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Use of Natural Products as Green Reducing Agents To Fabricate Highly Effective Nanodisinfectants

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Supporting Information

ABSTRACT: Disinfection of water using nanoparticles (NPs) can be achieved through selection of either metals (M) or transition metal oxides (TMO). In this research, 64 formulations of silver-titania nanocomposites (Ag/TiO_2) were prepared via a feasible wet-chemistry technique using different natural products as reducing agents. Four selected products successfully reduced Ag^+ ions to Ag, allowing Ag/TiO_2 composite to efficiently inactivate microbes found in the activated sludge. The degree of antibacterial activity was measured using zone of inhibition, which indicated all formulations inactivated the bacteria with high potency (0.01 I/6 h). The results from this study and comparison of literature values collectively suggest that light roasted coffee acted as one of the best natural reducing agents due to its low antioxidant index (LAI). Our selection framework also suggested any M/TMO with an oxygen reduction potentials (ORP) range of −0.41 to +1.23 V and any natural product with a LAI (<0.5) would be suitable as a reducing agent. Collectively, the high ORP and low AI provide effective disinfection of water-borne microbes.

KEYWORDS: *green reductants, nanodisinfectant, antimicrobial activities, high potency, morphological analyses*

INTRODUCTION

The United Nations has designated 2005−2015 as the “Water for Life” decade and estimates that one-fifth of the world population face a water shortage.[1] This complex problem requires a multifaceted approach in reducing water stress. The biological cost of water stress on microbes,[2] plants,[3] and animals is well-known.[4] Two generally employed strategies are water reclamation[5] and water treatment.[6] The latter can be undertaken through chemical,[7] photochemical,[8] or electrochemical[9] strategies. Most of these disinfection strategies have drawbacks such as incomplete disinfection, for example, viability of *Cryptosporidium* and *Giardia* post-treatment or disinfectant byproducts, some of which are toxic.[10] Examples of chemical disinfectants include oxidizing agents (bleach, chloramine, hydrogen peroxide, peracetic acid, and potassium permanganate), other chemicals (isopropanol, glutaraldehyde, phenols, quaternary ammonium salts biguanide biopolymer), high energy (ultraviolet, gamma irradiation), and metals (silver, copper). Oxidizers are thought to break membrane bonds, causing lysis and inactivation, and are the “first line of defense” in any use of disinfectant. However, there is no single measurement reported in the literature in which a priori assessment can identify effective disinfectant. Hence, we intend to provide baseline parameters on which physiochemical properties are significant in selection of agents as disinfectants.

Developments in material science have allowed nanomaterials to be examined as possible disinfectants, due to their unique properties, such as ultra high surface area and volume. These properties allow for increased activity due to a dramatic increase in the number of atoms in the surface,[11] due to the quantum size effect. For example, when the bulk material size is reduced to about 10 nm dimension, the surface atoms account for 20% of the total atoms composed of the perfect particles. If this size is further decreased to 1 nm, the surface atoms account for up to 99% of the total number of atoms.[12] Because of the lack of adjacent atoms, there exist a large amount of dangling bonds, which are not saturated. Those atoms will bind with others to be stabilized. This process results in lowered coordination number and increased surface energy, collectively resulting in high chemical reactivities of the generated nanomaterials.[13] Therefore, development of new materials for water treatment has steadily increased in the last few decades, due to the numerous advantages of nanoparticles (NPs) as mentioned above.[14]

One type of NP is transition metal oxides (TMOs). These materials can behave as semiconductors, a property that can be exploited in designing efficient disinfectants. TMO electrical properties are better understood in terms of movement of electrons across the band gap barrier. For a TMO,[15] electron−hole pair generation may occur upon absorption of energy, sufficient to cause electron transfer between the conductance

