel wool.
- Create relevantly sized microorganism waterborne/airborne environments (surrogates) and filters, the ability to entrain them in fluid streams, and a way to reliably detect them with the surrogate to deactivate them and determine the efficacy of the treatment.

REVIEWS AND APPROVALS

1. Authors:

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Name and Signature Date

2. Technical Review:

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Name and Signature Date

3. PI's Manager Signature:

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Name and Signature Date

4. Intellectual Property Review:

This report has been reviewed by SRNL Legal Counsel for intellectual property considerations and is approved to be publicly published in its current form.

SRNL Legal Signature

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Name and Signature

Introduction

Controlling and preventing air-and water-borne infections from exposure to pathogenic microorganisms is of global concern. The COVID-19 pandemic highlighted the need for development of novel filtering technologies for deactivation of human viral pathogens and surrogates present in airborne, foodborne and/or waterborne environments. Herein, we address fundamental questions to develop an effective means for the capture, removal and deactivation of human pathogens and surrogates through attraction to activated nanoparticles (NPs), with subsequent destruction and decontamination. The small particle size and high surface areas of nanomaterials are attractive additives as they can dramatically alter performance at low loadings. Metal and metal oxides, e.g., silver (Ag) and cuprous oxide nanoparticles, have been known for decades to display antiseptic characteristics, including antibacterial, antifungal, and antiviral properties.[1] They are efficient in wound management, various coatings for medical devices, and impregnating textile fabrics. Moreover, it has been shown to have increased efficacy at limiting viral infectivity on material bound viruses in clinical trials. Prior reports have indicated the Ag nanoparticles at 3-6 nM concentration had antimicrobial effects on yeast (isolated from bovine mastitis) and *Escherichia coli* (*E.coli)* O157:H8 (ATCC 43886). A higher concentration (33 nM) was required for inactivation of *Staphylococcus aureus* (ATCC 19636).[2] The mechanism by which Ag nanoparticles are thought to function as antimicrobial agents is linked to damage caused to the microorganisms cell membrane either through electrostatic forces[3] or by internal physical damage by passage of the nanomaterials through cell membranes or by the formation of pits leading to leaking of the cells.[4] We investigated Ag and gold (Au) nanomaterial bound to stainless-steel filter materials, as well as unbound nanomaterials, for their antimicrobial activity in both airborne and waterborne environments.

Approach

Airborne pathogen approach:

Initial tests were completed to determine the optimal distance from nebulizer and amount of time required for collection and formation of colony forming units (CFU). Varying distances and timepoints were collected on tryptic soy agar plates and incubated overnight to determine a dilution factor that would allow for countability (between 50-200 colonies/plate) pf the CFUs.

E. coli K-12 (ATCC, Manassas, VA), were diluted in deionized (DI) water (1:250,000 from stock culture) and loaded into a nebulizer to generate an aerosol stream. The nebulizer fan was set at 50% sonication to deliver the aerosol stream containing *E. coli* at 11.2 L min^{-1}. The aerosol stream containing *E. coli* was set up 1 inch away from the filter material covering a tryptic soy agar plate (**Figure 1**). Each agar plate was exposed for 30 seconds, and all experimental conditions were performed in triplicate. Filter material tested included spherical silver (Ag) nanoparticle-coated 316 stainless steel, spherical gold (Au) nanoparticle-coated, 316 stainless steel without nanoparticles bound, and no filter material as a positive control. Aerosolized *E. coli t*hat had traveled through the filter material was collected onto agar plates and incubated overnight. Colonies were allowed to form for 24-h post collection at 37°C and CFUs were imaged and counted using a AlphaImager.

Waterborne pathogen approach:

E.coli were diluted in DI water or Tryptic Soy Broth (TSB) growth media (1:250,000 from stock culture) and 10 mL of diluted culture were placed in a petri dish and incubated with varying filter materials: Ag nanoparticle-coated stainless steel, Au nanoparticle-coated stainless steel, stainless steel without nanoparticles bound, and no filter material as a positive control, or unbound spherical nanoparticles (Ag and Au) while shaking on a rocker table (**Figure 2**). Timepoints were taken over the course of incubation

by taking 20 µL of the incubated *E. coli* culture and plating it onto a tryptic soy agar plate. Colonies were allowed to form for 24-h post plating at 37°C and CFUs imaged and counted using an AlphaImager.

