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Three-Dimensional Coating of Porous Activated Carbons with Silver Nanoparticles and its Scale-up Design for Plant Disease Management in Greenhouses

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Abstract

Greenhouse vegetable production is significantly impacted by pathogens that cause diseases to the roots of the plants. These diseases are increasingly problematic in hydroponic vegetable production. Standard commercial practices in modern vegetable production facilities reuse the nutrient solution to reduce costs, or use dugout water for greenhouse irrigation in rural areas. This practice of recycling water may introduce or spread pathogens. Once pathogens contaminate water systems, they can spread quickly and cause dramatic losses to yield. Water filters have been used, but do not effectively kill fungi and bacteria. Therefore, better water treatment solutions are urgently needed to manage plant disease, especially for hydroponically grown vegetables. Numerous studies have demonstrated the efficacy of silver ion (Ag^+) and silver-based compounds for disinfection of a wide range of harmful microorganisms. In this article, we present a new filter material based on three-dimensional (3D) silver nanoparticle (AgNP)-coated substrates for water treatment. We prepared AgNP-coated active carbon materials and tested their antimicrobial efficacy against phytopathogenic bacterial and fungal spores, such as *Pseudomonas sp.*, and *Fusarium sp*. We then conducted large-scale tests in a dynamic flow setting and evaluated the effect of the filter on Pythium root rot control of hydroponically grown cucumbers. Results indicated that killing efficiencies of 3D coating were greater than 95% in the laboratory and that cucumber plants had no root infection in AgNP-AC filter treatment. The developed technique is approved to be a very efficient approach and has a great potential to be used in the greenhouse to manage plant root diseases.

Keywords: Water filtration; Plant disease management; Greenhouse vegetable production; Silver nanoparticle; Activated carbons; Greenhouse pathogens; Antibacterial filters; Hydroponic systems

Introduction

Root diseases are increasingly problematic in hydroponic vegetable production, because pathogens can quickly spread and cause losses to yield of agricultural products once they enter water systems. Highly destructive plant diseases, such as *Phytophthora* late blight, have become substantial risks to greenhouse tomatoes because of their presence in farm fields and home gardens. Pathogens are often introduced from dugout water used for greenhouse irrigation. The standard practice in modern vegetable production uses recycled nutrient solutions that further increase risk by spreading disease from infected plants throughout the entire greenhouse. Hong and Moorman [1] pointed out the challenges and opportunities of plant pathogens in irrigation water. Stewart-Wade [2] reported the detection and management of plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses. Various techniques have been applied to treat recycled nutrient solutions in greenhouses, including heat treatment, ozone treatment, UV disinfection, H_2O_2 treatment, and biofilters [1,2]. Biofilters are used in greenhouses to treat recycled nutrient solutions, but their main purpose is to convert ammonia to nitrogen gas and remove carbon dioxide and various organic contaminants, with less in-built design mechanisms for removing pathogens [3,4]. Better water treatment solutions are needed for plant disease management in the domain of hydroponically grown vegetables.

Silver nanoparticles (AgNP) range in size between 1 nm and 100 nm in diameter. Silver ions (Ag+) have been studied for many years for the disinfection of various harmful microorganisms because of their multiple modes of inhibition. Safavi et al. [5] reported that nano-silver could remove bacterial contaminants in plant tissue media. Dibrov et al. [6] showed that low concentrations of Ag^+ induce a massive proton leakage and result in a high degree of *Vibrio cholerae* death. Mazurak et al. [7] illustrated how silver-coated dressings can help with healing skin wounds. Maiti et al. [8] and Zahir et al. [9] demonstrated that AgNP can be synthesized by using eco-friendly reducing agents: red tomato juice and *Euphorbia prostrate* leaves, respectively. Tuan et al. [10] presented a modified sono-electrodeposition technique for making non-toxic nanosilver colloids. Karumuri et al. [11] discussed the coating of AgNP onto hierarchical structures fabricated by grafting carbon nanotubes to increase specific surface area. Richter et al. [12] explained the design of lignin nanoparticles infused with silver ions and coated with a cationic polyelectrolyte layer, which is a biodegradable and environmentally friendly alternative to silver nanoparticles.

Recently, several promising reports on using silver nanoparticles (AgNPs) against plant pathogenic fungi have been published. Jo et al. [13] and Kim et al. [14] showed various forms of silver ions and nanoparticles in killing two plant-pathogenic fungi: *Bipolaris sorokiniana* and *Magnaporthe grisea*, *Raffaelea* sp., and the other eight plant pathogenic fungi [15], respectively. Lamsal et al. [16] demonstrated in field tests, silver nanoparticles of 100 ppm reach the

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highest inhibition rate before and after disease outbreaks on cucumbers and pumpkins. Nasrollahi et al. [17] examined the effect of AgNP on killing fungi (*Candida albicans*), and yeasts (*Saccharomyces cerevisiae).* To prevent the leakage of AgNP, Karumuri et al. [18] and Tuan et al. [10] proposed to coat AgNP on porous carbon structures. A review article on the antimicrobial action, synthesis, medical applications, and toxicity effects of AgNPs was published in 2012 [19]. In addition to AgNPs, Wani et al. [20] showed that zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles also exhibit antifungal effects. The mechanisms and potential application of synthesized AgNPs as alternatives to pesticides have been reviewed [21,22], but there have been few attempts to leverage AgNP technology for practical use.

In this article, we present the procedure to develop a new water filter material using an AgNP-coated substrate. Additionally, we show the results of efficacy evaluation of the treated filter substrate *in vitro* and in a growth chamber trial. The substrate was characterized using state-of-the-art equipment (i.e. Transmission Electron Microscopy, TEM). We subsequently evaluated the *in-vitro* antifungal effect of the designed filter against common bacterial and fungal pathogens. Since there are many plant pathogens in irrigation water [1], which can cause diseases in various greenhouse vegetables, it is almost impossible for us to test all pathogens. In this study, we chose bacteria *Pseudomonas* sp., *Paenibacillus* sp. and a fungus *Fusarium oxysporum* as examples to show the antimicrobial effectiveness of our designed bio-filtration material.

