

**EVALUATION OF IMMOBILIZED TITANIUM DIOXIDE-SILVER-
HYDROXYAPATITE NANOPARTICLES
AND COLLOIDAL SILVER FOR WATER DISINFECTION**

By

IRENE LIANG

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Approved by:



Dr. Charles P. Gerba

Department of Soil, Water, and Environmental Science

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ABSTRACT

Titanium dioxide nanoparticles combined with silver and hydroxyapatite (TiO₂-Ag-Hap) form a photocatalytic composite capable of oxidizing and mineralizing a wide spectrum of microbiological and chemical contaminants in water, while silver nanoparticles have long been employed for their antimicrobial properties. These materials were evaluated through an iterative series of experiments that evaluated microbial reduction, material formulation, method of application, surface-interface interactions, and reusability. The TiO₂ formulation was assessed as an antimicrobial film coated onto fabric and ceramic beads in three experimental designs: a gravity filtration column, a portable treatment capsule, and a static chamber. Colloidal floating Ag nanoparticles in solution were also assessed. Reduction of *Escherichia coli*, *Klebsiella terrigena*, MS2 bacteriophage, and Rotavirus was evaluated through standard culture-based methods. Significant microbial reduction was only observed in the static open pan design for the TiO₂-Ag-HAP materials. Colloidal silver was more effective and caused a 5 log reduction of *K. terrigena*, within 60 minutes, and a 5 and 4 log reduction of MS2 within 120 and 90 minutes respectively in initial trials. The anti-microbial properties of both materials were apparent, but further investigations are necessary to assess the potential of the materials for integration and development in water treatment technologies.

INTRODUCTION

Oxidative techniques have long been employed in water treatment to mineralize potentially hazardous organic chemicals and pathogens. Reactive oxygen species can be generated in-situ from ozone, hydrogen peroxide or oxygen, and the addition of an energy input such as UV light or heat (Li, 2010). Reactive oxygen species may also be generated photo-catalytically with titanium dioxide, TiO₂. Titanium dioxide was first found to photo-catalytically split water molecules when irradiated with ultraviolet light in 1972 (Chen, 2007). In 1985, new research proved that the hydroxyl radicals generated by TiO₂ photocatalysis could be used for microbial disinfection (McCullagh, 2007). Intense research has since been on-going to further explore and advance the

novel application of TiO₂ for microbial disinfection. Recent literature shows that titanium dioxide nanoparticles effectively and completely mineralize a wide spectrum of organic chemicals and microbial agents such as bacteria, viruses, algae, and fungi (Nadtochenko, 2005). TiO₂ will progressively damage cell walls and membranes, followed by intracellular components (Huang, 2000).

Nanoparticle research for application in water treatment and pollution mitigation is also expanding. Silver nanoparticles have long been recognized for their antimicrobial properties and have been employed in many applications. Silver is commonly used in water disinfection systems. For instance, it is commonly combined with other filter material in home point-of-use purification

units to prevent microbial growth and biofilm formation within the filter and unit (Gupta, 1998). Silver ions reduce microbial numbers by binding to cell walls and disrupting metabolic processes leading to cell death (Silvestry-Rodriguez, 2007).

A Titanium Dioxide, Silver, and Hydroxyapatite Composite

Titanium dioxide is often doped with other materials to expand and enhance desired properties (Chen, 2007). The addition of a metallic conductor can expand the electromagnetic range necessary to activate the TiO₂ photocatalyst. Silver is an effective choice as a semi-conductor. It allows the TiO₂ to be induced in visible light, is resistant to oxidation, and has demonstrated anti-microbial properties of its own (Foster, 2011). Furthermore, the photocatalytic reaction damages cell walls and enhances the damaging effects of the heavy metal ions (Hashimoto, 2005). This synergistic effect allows for Ag-doped titanium dioxide to be effective in both light and dark conditions, and the mixture of nanoparticle TiO₂ and Ag has greater antimicrobial properties than either form alone (Chen, 2010). The positive charge of the silver ions also enhances charge separation of the negatively charged reactive oxygen species, allowing them to persist longer in solution and interact with target contaminants (Sökmen, 2001).