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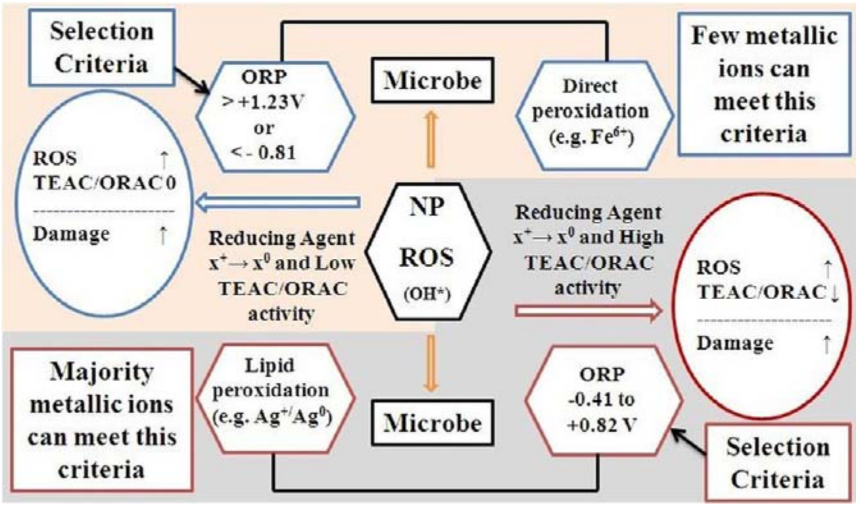

Figure 1. Mechanistic processes of antioxidation magnitude, ROS and net contribution toward disinfection. The strength of antioxidant quotient and ROS on net disinfection is summarized as promoting disinfection (↑), no net effect (0), or no/low disinfection (↓). Noting relative ORPs* for chemical disinfectants ranging from 0.57 (Br) to 2.25 (F), such as 2.05 ($^{\bullet}OH$), 1.30 (H_2O_2), and 1.10 (HOCl), *www.eclear.co.za.

band (CB) and the valence band (VB). Water or molecule adsorption on the surface of the TMO with the valence band hole would lead to generation of anion or radical species.[16] The generated photoelectrons or the adsorbed molecule would be expected to be at the surface of the TMO, which in turn would be reduced. Absorbed molecules such as oxygen at the reduced TMO sites would result in generation of superoxide radical via charge transfer.[17] Addition of a metal to the TMO would be expected to lower the band gap energy, resulting in generation of radical(s) at lower energies, for example, by going from ultraviolet (UV) activation to activation by visible light. The generation of various reactive species is summarized in the following eqs 1−4:

$$TMO_x^n + h\nu \rightarrow \bar{e}_{CB} + h_{VB}^+(TMO_x^n) \quad (1)$$

$$H_2O + h_{VB}^+ \rightarrow {}^{\bullet}OH + H^+;\ h_{VB}^+ + OH^- \rightarrow {}^{\bullet}OH \quad (2)$$

$$TMO^n_{(x+(x-1))} + \bar{e}_{CB} + xH_2O \rightarrow TMO^{n-1}_{x-1} + 2xOH^- \quad (3)$$

$$TMO^{n-1}_{x-1} + xO_2 + \bar{e}_{CB} \rightarrow TMO^{n+1}_{x-1} + xO_2^{\bullet -} \quad (4)$$

noting that x represents number of oxygen atoms, hν represents the quanta of energy (in eV), $\bar{e}_{CB}$ the electron arising from the conductance band, h^+_{VB} the corresponding valence band hole, hydroxyl radical ($^{\bullet}OH$) and superoxide radical ($O_2^{\bullet -}$), collectively known as reactive oxygen species (ROS).[18,19]

This study aims to find a feasible and effective solution to eliminate biological impurities from contaminated water. We report the bactericidal effect of Ag-inserted TiO_2 NPs generated using various natural products as reducing agents against a variety of microbes found in water (in the form of activated sludge). This comparative framework would allow for a more intelligent design approach to be utilized in a system biology context, exemplified by rationale design, fabrication, and evaluation of nanodisinfectant using natural products as green reducing agents. This research focused on M/TMO fabrication parameters, selection of natural products, and the tools to evaluate oxidation state. The various physiochemical properties of the green reducing agents are discussed. Finally, an operational hypothesis is given on which parameters influence disinfection the most (summarized in Figure 1).