Materials and Methods

Sample preparation and characterization

Activated carbon (AC) has been successfully used for water purification over the past decades as an inexpensive and efficient filter material. We have combined the antibacterial properties of silver with the absorption properties and large surface area of activated carbon for water purification to kill bacteria and fungi that infect greenhouse crops. Considering the specific characteristics of AC, it was employed as the filter material of choice to be coated with silver nanoparticles. Two types of AC materials were tested - larger granules of AquaSorb® 1500 (Jacobi) from Bituminous coal, water washed (size 0.6 - 2.36 mm); and smaller diameter AC - CR1230C-AW (Carbon Resources manufacturer), from coconut shell, acid washed (size 0.6-1.7 mm).

We evaluated two types of coating methods, single coating and double coating, in order to determine which has better killing effects. Sample preparation was done according to the following protocols:

Single coating: A range of AgNP concentrations were tested to determine the optimum concentration. $AgNO_3$ solutions (3 mL) of different concentrations (3, 8, 11, 17, 20, 23 and 33 g L^{-1}) was mixed with 2 g of activated carbon, gently shaken and left overnight to penetrate into pores and undergo absorption by the carbon particles. The remains of the solution were then removed, and 10% HCl was used in an analytical reaction for Ag+ detection to prove that all silver ions were absorbed and none were left in the solution. Three millilitres of $NaBH_4$ solution (5 g L^{-1}) was added into the above AC to initiate a reduction reaction [23], and shaken evenly. Samples were then washed using deionized (DI) water.

Double coating: A single coated sample was used as the precursor. Two grams (wet weight) of the previously coated sample was mixed with 1.6 mL of $AgNO_3$ solution (10.8 g L^{-1} concentration). Samples were shaken gently and left for an hour at room temperature. Samples were then washed using DI water. A total of 1.6 mL $NaBH_4$ (6 g L^{-1}) solution was quickly added to the sample and shaken until bubbles disappeared. Samples were then washed three times with DI water and treated for removal of any remaining liquid.

Three types of sample-drying treatments were evaluated: (a) removal of the liquid using a pipette; (b) freeze-drying under vacuum; and (c) drying in the oven (60°C) for 2-4 hours. AC mixed with DI water without $AgNO_3$ was used as a control.

The coated materials were characterized using standard methods, including Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and X-ray diffraction analyses.

SEM: AgNP treated activated carbon particles were mounted onto the stubs and air-dried at room temperature in the dark, then coated with evaporative carbon in a Leica EM SCD005. Samples were observed with a Field Emission scanning electron microscope (JEOL 6301F).

TEM: Samples of AC pre-treated with AgNP were air-dried and embedded in plastic. Thin cuts (80-100 nm) were made using a glass knife to extract sample slices from this substrate. TEM images were taken using a Philips-FEI Morgagni 268 instrument, operated at 80 kV.

X-Ray: Diffraction analysis was performed on the Rigaku Ultima IV Powder X-Ray diffractometer. Samples were air-dried at room temperature and prepared in the same way as for SEM imaging.

ICP-MS: Detection of silver (Ag^+) ions in the filtrates was performed using ICP-Mass Spectroscopy. Samples (0.5 g) were mixed with 2 mL of DI water and shaken overnight. Subsequently, the solution was filtered through a 0.22 μm millipore filter to remove remnants of carbon and then tested for Ag^+.

Evaluation of the developed filter materials

Common fungal pathogens, *Botrytis* sp., *Fusarium* spp., *Pythium* spp., *Rhizoctonia* sp., and *Sclerotinia* sp. as well as bacteria (*Pseudomonas* sp.) were collected from greenhouse cucumbers, lettuce, tomatoes, and peppers. Infected plant tissue was surface disinfected in 0.5% bleach, rinsed in sterile distilled water and placed on potato dextrose agar (PDA). Fungal/bacterial isolates were purified by single spore/single colony culture techniques and stored at 4°C.

Screening test

Preliminary efficacy of AgNP-treated AC samples was tested against yeast cell culture (*Saccharomyces cerevisiae*) at the lab scale to determine the best drying condition and optimum AgNP concentration for different treatment durations as well as for selection of AC material. One colony of two-day-old *S. cerevisiae* culture was transferred from yeast-extract peptone-dextrose (YPD) agar plate to 100 mL of YPD broth and incubated in a shaker at 180 rpm at 30°C for 24 hours. One mL of diluted (OD_{600} = 0.1) overnight-grown yeast culture was added to a 20 mL vial containing 0.5g of AgNPs-AC (wet weight). The mixture was shaken for 3 to 24 hours, and then 100 μL of the culture was plated on YPD agar plates, duplicated and incubated at 30°C for 24 hours and the colonies per plate were counted and recorded.