Since titanium dioxide requires sufficient time to generate reactive oxygen species and to eliminate contaminants of concern, the necessary treatment time is a major constraint in the ideal, continuously flowing system in which contaminated water passes through quickly and is not re-circulated.

This issue can be addressed by enhancing titanium dioxide with an adsorbent material. Hydroxyapatite, a calcium-phosphate material, is known to be adept at adsorbing bacteria and viruses. The incorporation of hydroxyapatite particles will allow for microorganisms to be trapped and retained in the treatment column or filter until they are mineralized by the electrochemical reactants (Sakurada, 1999). Titanium dioxide nanoparticles combined with silver and hydroxyapatite (TiO₂-Ag-HAp) form a photocatalytic composite capable of treating water in visible light by adsorbing and mineralizing a wide spectrum of organics.

Low Temperature Melt injected TiO₂/Ag +HAp/Ag Film

The methods evaluate a mixture of fine titanium dioxide, silver, and hydroxyapatite nanoparticles (Shinshu Ceramics Co., EarthPlusTM) combined and immobilized on cloth filters and small, ceramic spheres. The nanoparticle composite is a low temperature melt injected antimicrobial film produced by melting the particles and propelling them in a high velocity flame onto the fibrous or ceramic substrate (Sakurada, 1998). The molten mist rapidly solidifies and results in a thin film with a large specific surface area. The film is durable, porous, flexible, and does not require a binder to adhere to the surface. The high porosity of the material and the ability to adhere without the aid of a binder enhance the available, active surface area of the TiO₂/Ag + Ag/HAp film.

The fabric substrate was an unwoven acrylic cloth electroplated with a silver conductor. When coated and impregnated with the TiO₂-Ag-HAp film, the fabric maintains its

porosity and flexibility, and can be used as a filter or packed into a treatment column. The ceramic spheres were initially composed of pumice, but were reformulated with a clay polymer material. This second formulation adds improvements of stability and greater resistance to erosion. The coated spheres can be packed into a filtration column, allowing for contaminated water to pass through and interact with the antimicrobial material.

Colloidal Silver Suspension

The methods also evaluate a colloidal silver suspension with 0.36% colloidal Ag and 99.64% bio-distilled water (Silverdyne®).

Point of Use Water Treatment with TiO₂-HAp-Ag and Colloidal Ag Suspension

Titanium dioxide is a very effective photocatalytic oxidant and silver nanoparticles are effective antimicrobials; however, the antimicrobial activity of both substances are limited in highly contaminated or turbid waters. Furthermore, immobilized TiO₂ is more manageable, but is less active than suspended TiO₂ (Foster, 2011). For these reasons, titanium dioxide and silver nanoparticle based systems would best be employed for treatment of less contaminated water. They would be ideal for point-of-use devices such as in a home or personal treatment system. They may also be valuable as a portable means of solar water disinfection, such as for outdoor recreation, travel in a remote location with less reliable potable water sources, or in emergency situations. The technologies may also be applied in a municipal drinking water treatment system or in the final, purifying stages of wastewater treatment.

MATERIALS AND METHODS

Treatment System Designs: The following system designs were used to evaluate the antimicrobial effectiveness of the TiO₂-Ag-HAp film and colloidal Ag.

(1) Passive Reactive Chamber: The TiO₂-Ag-HAp coated materials and free floating silver suspension were tested in a simple vessel that allowed the antimicrobial materials to interact with contaminated water. The TiO₂-Ag-HAp coated material formed a stationary treatment layer on which a shallow depth of water was applied and tested.

(2) Filtration Column: A treatment column was formed as might be used in a household to treat and use municipal tap water. The column was packed with the TiO₂-Ag-HAp coated material and irradiated with visible light from a fluorescent bulb source. Contaminated water was allowed to trickle through by gravity flow and the effluent was assayed.

(3) Portable Travel Capsules: Capsules (1.8 cm diameter, 5.8 cm length) packed with the immobilized TiO₂-Ag-HAp materials were evaluated. The capsules enclosed the material in an outer plastic frame that allowed the free flow of water. In one, the TiO₂-Ag-HAp film coated acrylic cloth was rolled into a cylinder and enclosed in the capsule. In the other, the TiO₂-Ag-HAp film coated ceramic spheres were structurally maintained with a wire mesh column. The capsules could be inserted in contaminated water and then removed to be used again.