Accomplishments

- Airborne: A log-reduction (~90%) in CFUs of aerosolized *E.coli* in DI water was measured with filter materials embedded with Ag nanoparticles versus the control with no filter present (T-Test one-tail *p*-value <0.01) . The stainless-steel filter alone and stainless steel embedded with Au reduced CFU by ~70-79% and was measured to be statistically significant vs the control no filter test (*p*-values = 0.01, *p*-value = 0.03) respectively. A marginal difference was measured in comparing the antimicrobial effect of the stainless-steel filter alone vs the stainless-steel embedded with Ag (T-Test one-tail *p*-value=0.05) (**Figure 3**).
- Waterborne: Statistically significant reductions ($p<0.05$) in colony formation were observed with filters embedded with spherical Ag nanoparticles at a minimum of 1 h of exposure. Exposures performed using dilute TSB growth media containing salts showed little to no reduction in CFUs. Salts are known to cause agglomeration of nanoparticles[5] and could have reduced their efficacy by not allowing the Ag to pass through the cell membrane to cause damage. Efficacy of Au is driven by the size and shape of the nanoparticles, with rod shapes being the most effective[6], and in this study the spherical shape and size were not as effective for bacterial reduction (**Figure 4**).
- Unbound nanoparticles alone were tested at concentrations of 450 nM, 4.5 µM, 45 µM in DI water for 6 h with 100% reduction of CFUs with the stainless steel + Ag filters and no statically significant difference in CFUs measured vs the control ($p>0.05$). Using a dilute concentration of nanoparticles (30 nM, 300 nM and 3µM Ag and Au) in TSB growth media containing salts no statistically significant reduction in CFUs vs the control ($p>0.05$) was measured (**Figure 5**).

Future Directions
None

FY 2021 Peer-reviewed/Non-peer reviewed Publications
None

Intellectual Property
None

Total Number of Post-Doctoral Researchers
One – Dr. Candace Langan (SRNL)

Total Number of Student Researchers
None

References

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3. Dibrov, P.; Dzioba, J.; Gosink, K.K.; Häse, Chemiosmotic mechanism of antimicrobial activity of Ag^+ in *Vibrio cholerae*. *Antimicrob Agents Chemother*. **2002**, 46(8), 2668-2670.
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5. Williams, D.N.; Ehrman, S.H.; Pulliam Holoman, T.R., Evaluation of the microbial growth response to inorganic nanoparticles. *J Nanobiotechnology*. **2006**, 4:3, 1-8.
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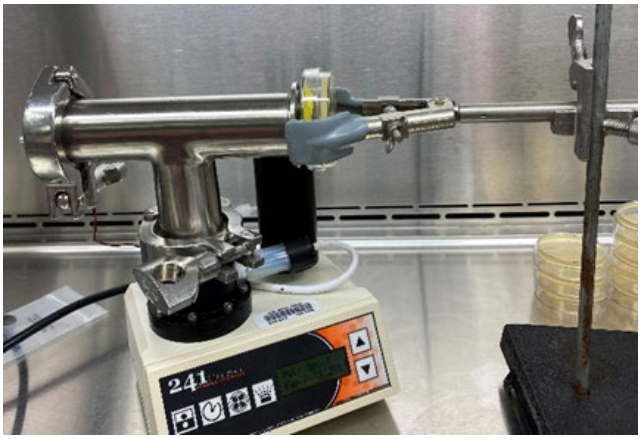


Figure 1: *E. coli* aerosol delivery to the filter material covering an agar plate.

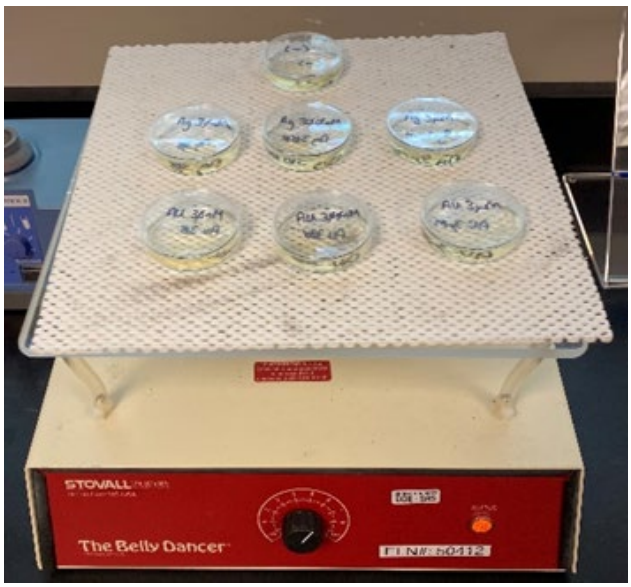


Figure 2: *E. coli* waterborne incubation with bound nanoparticle filter materials and unbound nanoparticles at room temperature on shaker.