AgNP-AC against pathogenic bacteria and fungi (rotation test)

Activated carbon treated with silver nanoparticles (20 g L^{-1} or 23 g L^{-1}) were evaluated for their effect against a bacterium *Pseudomonas* sp., and a fungus *Fusarium oxysporum* followed the method described by Karumuri et al. [18]. 0.5 g of each sample was added to 5 mL spore suspension (approximately 3×10^6 spores mL^{-1}) in a test tube. AC treated with water was included as a control. Tubes were incubated on a shaker at 200 rpm at room temperature. A drop (10 μL) of the culture mix was

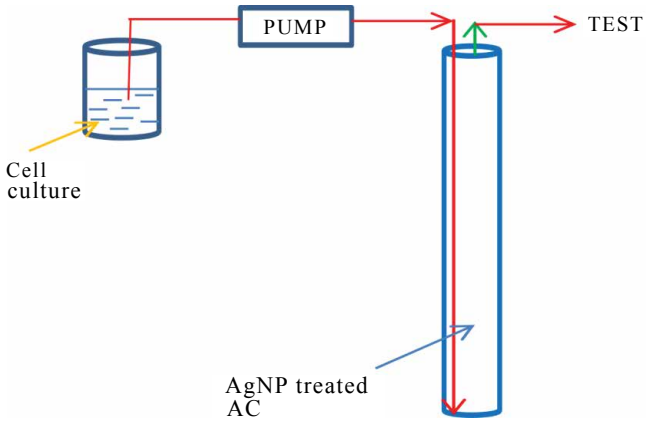

Figure 1: Schematic drawing of a filtration system used for dynamic filtration tests of the AgNP treated AC material. *Paenibacillus* spore suspension was pumped at a flow rate of 70-75 mL h^{-1} to the bottom of the 100 mL column filled with the developed material and the filtrated water samples were collected on the top of the column for plating on agar and further CFU count.

Figure 2: A large scale-up filtration set up, we used a commercial filtration cartridge that was filled with AgNPs-coated AC and then put into the housing unit. A cartridge filled with uncoated AC was used as a control.

plated to a PDA plate after growing for 1 h and 24 h, respectively, with four plates per treatment. Plates were then incubated for 3 days at 25°C in an incubator and the colonies per plate were counted and recorded to calculate the killing efficiency of the filter materials. The percent reduction of colony forming units (CFU) efficiency was calculated based on the CFU counts of bacteria on control plate:

*Efficiency (%) = (CFU of Control – CFU of Treatment)/CFU of Control * 100* (1)

AgNP-AC against bacteria (small-scale filtration test)

Smaller size carbon (CR1230C-AW) had larger cumulative surface area and showed better results on small samples in the rotation test, thus we used it for filtration tests. Sample preparation was performed using the same protocol as that at a rotation test. Dynamic-flow filtration testing was performed using bacterium *Paenibacillus polymyxa* that is morphologically different from other bacteria, with untreated AC as the control. A cylindrical column (100 mL) was filled with active carbon (CR1230C-AW) pre-treated with a single or double coating of AgNP. Scale-up tests were performed with the following system for dynamic tests (Figure 1). Bacterial (*Paenibacillus*) suspension with a concentration of about 10^5 CFU/mL was filtered through the column at a flow rate of 70-75 mL h^{-1}. The test was run non-continuously for 6 days for a total of 6 hours per day. Filtrates were collected hourly for the first 3 days, then every 3 hours on day 4, then every 6 hours on day 5 and day 6. Collected samples were plated on PDA plates and total CFU per plate was counted after incubation for two days. This test was repeated three times. The percent of antibacterial efficiency was calculated based on the original bacterial concentration:

*Efficiency (%) = (1 - bacterial concentration in filtrate/original bacterial concentration)*100* (2)

AgNP-AC against bacteria using a commercial filtration cartridge

In the final scale-up filtration test, we used a commercial filtration cartridge and filled it with our developed AgNP-AC material. Based on the result of dynamic testing for 100 mL, we chose double-coated material for commercial sized scale up testing. A total of 1.7 L AC (CR1230C-AW) was double-coated with AgNPs, and then was filled in a commercial RFF-Series Refillable Cartridge (200701, 2.5 × 20 inch, AXEON Water Technologies) that was then inserted into a Slim Line Series Filter Housing (207296, AXEON Water Technologies). Bacterial (*Paenibacillus*) suspension with a concentration about 10^4 CFU mL^{-1} was filtered through the filter device. A pump (Mandel Watson 505U) was set at 2 rpm and a flow rate at about 800 mL per hour (Figure 2). Filtrate samples were collected hourly and plated on PDA plates, which were incubated at 25°C in the dark. CFU was counted 2 days after plating. A cartridge filled with uncoated AC (CR1230C-AW) was used as a control and tested using the same procedure. The percent of antibacterial efficiency was calculated based on the original bacterial concentration using the formula (2) described above.

Growth chamber trial

Preparation of filter: In the growth chamber test, we used the commercial filtration cartridge and filled it with the freshly prepared AgNP-double-coated AC material. A total of 1.7 L AC (CR1230C-AW) was double-coated with AgNPs, and then was filled in a commercial RFF-Series Refillable Cartridge (200701, 2.5 × 20 inch, AXEON Water Technologies) that was then inserted into a Slim Line Series Filter Housing (207296, AXEON Water Technologies). A filter filled with untreated AC was used as a control.

Preparation of cucumber seedlings: Cucumber seeds ('Marketmore') were seeded to 1-inch rockwool cubes and incubated at 25°C 12 h/12 h light/dark in a growth chamber for 7 days. Seedlings were transplanted to 4-inch rockwool blocks one week after seeding, watered with nutrient solution and incubated at the same condition for 10 days.

Preparation of *Pythium* inoculum: *Pythium ultimum* was cultured on potato dextrose agar for 5 days. The culture agar was cut into strips that were transferred to clean plates containing autoclaved distilled water. Plates were incubated at 25°C in the dark for 7-10 days until numerous sporangia were formed. The agar stripes were then blended to fine pieces and used as an inoculum to the nutrient solution.