■ MATERIALS AND METHODS

Materials. All chemicals were purchased from EMD Chemicals (Gibbstown, NJ) unless otherwise specified. Doubly distilled water was used, and the natural products were purchased from a local grocery store in Berkeley, CA, USA. The bactericidal experiments were carried out using microbes from activated sludge samples (collected from Lawrence Berkeley National Laboratory) known as ash bud 1, 3, and 5 in nutrient media, composed of peptone and NaCl (5 g/L), yeast and beef extract (1.5 g/L). The solid media contained 2% agar as solidifying agent. Four natural products used as reducing agents were purchased locally in Berkeley: jasmine tea (20 ct, Trader Joe's), coffee (Starbucks House Blend Ground Coffee, 20 oz, Wal-Mart), vitamin C (as ascorbic acid, EMD), and prune juice (Pure Prune Juice, 48 fl oz, Wal-Mart). Four stoichiometric ratios, 1:1, 1:2, 1:4, and 1:8 were used between the Ag and reducing agent. In addition, two different concentrations of reducing agents were used, corresponding to a molar concentration of 5 μM and 25 μM for vitamin C and for the other natural products. (Although an approximation, 160 mg of caffeine was assumed to be in the light-roast coffee and 20 mg in tea per 8 oz. The volume was adjusted to give approximate concentrations of 5 and 25 μM as described.) In total 64 formulations were generated and a subset of 16 were evaluated as disinfectants (144 runs). Each nanoparticle's formulation was evaluated three times (m = 3) with three replicates (n = 3). The standard derivation for the inhibition study for each replicate was less than 4%. For comparison purposes, literature values for orange juice (O) were used in place of our vitamin C reducing agent. Other literature values for prune juice (P), medium-roast coffee (C), and jasmine tea (J) were used to match the natural products used in this study.

Nanoparticle Synthesis. The Ag/TiO_2 NPs were prepared as previously described,[20] except natural reducing agents were used instead of sodium borohydride. Briefly, 70 mM $AgNO_3$ in water was prepared at 60 and 80 °C. The solution was allowed to cool at room temperature and during cooling TiO_2 was added incrementally. The TiO_2 feedstock was prepared using titanium(IV) butoxide in butanol with acetic acid and ammonia to control the hydrolysis rate. The reagents were mixed with dropwise addition (molar ratio of Ag/Ti = 1:20). The binary solution was continuously mixed and reduced with various molar ratios of reducing agents for 2 h. The so-prepared NPs were washed twice with deionized water.

Antimicrobial Disk Diffusion Test. Agar plates were prepared by applying a bacterial suspension of ca. 100 μL of 10^5 CTU/mL. The