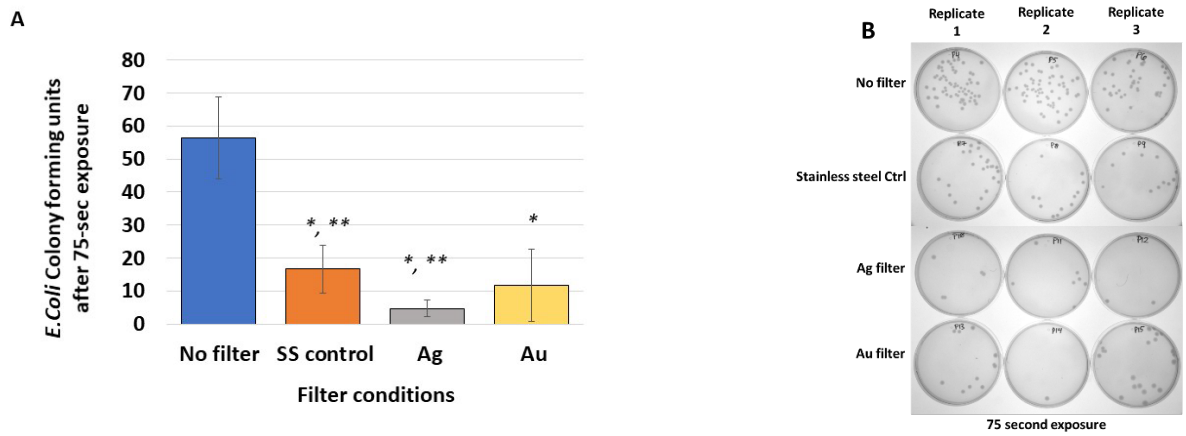
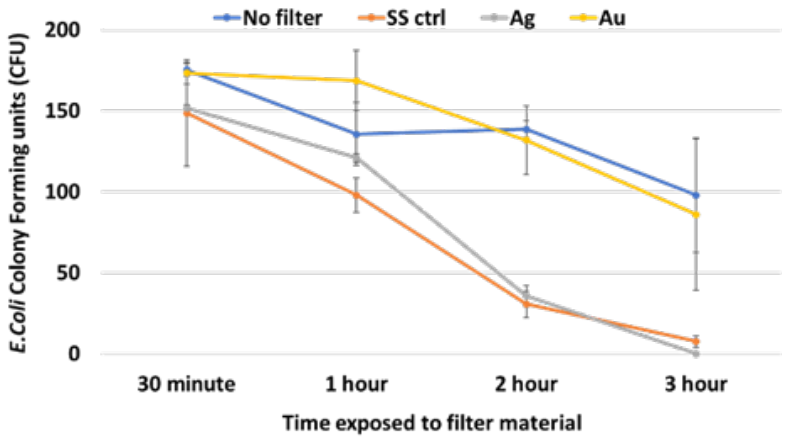


Figure 3: (A) Colony Forming Units (CFUs) of aerosolized *E. coli* exposed to filter materials containing embedded silver (Ag) or gold (Au) spherical nanoparticles compared to stainless steel (SS) filter control and no filter. **(B)** Post-24hr collection of exposed *E. coli* to filter material on agar plates. Nebulizer positive controls were included (n=3).
*All filter conditions were statistically significant vs the no filter control (AVOVA *p*-value <0.01).
**A marginal difference was measured between the SS control and the Ag filter (*p*-value = 0.05) and no difference between the Ag and Au filters (*p*-value >0.05)