Application of treatments: Cucumber seedlings (2-week-old) were transplanted to coconut coir slabs in a growth chamber programed at 24°C/20°C, 16h/8h light/darkness. Plants were irrigated with nutrient solution (NS) of following treatments (1) NS contaminated with *Pythium* spores/mycelia, filtered through the AgNPs treated AC filter, 10 plants; (2) NS contaminated with *Pythium* spores/mycelia, filtered through the AC filter without AgNPs treatment, 10 plants. Two extra controls were also included (1) NS contaminated with *Pythium* spores/mycelia, no filter, positive control, five plants and (2) clean NS, no filter,

negative control, five plants. There were five plants per slab per trough. The feeding system was set up to 500 mL water per plant per day at the first week, and then increased to 1 L per plant per day.

Data collection: Plant growth was observed weekly. Biomass and root rot disease were recorded at the end of the experiment (4 weeks after transplanting into the chamber).

Results

Pre-screening AgNP killing efficacy

Since we are developing a technology to treat greenhouse water, the common greenhouse microorganisms should all be employed in testing. In this study, we chose a range of different microorganisms (yeast, fungi and bacterium) to show the broad spectrum of applicability of AgNPs in killing any type of living species which threaten greenhouse vegetable production. For simplicity, we tested yeast (*Saccharomyces cerevisiae*) to obtain preliminary data used to optimize the synthesis.

In our studies, evaluating multiple concentrations of AgNP efficacy against yeast *S. cerevisiae* increased with silver concentration from 3 g L^{-1} to 23 g L^{-1} (data not shown here), and subsequently decreased when concentration was raised further. Evidently, there is an optimal ratio of AgNPs that can be coated on a given amount of activated carbon particles due to constraints on the limited surface area of activated carbon that AgNPs can be deposited on. Beyond this critical density, additional AgNPs can easily fall off or are easily removed. Based on our experimental results, efficiency sharply declines when the concentration of AgNPs reaches 33 g L^{-1}. Optimal efficacy was reached at concentration of 20-23 g L^{-1}, thus chose as the range over which to test against other pathogens (*Pseudomonas* and *Fusarium*).

Determining treatment duration

Time (treatment duration) was another important factor impacting antimicrobial efficacy of filter material. While 20 to 24 hours of treatment yielded excellent results, shorter times did not (data not shown here). Therefore, we tested another type of AC material with smaller particle size and larger surface area to address this problem. Another approach to increase exposure to silver was by a second coating the material. Results showed that smaller size carbon had better cell killing efficacy. Whereas larger sized AC required 20 to 24 hours of treatment, 3 hours was enough when using smaller AC granules. The larger surface area of the carbon with smaller particle size can easily explain this phenomenon. Second coating also improved results with the larger sized AC, but smaller AC-AgNP still showed better results.

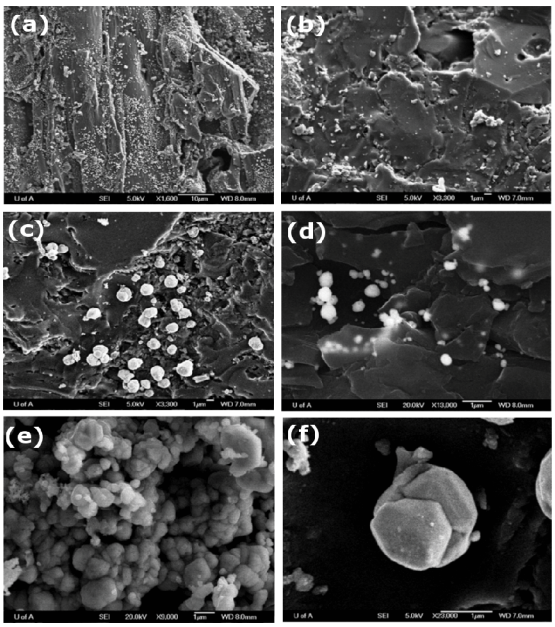

Figure 3: Scanning Electron Micrographs (SEM) of single-coated samples: (a) AgNP (20 g L^{-1})-treated activated carbon (AC); (b) untreated AC; and (c) – (f) AgNPs (23 g L^{-1}) treated AC. AgNPs are shown with red arrows.

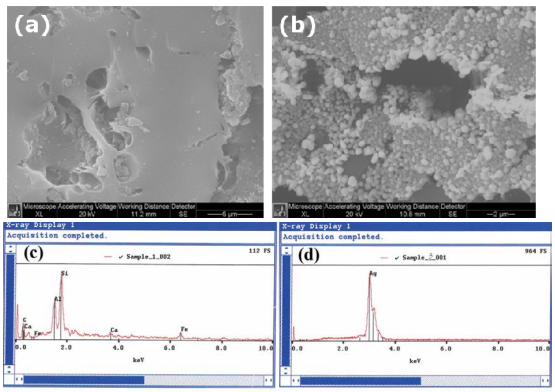

Figure 4: Scanning Electron Micrographs (SEM) of double-coated sample of CR1230C-AW carbon (20 g L^{-1}) (a) control, (b) AgNPs on the surface of AC and inside the pore. AgNPs are shown with red arrows. X-ray analysis showing the major elements in the samples: (c) Untreated AC had high levels of silicon, aluminum and some carbon, calcium and iron; (d) AgNP treated AC had high level of silver.