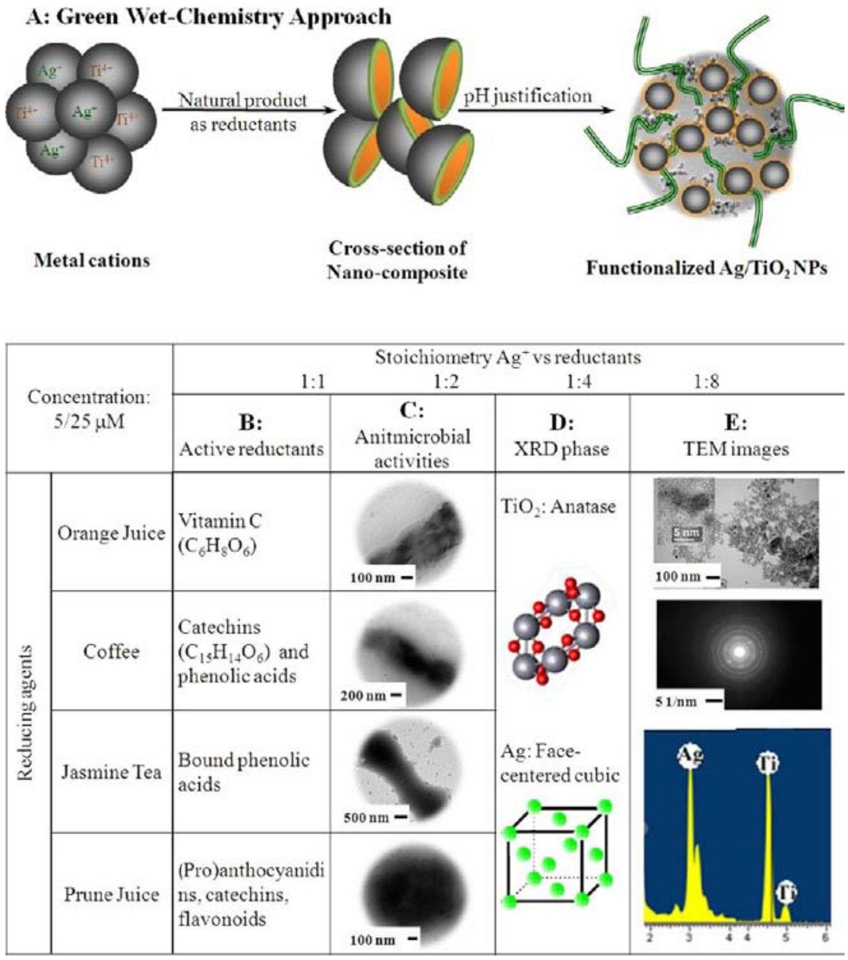

Figure 2. (A) Green wet-chemistry was employed to produce series of Ag/TiO_2 nanodisinfectants using natural products as reducing agents; (B) active chemical components of each natural product are listed; (C) all disinfectants displayed high potency of antimicrobial activities when used to treat activated sludge specimens; (D) TiO_2 and Ag display anatase and face-centered cubic crystalline phase structure, respectively; and (E) selected TEM images and EDS Ag and Ti elemental composition analyses for demonstration of NPs, which are highly crystallized as shown in lattice fringe and ring patterns.

plates were then incubated at 35 °C overnight. An impregnated disk was placed on each plate, similar to evaluation of antibiotics. Approximately 25 μL of a colloidal suspension was placed on 6 × 1 mm disks. On the grown microbe colonies, four disks impregnated with disinfectants were placed and left for 8 h. After 8 h, the plates were removed, and the average diameter (in mm) of the zone of inhibition (ZoI) was measured. Control samples used were the same suspension minus the nanoparticles. Four disks were placed per plate, with each disk with Ag/TiO_2 nanodisinfectants. The mean and standard deviation of the ZoI for each formulation were recorded, noting the maximum percent error was less than 1.1%.

Morphological Analyses. The activated sludge, from which *Escherichia coli* was identified, was treated using Ag/TiO_2 nanodisinfectants. The morphology and elemental composition of these samples were characterized using a transmission electron microscopy (TEM)[20] (FEI Company, Tecnai F20-G^2 Hillsboro, OR). This TEM was equipped with X-ray energy dispersive spectroscopy (EDS) and energy electron loss spectroscopy (EELS) for elemental composition and distribution analyses. The particle size range was the same for the replicate formulations, with a distribution error of <7%.

■ RESULTS AND DISCUSSION

Green Synthesis of Ag/TiO_2 Nanodisinfectants. Green wet-chemistry was employed due to its significant advantages compared to traditional fabrication processes (Figure 2). The main considerations of green synthesis are use of less-toxic reagents and renewable precursors to generate low waste, without compromising yield and purity of the products (Figure 2A). When a chemical process incorporates these principles into NPs fabrication, the hazardous waste was reduced, whereas energy efficiency was enhanced, or at least not compromised.[21] This approach also has additional benefit of generating minimal waste, offering low manufacturing costs, and ease of operation.[22] A practical approach in the green synthesis of NPs is wet-chemistry bottom-up synthesis under moderate conditions. This approach displays advantages of controlling materials from the molecular level and providing high homogeneity. Further, it will guide the development of nanoscaled materials with the maximum benefit for society and the environment.[23] The green synthesis used in this research would require the "agents" to sufficiently reduce oxidative states without any toxic byproducts.[24] Previous approaches, which have used pepper extracts,[25] vitamin C,[26] sugars,[27] starch,[28] polysaccharides,[29] and plant extracts[30] have been shown to be effective antibacterial agents and generate an antibacterial effect.[31] In this study, four natural products, jasmine tea, coffee, ascorbic acid (vitamin C), and prune juice (Figure 2B) were used to produce highly effective nanodisinfectants (64 formulations, Supplemental Table 1, Supporting Information).