Preparation and Assay of Test Water: A new batch of test water for the specified organism was prepared for each individual experiment and replicate experiment as described below.

(1) *Escherichia coli* or *Klebsiella terrigena*; 1000mL, $\sim 10^{6-7}$ CFU/mL; Assayed using the Spread Plate Technique:

An overnight culture of *Escherichia coli* ATCC 25922 (American Type Culture Collection; Rockville, Md.) or *Klebsiella terrigena* ATCC 33629 was prepared by inoculating a loop-full of the organism into 100 mL Trypticase Soy Broth (TSB) (EMD Chemicals Inc., Gibbstown, NJ), and then incubating on a shaker (Lab-Line® Orbit Shaker 3590, Melrose Park, IL) (130 rpm, 37°C, 24 h). The overnight culture (100 mL, $\sim 10^8$ CFU/mL) was centrifuged (Beckman CS-6 Centrifuge, Tucson, AZ) (3500 rpm, 20 min) to pellet the cells, washed in sterile physiological saline (50 mL, 85% NaCl), centrifuged to pellet again, and then reconstituted in sterile physiological saline (10 mL, $\sim 10^9$ CFU/mL). The reconstituted bacteria (1 mL, $\sim 10^9$ CFU/mL) was used to seed dechlorinated water (999mL in a sterilized beaker) to obtain a concentration of $\sim 10^6$ CFU/mL. Continuous stirring was maintained with a magnetic stir bar.

Samples collected after the treatment phase were assayed through serial dilution in sterile physiological saline (85% NaCl) and enumeration on MacConkey Agar (MAC, EMD Chemicals Inc.). The spread plate technique (Standard Methods 9215C) was used to distribute the sample dilutions over

the plates, and colonies were counted after an incubation period (37°C, 24 h).

(2) MS2 bacteriophage, 1000 mL, $\sim 10^5$ PFU/mL; Assayed with the Double Overlay Plaque Forming Units Method:

The MS2 bacteriophage ATTC 15597-B1 was prepared and assayed with the suggested host, *E. coli* strain C3000 ATTC 15597 (EPA Method 1601 and 1602). For each assay, *E. coli* was inoculated into and incubated in TSB (100 mL, 130 rpm, 37°C, 3 h) to achieve log phase growth.

Tubes with the soft overlay agar (5 mL) were prepared in advance with 3% TSB and 1% agar-agar (EMD Chemicals Inc.). When ready to use, they were melted in a steamer (10-15 min), and then maintained in a water bath (Fisher, Versa-bath®, Fairlawn, NJ) (50°C).

The MS2 stock was maintained by preparing five replicate plates of $\sim 10^5$ PFU/mL MS2. The *E. coli* host suspension (1 mL) and the appropriate dilution of MS2 (0.1 mL) were added to the soft agar tube, gently swirled to mix, and poured over room temperature Trypticase Soy Agar (TSA) (EMD Chemicals Inc.) plates. The plates were swirled to evenly disperse the overlay agar, allowed to solidify, and then incubated (35°C, 24 h). MS2 was harvested from the replicate $\sim 10^5$ PFU/mL plates by allowing the phage to diffuse off the plates into TSB (6 mL, 2 h). The liquid fraction was aspirated, centrifuged (1000 rpm, 10 min), and filter sterilized using a syringe filter (0.22 μ m pore size) moistened with 3% beef extract (EMD Chemicals Inc.). The concentrated MS2 stock was titered and

stored (4°C, up to 2 weeks). The concentrated MS2 stock was used to seed dechlorinated water in a sterilized beaker to obtain a final volume of 1000mL and concentration of $\sim 10^6$ PFU/mL. Continuous stirring was maintained with a magnetic stir bar.