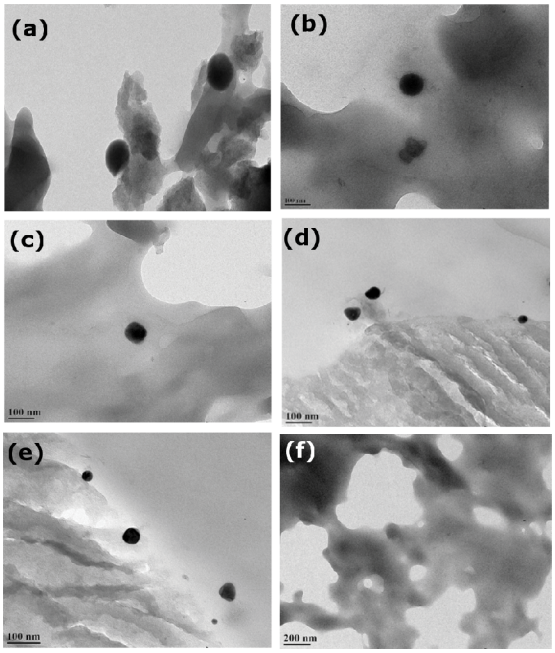

Figure 5: Transmission Electron Microscopy images: AgNP (20 g L^{-1}) treated activated carbon (AC) sample **(**a)-(b) bigger sized Aquasorb carbon with single coating, and (c)-(e) smaller sized CR1230C-AW with double coating, (f) control (No nanoparticles coating). Pictures show AgNPs on the surface (a), (d), (e) and embedded in the structure of AC (b) and (c).

Characterizing AgNP coated AC

Scanning Electron Micrographs (SEM) showed that the distribution of AgNPs was quite uniform on the surface and inside pores of activated carbon (Figures 3a and 4b) while there were no AgNPs found on the

untreated AC (Figures 3b, 4a). AgNPs of different sizes (Figure 3c) can be found inside the porous area (Figure 3d) or can aggregate (Figure 3e) to form larger particles (Figure 3f). The micrograph samples with double and single coating exhibit similar appearance (Figures 3 and 4). There was no obvious difference between samples of smaller and larger sized AC (we used the same concentration of silver to coat them in both cases). X-ray diffraction analysis confirmed the presence of AgNPs on the surface of treated active carbon (Figure 4d) and no AgNPs were found on untreated AC (Figure 4c).

Transmission Electron Microscopy (TEM) images showed that while the carbon structure was destroyed during the process of cutting in preparation for TEM, AgNPs were still present on both the surface and in the pores of the carbon (Figure 5). There was no visible difference between samples of single-coated and double-coated AC-AgNPs. Though some AgNPs fall off during the cutting, from Figure 5d we can see how AgNPs remain attached to the surface of AC on the edge of AC and plastic. These particles were within the range of 20-100 nm, generally round in shape, and could be distinguished on the micrographs by possessing a much higher density compared to that of carbon.

Analytes	Ag (ppm)
Detection Limits (DL)	0.00001
Control	<DL
SD	0.0273
SS	0.00354

Table 1: ICP-MS testing results of silver in filtrate.

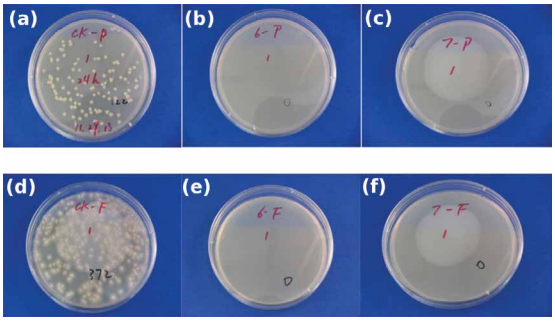

Figure 6: *Pseudomonas* sp. treated with AgNP-coated AC for 24 hours on a shaker at 200 rpm at 25°C, 3 days after plating. (a) Control; (b), (c) filtered with 20 g L^{-1} and 23 g L^{-1} treated AgNP-AC, respectively. *Fusarium oxysporum* treated using AgNP-coated AC for 24 hours on a shaker at 200 rpm at 25°C, three days after plating. (d) Control; (e), (f) filtered water with 20 g L^{-1} and 23 g L^{-1} treated AgNP-AC, respectively.

Microbe	Treatment	Mean CFU per Plate (n = 4)	
		1 hour	24 hours
Pseudomonas	20 g L^{-1}	0.0	0.0
	23 g L^{-1}	0.0	0.0
	0 g (CK)	102.3	95.0
Fusarium	20 g L^{-1}	12.5	0.5
	23 g L^{-1}	66.8	0.0
	0 g (CK)	500.0	472.8

Table 2: Bioassay of AgNPs-coated activated carbon against *Pseudomonas* and *Fusarium* treated for one or 24 hours, and also counted three days after plating and incubating at 25°C.

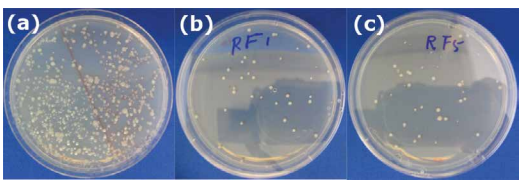

Figure 7: Agar plates two days after plating with the filtrated water collected from the dynamic scale-up test. (a) Control, original bacterial suspension; (b), (c) filtrates collected after one hour and 5 hours of filtration through AgNP-AC material, respectively.

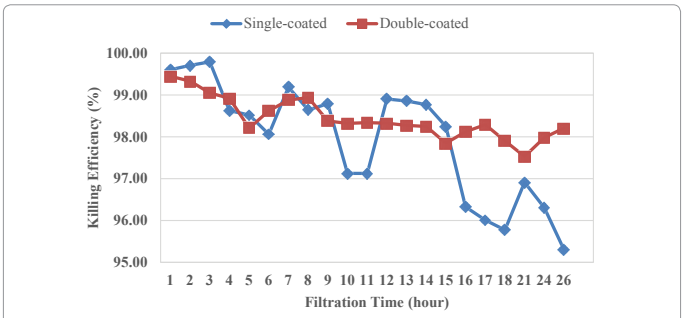

Figure 8: Efficiency test using the scale-up (100 mL) dynamic flow system to treat *Paenibacillus* spore suspension with single-coated vs. double-coated material.