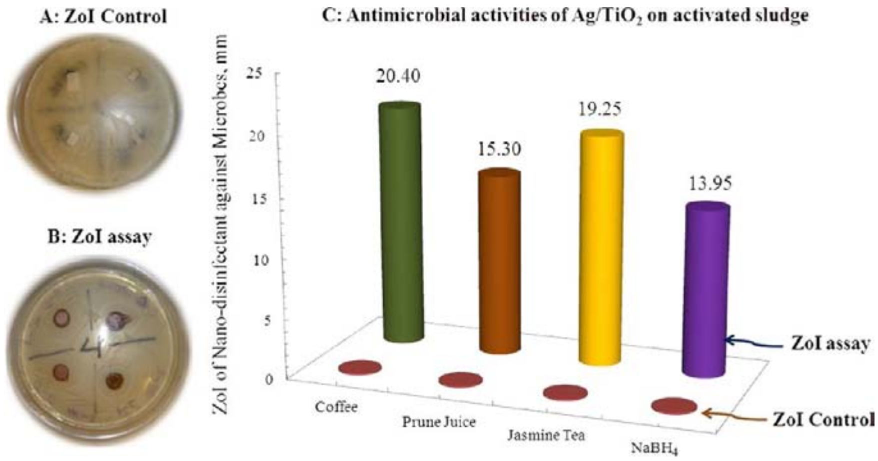

Figure 3. The zone of inhibition (ZoI, mm) on activated sludge (AS) was measured for Ag/TiO_2, where Ag^+ was reduced using natural products (ascorbic acid, light roasted coffee, or prune juice). A: ZoI control; B: ZoI assay; and C: antimicrobial activities of Ag/TiO_2 on activated sludge and literature comparison (ref 33). Relative standard deviation error of three replicates <1.8%. [$\bar{x}$ 20.4, RSD; 1.8; $\bar{x}$ 15.3, RSD 1.7; $\bar{x}$ 19.25, RSD; 0.68; $\bar{x}$ 13.95, RSD; N/A].