Samples collected after the treatment phase were serially diluted in sterile physiological saline (85% NaCl) to be assayed through the same overlay plaque forming units as described above. The overlay agar was inoculated with the *E. coli* host suspension (0.5 mL) and the sample (0.1 mL for a 10^{-1} dilution, 0.01 mL for a 10^{-2}), and then plated and incubated (35°C, 24 h) for enumeration.

(3) Rotavirus, 100 mL, 10^5 PFU/mL; Assayed with the TC-ID50 Method:

A monolayer of Rhesus monkey epithelial cells MA104, ATCC CRL-2378.1 was grown in a 225 cm² flask to propagate the rotavirus. The cells were rinsed with Tris-buffer saline (EM Industries, Inc.), and then seeded with the Rotavirus SA11 stock ATTC VR-1565 (1 mL, 10^6 PFU/mL) and enough Eagle Minimum Essential Medium (EMEM + 0% serum, Mediatech Inc., Herndon, VA) to cover the cells for incubation (37°C, 5% CO₂, rocking, 30 min). The cells were then incubated with additional EMEM + 0% serum with Trypsin (0.5 µg/mL, 60 mL, HyClone Laboratories Inc., Logan, UT) (37°C, 5% CO₂, rocking, ~ 7 d) and observed every 24 h until cytopathic effects (CPE) destroyed 90% of the monolayer. The virus was liberated from the flask through a freeze-thaw cycle (-20°C, 25°C, 3 times), centrifuged (2500 rpm, 10 min) to isolate the

supernatant, and then concentrated through Polyethylene glycol (PEG) (Thermo Fisher Scientific Inc., Fairlawn, NJ) precipitation (9 g/100 mL, 4°C, 24 h, stirring). The virus was pelleted (8000 rpm, 30 min), and re-suspended in phosphate buffered saline (PBS, Sigma-Aldrich, St. Louis, MO). The virus was extracted and micro-dispersed with the addition of Ventrel XF (MicroCare Corp., New Britain, CT) in a 1:1 ratio. The mixture was subjected to shaking (15 min) and then centrifuged (7000 rpm, 15 min, 4°C). The top layer was drawn off with a pipette and aliquoted into cryogenic tubes for storage (-70°C), keeping an aliquot (0.5 mL) out to titer.

Cryogenic tubes were thawed and used to seed ultrapure water (Barnstead Nanopure Ultrapure Water System) to achieve a final volume of 100 mL and concentration of $\sim 10^5$ PFU/mL. Stirring was maintained with a magnetic stirbar. The TC-ID50 Assay Method was used to evaluate the samples after the treatment phase. In preparation, MA104 was grown in EMEM with 0% serum and 0.5 µg/mL Trypsin in 96-well plates (37°C, 5% CO₂, rocking, 24-72 h) to obtain 80-90% confluent cell monolayer. The plates were then emptied of media and rinsed with warm Tris-buffered saline (0.1 mL, 28-37°C). The water samples were serially diluted in EMEM and then inoculated (0.1 mL) into the wells in replicate. The 96-well plates were briefly incubated (37°C, 5% CO₂, rocking, 1 h). Then MEM (0.1 mL) was added to each well, and the plates were returned to the incubator (37°C, 5% CO₂, rocking, 7 days). The 96-well plates were observed every 24 h under a light microscope for CPE.

Sample Treatment Methods

(1) TiO₂-HAp-Ag Portable

Decontaminating Capsules: *E. coli* test water (1000 mL, 10⁶ CFU/mL) was prepared in three replicates. The capsules containing the coated electroplated acrylic cloth and the capsules containing the coated ceramic spheres were each placed in a replicate beaker of the test water. The third beaker served as the control. Aliquots (1 mL) of solution were collected into DE neutralizing broth (EMD Chemicals Inc.) in a 1:1 ratio at three time points: 0 min, 15 min, 60 min.

(2) TiO₂-HAp-Ag Packed Treatment

Column: A 15 mL graduated glass funnel column from a microfiltration assembly (column, fritted glass support base, clamp, and vacuum flask) was packed with the TiO₂-HAp-Ag coated ceramic spheres (5.7 g) to create a 3.5 cm long column with a 1.7 cm diameter. The treatment column was conditioned by passing through tap water (50 mL). *E. coli* test water was prepared and 50 mL were passed through the column, collecting the last 5 mL fraction to assay. The experiment was repeated twice with a longer column (9.0 g beads, 4.4 cm long, 1.7 cm in diameter).