Testing filter materials against plant pathogens (Rotation test)

Our main objective was to develop a material, which is effective in killing major plant pathogens in greenhouse water. After the preliminary screening studies (for killing *S. cerevisiae* yeast cells), tests against a bacterium *Pseudomonas* sp. and a fungus *Fusarium* sp. collected from greenhouses were performed. Both (20 g L^{-1} and 23 g L^{-1}) AgNP-coated ACs completely inhibited the growth of *Pseudomonas* after one-hour treatment while the ACs significantly reduced the CFU counts after one hour treatment and almost completely killed the *Fusarium* spores after 24 hours of treatment (Table 1 and Figure 6).

Our testing results showed that the developed AgNP-AC material was very successful in killing living cells of pathogenic bacterial and fungal species in the small-scale setting of the lab. The next step was to test the developed material in dynamic flow with the expectation to implement the design in greenhouses (Table 2).

Testing in a dynamic scale-up setting (Filtration test)

Because smaller size carbon (CR1230C-AW) had larger surface area and showed better results on the small scale, we selected it for scale-up tests. Sample preparation was performed using the same protocol as for the smaller-scale tests. Dynamic testing was performed using a bacterium *Paenibacillus* as a model and untreated AC as a control. The results showed up to 90% killing efficiency (Figures 7 and 8).

Testing using commercial filtration unit

In the scale-up filtration test, we used a commercial filtration cartridge and filled it with our developed AgNP-AC material. Results showed that more than 99% of initial bacterial population were killed in the first hour and continued for 11 hours with the AgNP-coated AC substrate. The efficiency remained at 97% after 30 hours of filtration (Figure 9a). In the control (filter with AC substrate without AgNP coating), it was observed that bacteria also reduced by more than 90% in the beginning, but the efficiency rapidly dropped to about 30% after 10 hours filtration (Figure 9b) because the bacteria gradually obstructed the porous carbon. Consequently, the AC only filter has a very short life span. Results demonstrated that the addition of AgNPs to the filter substrate can significantly increase the filter life span and antimicrobial efficiency by killing bacteria which have adhered onto the filter surfaces. Again, no Ag+ was detected in the filtrated water.

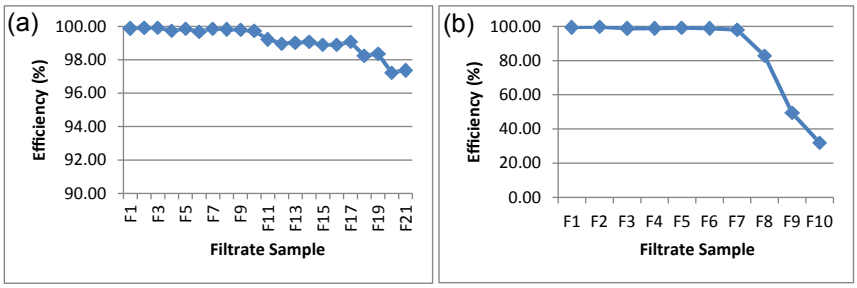

Figure 9: Summary of the scale-up test using commercial cartridge filled with 1.7 L AgNP double-coated AC material against a bacterium Paenibacillus. (a) Filtrate samples were collected hourly for the first 17 hours, and then collected every 3 hours between 18 to 21 hours; (b) Control: Filter cartridge filled with uncoated AC, filtrate samples were collected hourly for the first 10 hours. Filtrates were plated on PDA and incubated at 25°C for two days.

Figure 10: Six-week-old plants watered with *Pythium*-contaminated nutrient solution (NS) filtered through AgNP-AC filter (Treat) and watered with *Pythium*-contaminated NS filtered through AC without AgNP coating filter (CK) in the growth chamber trial (a); cucumber crown root showed discoloration watered with *Pythium* contaminated NS filtered with AC without AgNPs treatment (b) and healthy cucumber roots watered with *Pythium* contaminated NS filtered with AC with AgNPs treatment (c).

Treatment	Root Discoloration Rating	Mean Fresh Shoot Weight (g)
AgNP-AC Filter, Pythium Contaminated NS	0.0 a*	139.6 a
AC Filter, Pythium Contaminated NS	0.3 a	139.1 a
No Filter, Pythium Contaminated NS	1.0 a	127.8 a
No Filter, No Pythium clean NS	0.0 a	142.2 a
*Values followed by the same letter are not significantly different by LSD test (P=0.05).		

Table 3: Root infection rating and fresh biomass of cucumber plants in the growth chamber trial (n=10).

Growth chamber trial

Cucumber crown root showed discoloration four weeks after the plants were watered with *Pythium* contaminated NS without filtration while slight discoloration was observed on plants filtered with AC without AgNP treatment. There was no root infection observed on plants watered with AgNP-AC treated NS and control plants (Figure 10). This growth chamber trial demonstrated that the filter treatment reduced the Pythium root disease on cucumbers although the difference on root infection was mild between treatments. Plants of positive control had higher fresh shoot biomass but the difference was not significant between treatments (Table 3).

Discussion

Nanotechnology is an emerging field of science and has been applied in various medical procedures including imaging, diagnostic, therapeutics, drug delivery and tissue engineering [24,25]. It also has great potential in controlled environmental agriculture (CEA), an advanced and intensive form of hydroponically based agriculture [26]. Because AgNPs have strong antimicrobial/anti-fungal properties, they have attracted great research attention in agricultural applications, such as the reduction of root diseases and the improvement of the growth and health of various plants [27]. It can also be used to clean ground water. Argonide Corporation based out of USA, used 2 nm diameter aluminum oxide nanofibres (NanoCeram) as a water purifier that can remove viruses, bacteria and protozoan cysts from water (http://nanotechweb.org/articles/news/3/4/7). BASF is one of the largest chemical producers in the world and has devoted a significant proportion of its nanotechnology research fund to water purification techniques. Many countries in Europe, Asia and America have worked on the development of nanoscale materials for water purification [26]. Nanotechnology has been used in municipal and rural water treatment to remove bacteria. Nanosilver-coated polypropylene water filters have been designed and evaluated to treat *Escherichia coli* contaminated drinking water [28]. AgNP-coated materials were investigated for the removal of bacteria from groundwater [29]. Potentially, these nanomaterials can be applied to hydroponic production to control plant diseases. Furthermore, they can be applied to treat dugout water for plant irrigation in greenhouses in rural areas.