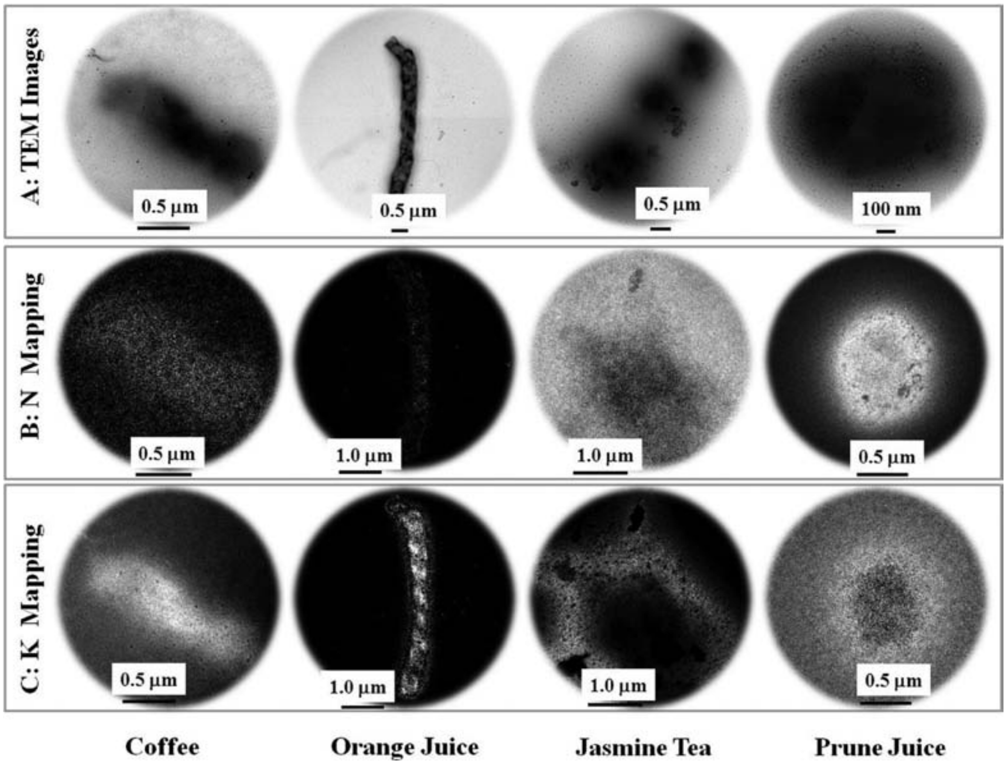

Figure 4. Morphological and elemental composition analyses of damaged microbes from the activated sludge. A: STEM image of cluster of microbes in varying stages of cell damage; B: nitrogen (N) elemental mapping to demonstrate the leakage from the cell membrane; and C: potassium (K) elemental mapping to demonstrate the leakage from the cell membrane, the mapping region was randomly selected. Note ascorbic acid was used experimentally, but literature values were for orange juice.

All of the natural products successfully reduced silver ions (Ag^+) to zero-valence oxidation state (Ag^0), allowing formation of Ag/TiO_2 composite. Ag/TiO_2 particle size varied from 10 to 25 nm. The stoichiometric molar ratio of Ag:Ti was evaluated to be 1:20. The NPs are effective at inactivating microbes existing in the activated sludge (Figure 2C) due to their high chemical activity. Both TiO_2 (anatase, PDF 00-021-1272, a = 3.7852 Å, c = 9.5139 Å; α = 90°) and face-centered cubic Ag (fcc, PDF 00-004-0783, a = 4.085 Å; α = 90°) were highly crystalline with distinctive grain boundaries (Figure 2D). This favors the rapid cell membrane rupture due to the ROS generation (discussed next). It can be concluded that NPs offer an approach to engineer effective disinfectants, through design of specific band gap (as measured by LAI/ORP), specific particle shape, and size (Figure 2E).

Antimicrobial Activities of Ag/TiO_2 Nanodisinfectants. The inhibition was carried out on microbes from activated sludge, which consists of nutrients from wastewater, protozoans, bacteria, and filamentous microorganisms.[32] A practical demonstration of the effectiveness of Ag/TiO_2 nanodisinfectants was shown as in Figure 3. It can be seen that the average measured ZoI with diameters of 18.7 mm, 15.3 mm, 20.4 mm