(3) TiO₂-HAp-Ag Passive Reaction

Chamber, Surface Area= 754.8 cm²: The bottom surface of a shallow glass pan (22.86 cm x 33.02 cm) was layered with TiO₂-HAp-Ag coated beads (100 g). *E. coli* test water was prepared and applied in a shallow depth (250 mL) over the bead layer to maximize surface area interactions and light penetration. Movement of the water over the TiO₂-HAp-Ag surface was maintained with

a shaker (Gyrotory® Shaker G2, Edison NJ) (50 rpm), and a light source was applied. Samples (1 mL) were collected into DE neutralizing broth in a 1:1 ratio at the following time points: 0 min, 15 min, 30 min, 1 h, 3 h, 6 h. The experiment was conducted in replicate.

(4) TiO₂-HAp-Ag Passive Reaction

Chamber, Surface Area= 33.2 cm²: The base of a 250-mL beaker was layered with TiO₂-HAp-Ag coated beads (10 g) and *E. coli* test water (20 mL). A replicate beaker was layered with uncoated clay-based beads to serve as a control. Gentle mixing was maintained with a shaker (Gyrotory® G2, 50 rpm), and a light source was applied. Samples (1 mL) were collected into DE neutralizing broth in a 1:1 ratio at the following time points: 0, 15, 30, and 60 minutes. A replicate experiment was conducted using 35 mL of test water containing *E. coli*. Replicate samples were collected at each time point.

(5) Ag Nanoparticle Solution: Test water was prepared with *K. terrigena* (1000 mL, 10⁶ CFU/mL). The Ag nanoparticle solution was added to the 1000-mL beaker (0.15 mL). Samples (1 mL) were collected into DE neutralizing broth (1 mL) at the following time points: 0, 60, and 90 minutes. A replicate experiment was conducted with 0.125 mL Ag solution.

The Ag nanoparticle solution was tested on the MS2 test water (1000 mL, 10⁶ CFU/mL). The Ag solution was added in volumes of 0, 0.125, 0.15, and 0.175 mL. Samples (1 mL) were collected and placed into DE broth (1 mL) after the following periods of time: 0, 60, 90, and 120 minutes.

A replicate experiment was conducted twice by addition of an Ag solution volume of 0.126 mL and time points of 0, 60, 120, 180, 240 minutes as well as 24 h.

The Ag solution was tested on the Rotavirus (100 mL, 10^5 PFU/mL). Ag solution was

added in volumes of 12.5 μ L and 15.0 μ L. Samples (3 mL) were collected into DE broth (1 mL) at the following time points: 0, 30, 60, 90, and 120 minutes. A replicate experiment was conducted.

RESULTS

Time (min)	Pan			250-mL beaker		
	Control (CFU/mL)	Treatment (CFU/mL)	% Reduction	Control (CFU/mL)	Treatment (CFU/mL)	% Reduction
0	6.3E+06	6.3E+06	0.00	8.20E+06	8.20E+06	0.00
		4.8E+06	23.81		8.10E+06	1.22
15	6.3E+06	2.3E+06	63.49	8.15E+06	4.95E+06	39.26
		1.5E+06	76.19		4.70E+06	42.33
30	6.3E+06	2.9E+02	>99.99	6.40E+06	4.45E+06	30.47
		2.1E+02	>99.99		3.80E+06	40.63
60	3.5E+06	1.7E+03	99.95	3.95E+06	1.32E+06	66.58
		9.0E+03	99.74		1.70E+06	56.96
180	4.6E+06	6.6E+02	99.99	N/A	N/A	N/A
		2.3E+02	>99.99		N/A	N/A
360	1.1E+07	1.0E+01	>99.99	N/A	N/A	N/A
		2.5E+03	99.98		N/A	N/A