Our results showed that, although AC coated with AgNPs is effective in killing microorganisms, the sample preparation methods had a distinct impact. We tested three drying methods in the sample preparation and tested the samples against *S. cerevisiae* cell culture. Results showed that all three drying methods exhibited some killing potential for AgNP-treated AC samples to kill the yeast cells. However, samples using vacuum or oven showed greatly reduced efficacy compared to liquid pipetting. The reason is that coated nanoparticles fell off from active carbon substrates during the process of vacuum or oven drying. Furthermore, oxidation can occur using the oven-drying method [30]. Therefore, we chose the pipetting method because the other two methods are much harsher on coated nanoparticles, producing unviable samples.

High anti-bacterial efficiency of the developed material suggested that it might have broader applications including drinking water filtration. However, the impact of AgNPs on humans is still unclear. Therefore, we designed another experiment to detect whether AgNPs were present in the eluent after filtration. We used an ICP-MS detection method for checking, and silver ions were not detected in the control or the sample (data not shown here).

Based on our small-scale tests using *S. cerevisiae*, *Pseudomonas* and *Fusarium*, 99% killing efficiency was observed for large size carbon and 99% to 100% killing efficiency for small size carbon during the first

24 hours of treatments using both single-coating and double-coating method. The bacterium is more sensitive to Ag^+ than the fungus. One possible reason may be due to bacteria being single-celled while *Fusarium* has multi-celled spores and mycelia. Results for the scale-up dynamic flow test using *Paenibacillus* spore suspension showed that in both cases (either for single-coated or for double-coated filtration material), over 95% of bacterial cells and more than 80% of bacterial cells were killed after the system ran for 29 hours and 40 hours, respectively. The antimicrobial efficiencies decreased over the period of testing (Figure 9). However, the flow rate did not change significantly during the whole filtration process (data not shown here). Killing efficiency of the designed filter has been demonstrated in the growth chamber test. Cucumber plants watered with AgNP-AC treated nutrient solution had no Pythium root rot while plants watered without AgNP-AC filter treated NS showed root discoloration in four weeks testing period.

With respect to the environmental consequences of AgNP, so far no universal conclusion has been drawn about the toxicity of AgNP [31]. The only adverse effect in humans of chronic exposure to silver is argyria or argyrosis of the skin and/or the eyes [32,33]. Many studies show that only the release of ionic silver has been found to be toxic, but the release alone cannot be accounted for the toxic side effects. In the aqueous condition, the ionic silver tends to form silver chloride and silver sulphide, which are highly stable. According to the World Health Organization (WHO), the toxicity of trace amount of silver (0.2 - 0.3 μg liter-1) in drinking water is normally negligible. A recent publication [34] described that available data is inadequate for drafting a health-based guideline about the impact of AgNP in the drinking water.

Overall, our study showed that AgNP-coated AC material effectively killed pathogen cells during the filtration. Although AC with single-coated or double-coated AC had similar bacterial killing efficiency, double-coated AgNP-AC has a longer lifespan than single-coated AgNP-AC materials. In the scale-up tests, we loaded the 3D coated filtration materials into empty commercial filter cartridges and maintained the constant water flow. We observed up to 95% to 97% of bacterial cells were killed even after 29 hours of filtration. We believe that commercial filters for water treatment can be designed to fit the requirements of each greenhouse based on their water usage and contamination level.

Conclusion

Silver nanoparticles have previously shown great potential for killing many pathogenic microorganisms. In this study we have combined its disinfecting properties with an efficient, inexpensive, commonly used filter material - activated carbon. We developed a new filter material to address the problem of pathogen contamination of water in greenhouses. Our method of depositing silver nanoparticles on active carbon particles allowed for a three-dimensional coating of the activated carbon with nanoparticles covering both inside and outside surfaces of the pores, offering better antimicrobial efficiency during water filtration. The preparation of the material is easy and our tests showed that pathogen killing efficiency could reach as high as 90% to 99%. We have also tested the material in both a lab-scale setting and a scale-up dynamic setting for 100 mL and in the commercial large size cartridge. In our tests, we used four different types of microorganisms; three of them are typical pathogens in greenhouses: *Pseudomonas* sp. (G-negative bacterium), *Fusarium* oxysporum (fungus), and *Paenibacillus* (a G-positive bacterium). All tests showed that the developed AgNP-AC has great potential for plant disease management in greenhouse vegetable production. Such a design can be extended to kill other common bacterial and fungal cells/spores, such as *Erwinia* spp., *Phytophthora* sp. and *Pythium* spp., which often occur in greenhouse vegetables and irrigation water. Our solution is an alternative strategy to overcome pesticide resistance developed by pathogens, and is an alternative strategy to treat contaminated water in greenhouses to reduce pesticide related health risks and environmental contaminations.