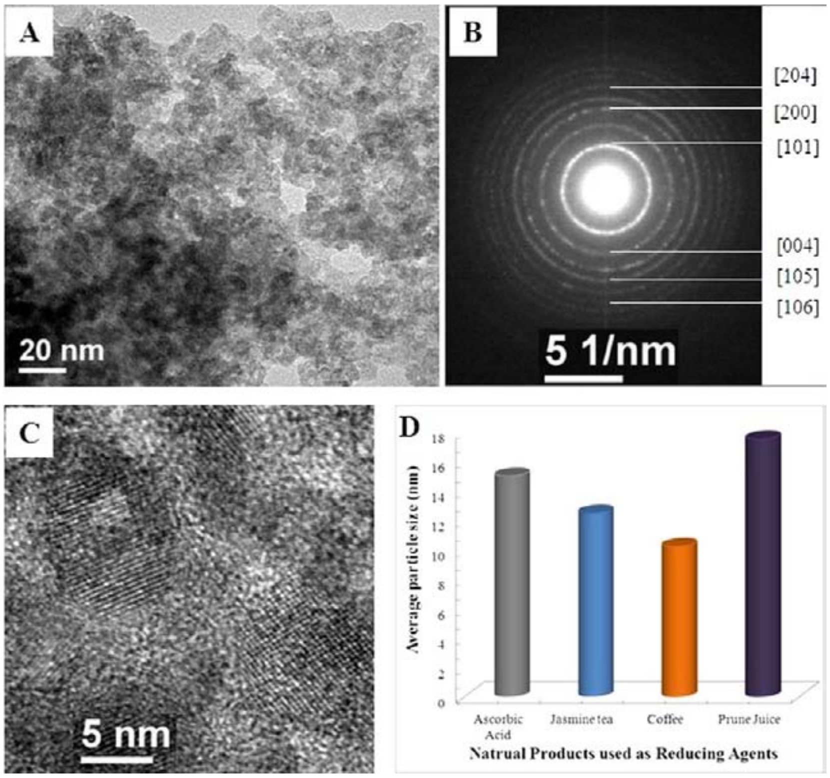

Figure 5. TEM image and particle size distribution of NPs using various reducing agents, (A) selected morphological analysis of Ag-TiO_2 NPs indicating the nanoparticles are pseudospherical; (B) the lattice fringe resulting from polycrystals; (C) the lattice fringe of the NPs, indicating the high crystallinity; and (D) the average particle range of NPs using four natural products (the NPs derived from coffee show smallest particle size. Standard error of three replicates <2.5%, largest σ_{est} at 95% probability <3.7).

(< ± 1 mm, Figure 3A,B) was larger than those obtained from single strains (diameter at 14 mm, Figure 3C),[33] reflecting the efficacy of the Ag/TiO_2 nanodisinfectants. From the observation, we can conclude the Ag/TiO_2 derived from reduction using four natural fruit juices or beverages successfully inactivate microbes in activated sludge. The degree of inhibition indicated all formulations of Ag/TiO_2 were of high potency (efficacy (I) = 1/% inhibition, 0.01 = I) within 6 h. Comparison of ZoI radii to other published works (up to 14 mm)[33] using Ag indicated that Ag/TiO_2 nanodisinfectants displayed superior antimicrobial activities. The ZoI measurement also indicated the efficacy of Ag/TiO_2 depends on the variation of natural products. To understand why different natural products have a variation in inhibition, the role of the M/TMO was examined with respect to redox potential, oxidation state, and other parameters.

Additionally, literature survey indicated that if the oxygen reduction potential (ORP) was above +1.2 V, any M/TMO will directly oxidize/reduce the phospholipids on the outer surface of the membrane resulting in lysis and inactivation of the microbe.[34] Examinations of the ORP highlighted Ag as a promising candidate suggested that Ag^+ could be reduced by the natural products and could pump the electrons to the surface of TiO_2, which would catalytically either generate ROS or oxidize membrane lipids/proteins of prokaryotes. A more practical approach was indirect inactivation of the microbe through generation of ROS such as hydroxyl radicals, which could be accomplished using M in zero or low oxidation states or M/TMO composites under visible light conditions (e.g., Ag/TiO_2 disinfectants). From the above discussion, it can be seen that nanodisinfection can be achieved through direction oxidation (ORP > +1.2 V; RP: −0.41 to +1.23 V), limiting the choice to a few M/TMOs. On the other hand, the pool of M/TMO as disinfectants can be increased through indirect oxidation of microbe membrane via generation of ROS ($\bar{e}_{CB}$ < 3.6 eV).

Morphological Analyses of Activated Sludge. The scanning transmission electron microscopic (STEM) images depict the effect of the composite on inactivation of bacteria existing in the activated sludge (Figure 4). Figure 4A shows images of the microbes, which were treated using 16 formulations of Ag/TiO_2 nanodisinfectants. Collectively, the microbe cell membranes were found to be damaged (but intact) upon addition of Ag/TiO_2 formed via four natural products. It is clear that the cell wall and membrane are intact (in the early stages) and that some cytoplasmic membrane peeling may occur as the incubation time was increased. The present observations are consistent with our previous and reported studies, in which it was shown that silver nanoparticles can induce the respiratory chain dehydrogenases of bacteria, such as *E. coli* into an inactive state, coupled with leakage of reducing sugars and proteins (determined by EELS). The latter was a result of changes in the permeability of the bacterial membranes.[35] In the same study, under STEM evaluation, certain disorganization was observed, phenomenologically similar to what is observed here.