Exposure Time (min)	Control	Fabric Based Capsule		Ceramic Spheres Based Capsule	
	CFU/mL	CFU/mL	% Reduction	CFU/mL	% Reduction
0	6.10E+06	6.20E+06	0.00	8.40E+06	0.00
15	6.00E+06	3.17E+06	47.17	3.40E+06	43.33
30	8.40E+06	6.70E+06	20.24	7.39E+06	12.02

	Control	Treated	% Reduction
	Pumice based spheres	5.69E+06	2.96E+06
5.60E+06		1.30E+06	76.79
Clay based spheres	6.50E+06	6.00E+06	7.69
	5.60E+06	4.40E+06	21.43

Ag (mL)	Time (min)	Control (CFU/mL)	Treatment (CFU/mL)	% Reduction
0.125	0	1.6E+07	1.6E+07	0.63
	60	1.7E+07	1.1E+04	99.94
	90	1.8E+07	1.6E+02	>99.99
	120	2.0E+07	<10	>99.99
0.15	0	1.5E+07	1.5E+07	0.67
	60	2.1E+07	40	>99.99
	90	2.0E+07	<10	>99.99

Ag (mL)	Time (min)	Control (PFU/mL)	Treatment (PFU/mL)	% Reduction
0.125	0	7.7E+05	7.7E+05	0.00
	60	3.2E+05	2.6E+04	91.88
	90	6.0E+05	2.8E+04	95.33
	120	6.8E+05	2.6E+04	96.18
0.15	0	7.7E+05	7.7E+05	0.00
	60	3.2E+05	3.1E+04	90.31
	90	6.0E+05	1.2E+02	99.98
	120	6.8E+05	< 10	>99.99
0.175	0	7.7E+05	7.7E+05	0.00
	60	3.2E+05	3.7E+03	98.84
	90	6.0E+05	1.1E+02	99.98
	120	6.8E+05	< 10	>99.99

Table 6: Ag Nanoparticles + Continuous Stirring: Inactivation of MS2 Bacteriophage

Time (min)	Control	Treatment: 0.125 mL Ag (CFU/mL)	% Reduction	Treatment: 0.15 mL Ag (CFU/mL)	% Reduction
0	3.50E+06	3.93E+06	0.00	3.50E+06	0.00
		3.95E+06	0.00	3.30E+06	5.71
60	4.60E+06	3.85E+06	16.30	4.80E+06	0.00
		5.11E+06	0.00	4.90E+06	0.00
120	5.70E+06	4.70E+06	17.54	4.90E+06	14.04
		4.77E+06	16.32	4.90E+06	14.04
180	5.50E+06	6.47E+06	0.00	N/A	N/A
		5.39E+06	2.00	N/A	N/A
240	5.30E+06	6.15E+06	0.00	N/A	N/A
		6.11E+06	0.00	N/A	N/A
300	3.50E+06	2.59E+06	26.00	N/A	N/A
		2.92E+06	16.57	N/A	N/A

Table 7: Ag Nanoparticles: Inactivation of Rotavirus

Ag (mL)	Time (min)	PFU/mL	% Reduction
0.125	0	1.50E+05	
	30	1.50E+05	0
	60	>1.50E+03	< 99
	90	>1.50E+03	< 99
	120	>1.50E+03	< 99
0.15	0	1.50E+05	
	30	1.50E+05	0
	60	>1.50E+03	< 99
	90	>1.50E+03	< 99
	120	>1.50E+03	< 99

DISCUSSION

The series of pilot tests showed positive antimicrobial effects for both the TiO₂-Ag-HAp Low Temperature Melt Injected film and the colloidal silver suspension; however, investigations are still in the utmost preliminary stages, and substantial additional research is necessary to further document the antimicrobial material. The current data represents an iterative series of explorations in treatment parameters, which are still in the exploratory phase and have not been optimized.

The TiO₂-Ag-HAp film coated fabric and ceramic beads caused reduction in microbial numbers. They were most effective when placed static in the bottom of the pan where maximum exposure to light could occur. A 99.9% reduction (Table 1) of the bacteria occurred within 30 minutes under these conditions. Beads packed in a capsule (Table 2) or column (Table 3) were not as effective. This is likely due to the limited penetration of light to the inner regions of these designs. Contact time between the antimicrobial material and the target microorganisms was also reduced.