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References

1. Hong CX, Moorman GW (2005) Plant pathogens in irrigation water: Challenges and opportunities. Crit Rev Plant Sci 24: 189-208.
2. Steward-Wade SM (2011) Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: Their detection and management. Irrig Sci 29: 267-297.
3. Tyson RV, Simonne EH, Treadwell DD, White JM, Simonne A (2008) Reconciling pH for ammonia biofiltration and cucumber yield in a recirculating aquaponic system with perlite biofilters. HortScience 43: 719-724.
4. Berghage RD (1996) Green' water treatment for the green industries: Opportunities for biofiltration of greenhouse and nursery irrigation water and runoff with constructed wetlands. HortScience 31: 690.
5. Safavi K, Mortazaeinzahad F, Estahanizadeh M, Dastjerd H (2011) The study of nano silver (NS) antimicrobial activity and evaluation of using ns in tissue culture media. International Conference on Life Science and Technology, Signapore.
6. Dibrov P, Dzioba J, Gosink KK, Häse CC (2002) Chemiosmotic mechanism of antimicrobial activity of Ag(+) in *Vibrio cholerae*. Antimicrob Agents Chemother 46: 2668-2670.
7. Mazurak VC, Burrell RE, Tredget EE, Clandinin MT, Field CJ (2007) The effect of treating infected skin grafts with Acticoat on immune cells. Burns 33: 52-58.
8. Maiti S, Krishnan D, Barman G, Ghosh SK, Laha JK (2014) Antimicrobial activities of silver nanoparticles synthesized from *Lycopersicon esculentum* extract. J Anal Sci Technol 5: 1-7.
9. Zahir A, Bagavan A, Kamaraj C (2012) Efficacy of plant-mediated synthesized silver nanoparticles against *Sitophilus oryzae*. J Biopest 5: 95-102.
10. Tuan TQ, Son NV, Dung HTK, Luong NH, Thuy BT, et al. (2011) Preparation and properties of silver nanoparticles loaded in activated carbon for biological and environmental applications. J Hazard Mater 192: 1321-1329.
11. Karumuri AK, Maleszewski AA, Oswal DP, Hostetler HA, Mukhopadhyay SM (2014) Fabrication and characterization of antibacterial nanoparticles supported on hierarchical hybrid substrates. J Nanopart Res 16: 1-14.
12. Richter AP, Brown JS, Bharti B, Wang A, Gangwal S, et al. (2015) An environmentally benign antimicrobial nanoparticle based on a silver-infused lignin core. Nat Nanotechnol 10: 817-823.
13. Jo YK, Kim BH, Jung G (2009) Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Dis 9: 1037-1043.
14. Kim SW, Lamsal K, Kim YJ, Kim SB, Jung M, et al. (2009) An *in vitro* study of the antifungal effect of silver nanoparticles on oak wilt pathogen *Raffaelea* sp. J Microbiol Biotechnol 19: 760-764.
15. Kim SW, Jung JH, Lamsal K, Kim YS, Min JS, et al. (2012) Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. Mycobiology 40: 53-58.
16. Lamsal K, Kim SW, Jung JH, Kim YS, Kim KS, et al. (2011) Inhibition effects of silver nanoparticles against powdery mildews on cucumber and pumpkin. Mycobiology 39: 26-32.
17. Nasrollahi A, Pourshamsian K, Mansourkiaee P (2011) Antifungal activity of silver nanoparticles on some of fungi. Int J Nano Dimens 1: 233-239.
18. Karumuri AK, Oswal DP, Hostetler HA, Mukhopadhyay SM (2013) Silver nanoparticles attached to porous carbon substrates: Robust materials for chemical-free water disinfection. Mater Lett 109: 83-87.

19. Prabhu S, Poulose EK (2012) Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett 2: 32.

20. Wani AH, Shah MA (2012) A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. J App Pharm Sci 2: 40-44.

21. Al-samarrai AM (2012) Nanoparticles as alternative to pesticides in management plant diseases - A review. Int J Sci Res Publications 2: 1-4.

22. Sahayaraj K, Rajesh S (2011) Bio-nanoparticles: Synthesis and antimicrobial applications. Science against microbial pathogens: communicating current research and technological advances, Mendez-Vilas A (ed), Formatex Research Center, 228-244.

23. Mavani K, Shah M (2013) Synthesis of silver nanoparticles by using sodium borohydride as a reducing agent. Int J Eng Res Technol 2: 1-5.

24. Srilatha B (2011) Nanotechnology in agriculture. J Nanomed Nanotechnol 2: 1-5.

25. Shanti V, Mrudula T, Deepth N, Venkateshwarlu S (2011) Novel applications of nanotechnology in life sciences. J Bioanal Biomed R1: 1-4.

26. Joseph T, Morrison M (2006) Nanotechnology in agriculture and food. Nanoforum Report by European Nanotechnology Gateway.

27. An NT, Dong NT, Hanh PTB, Nhi TTY, Vu DA, et al. (2010) Silver-N-carboxymethyl chitosan nanocomposites: Synthesis and its antibacterial activities. J Bioterr Biodef 1: 102.

28. Heidarpour F, Ghani W, Ahmadun F (2010) Nano silver-coated polypropylene water filter: II. Evaluation of antimicrobial efficiency. Dig J Nanomater Biostruct 5: 797-804.

29. Mpenyana-Monyatsi L, Mthombeni NH, Onyango MS, Momba MNB (2012) Cost-effective filter materials coated with silver nanoparticles for the removal of pathogenic bacteria in groundwater. Int J Environ Res Public Health 9: 244-271.

30. Gallardo OAD, Moiraghi R, Macchion MA, Godoy JA, Perez MA, et al. (2012) Silver oxide particles/silver nanoparticles interconversion: susceptibility of forward/backward reactions to the chemical environment at room temperature. RSC Adv. 2: 2923.

31. Yu SJ, Yin YG, Liu JF (2013) Silver nanoparticles in the environment. Environ Sci Process Impacts 15: 78-92.

32. European Commission (2014) Opinion on nanosilver: safety, health and environmental effects and role in antimicrobial resistance p: 103.

33. World Health Organization (1996) Silver in drinking-water. Guidelines for drinking-water quality, (2ndedn). WHO, Geneva.

34. World Health Organization (2011) Guidelines for drinking-water quality, (4thedn). WHO, Geneva, Chapter 8: 186.