A



B

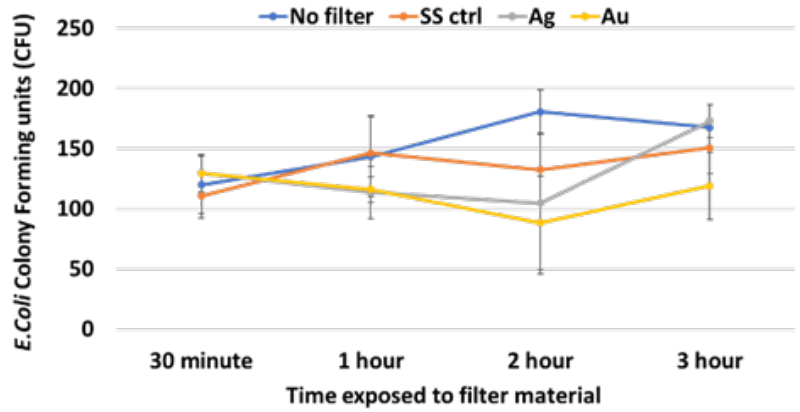


Figure 4: (A) CFUs of waterborne *E. coli* in DI water exposed to filter materials containing embedded silver (Ag) or gold (Au) nanoparticles compared to stainless steel (SS) filter control and no filter (n=3). **(B)** CFUs of waterborne *E. coli* in growth media exposed to filter materials containing embedded silver (Ag) or gold (Au) nanoparticles compared to stainless steel (SS) filter control and no filter (n=3).

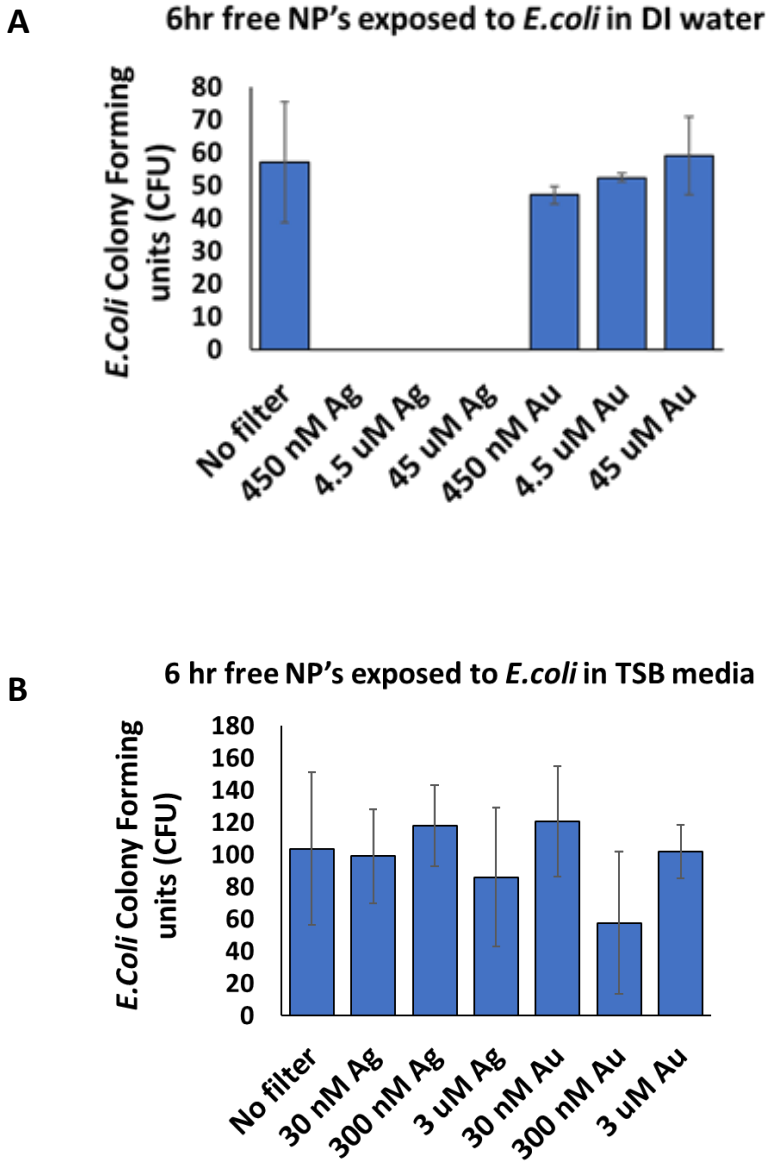


Figure 5: (A) CFUs of waterborne *E. coli* in DI water exposed to unbound spherical silver (Ag) or spherical gold (Au) nanoparticles compared to control culture with no nanoparticles (n=3). **(B)** CFUs of waterborne *E. coli* in TSB growth media exposed to unbound spherical silver (Ag) or spherical gold (Au) nanoparticles compared to control culture with no nanoparticles (n=3).