The adsorbent hydroxyapatite particulates bind microorganisms until they can be electrochemically mineralized. It is possible that the microbial load was too great for complete adsorption. Additional studies could be conducted utilizing lower initial microbial concentrations to determine the maximum capacity of the system and the amount of time necessary for the system to completely mineralize contaminants and to be re-generated. The effectiveness of the treatment column could also be evaluated

for its ability to degrade organic chemicals of concern such as pharmaceuticals and emerging contaminants in water systems.

A slurry of the TiO₂-Ag-HAp materials would be the most effective since the treatment reaction is surface area dependent. The material design of ceramic spheres generates a stable base for retention and management of the nanoparticle catalytic material, yet may have overly reduced the available active surface area. The film-coated fabric filter application can be used to produce a denser filtration column with more exposed TiO₂-Ag-HAp per volume, but is a less stable base that will degrade over time. The TiO₂-Ag-HAp coated materials in their current formulation are too ineffective for a water treatment system on their own. Fundamental advancements are needed at the nanoparticle level. The formulation of materials used to produce the Low Temperature Melt injected film might be altered, or other materials might be considered. The TiO₂ composite might be more valuable for synergistic effects with other technologies employed in water treatment. For instance, it might be combined with granulated active carbon systems. The carbon would enhance organics adsorption capacity and allow more time for the titanium dioxide to react, while the incorporation of titanium dioxide would add a mineralizing effect to the system that typically only retains organics.

The colloidal silver solution treatment was more positive. In the tests against *K. terrigena* (Table 4), a 99.999% reduction was obtained within 60 minutes with 0.15 mL (3 drops) Ag⁺ and within 90 minutes

with addition of 0.125 mL (2 drops), while a 99.9% reduction was obtained within 60 minutes with 0.125 mL (2 drops). Addition of 0.15 mL (3 drops) and 0.175 (4 drops) each caused an ~99.99% reduction within 90 minutes. In the tests against MS2 coliphage (Table 5), a 99.999% reduction was observed within 120 min with ≥ 0.15 mL Ag, and a 99.99% reduction was observed within 90 min with ≥ 0.15 mL Ag. A $> 90\%$ reduction was observed within 60 min with ≥ 0.125 mL Ag.

However, despite these positive results, replicate experiments with the silver solution to evaluate a longer time range (Table 6) and effectiveness against Rotavirus (Table 7) showed no observable phage or virus inactivation. These tests were conducted eight months after the initial experiments and conditions in these trials involved continuous stirring with a magnetic stir bar to maintain a homogenous solution. The initial tests did not include continuous stirring, and although silver is diamagnetic, it is possible that another component in the colloidal solution was affected by the magnetic field. Further investigations are necessary. Silver and nanoparticle solutions may also age, and fall out of solution as the particles aggregate. This also may have occurred, causing the reduced effectiveness and discrepancy in results.

More test are necessary to evaluate whether the magnetic field applied for stirring affected the antimicrobial properties of the silver colloidal solution or to determine whether the solution has a limited life span. If a magnetic field is confirmed to affect the

antimicrobial properties of the silver solution, it would be necessary to further quantify and evaluate the sensitivity of the solution. For instance, it should be tested whether antimicrobial properties can be restored after exposure to strong magnets, or if exposure permanently dampens anti-microbial properties. This second scenario would be of concern since the product may be deactivated and unwittingly still used for treatment.

Overall, most experimental designs evaluated were found to be inherently flawed, so they were not extensively replicated to achieve statistical significance. General conclusions have been deduced, but inconsistent results, substantial variation, and poor replication limit the depth of interpretations. The $\text{TiO}_2\text{-Ag-HAP}$ formulation was only found to be effective when tested in a wide, shallow pan to allow maximum light penetration. Such a system would not be practical in water treatment since the infrastructure cost would be too high and the water capacity too low. The Ag colloidal suspension was very effective in initial tests, but not in subsequent tests and further investigation must be conducted to determine the cause of the discrepancy.

Nevertheless, the proven anti-microbial properties of both materials show promise for future advancements and integrations into water treatment technologies, and for incorporation into synergistic applications.

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