EELS data were used to determine the atomic composition, chemical bonding, and semiconductor (valence/conductance band) properties. Both elements, leakage of nitrogen (N) and potassium (K), is considered indicators for cell death. Figure 4B,C indicated that permeability of nitrogen (N) and potassium (K) ions across the cell membrane into the extracellular matrix for microbes resulted in the cell death. Other elemental mapping on sulfur (S), phosphorus (P), calcium (Ca) also suggested these heterogeneous atoms across the cell membrane, causing inactivation of the microbes. This observation was due to the high intensity shown on the map. The mapping data confirm that Ag/TiO_2 nanodisinfectants are observed to be present around the microbe, but none incorporated within the cells. Additionally, the variation in intensity may be attributed to cell wall compositional differences between the microbes yielding differing degrees of interaction with the NPs, which in turn give different intensities.

Our data indicated that Ag/TiO_2 NPs reduced with coffee were more effective disinfectants than those reduced by jasmine tea and other reductants. To understand the reasons, the contributing parameters were considered: particle diameter, natural product composition, antioxidant quotient, and ORP. The relationship between these parameters including conceptual workflow is summarized in Figure 1. The antioxidant quotient was evaluated by two standard assays, the Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC), respectively. These TEAC and ORAC values are important in selection of natural reducing agents. The antioxidant quotient and ROS have opposite contributions toward inhibition of microbes. The former promotes cell survival, whereas the latter promotes the cell death. In general, the net contribution toward inactivation is the difference between the above two factors.

Effect of Nanoparticle Size on Antimicrobial Activities. The diameters of the green chemistry generated NPs[36] are known to strongly influence not only the degree of biocidal activity but also the speed of microbial inhibition. TEM analyses (Figure 5A) indicate that the particles are approximately 10−25 nm (±5 nm); therefore size (Figure 5B) is not the defining parameter in this instance (Figure 5C). Our previous work[37] has shown those smaller than 10 nm are more effective as disinfectants but are less stable,[38] whereas diameters of more than 30 nm are stable but less effective. An optimal range between 15 and 25 nm (±4 nm) yields the desired activity and stability (Figure 5D).[39] If the NPs were reduced and washed with water, the reducing agent components ought to be washed away. However, if some remained, composition of the natural product is important in understanding the observed trends. (Jasmine tea is a combination of Chinese green tea with aroma of jasmine flower petals, by forming interlayers of tea and jasmine leaves in six layers, such that the jasmine aroma is absorbed by the tea leaves, after which the tea leaves are processed in the usual manner for green tea, noting that these teas are unfermented.[40])

Effect of Nature of Natural Products on Antimicrobial Activities. The intrinsic "antioxidant quotient" of the reducing agent is related to composition. Composition is indirectly related to disinfection efficacy. It has been shown that natural products with high polyphenols are effective reducing agents, but poor disinfectants, because high polyphenol content promotes antioxidation and protects the microbe membrane against damage.

Compositional analyses of natural products indicate that the major components are polyphenols[41] and catechins.[42] Other components and their abundances are listed in Supplemental Table 2. The degree of antioxidation (measured by bioassays) for polyphenols from green tea has been shown to be concentration-dependent,[43] indicating that green tea would be good reducing agents but M/TMO reduced would exhibit poor disinfection. The major components in prune juice are α-aminobutyric acid, taurine, quinic acid, O-linked-phosphoethanolamine, and citrulline, in addition to polyphenols which can provide antioxidation protection.[44] Coffee although high in polyphenol content has a low percent of unbound polyphenols, which contribute toward antioxidation. The major types of polyphenols in ground coffee are hydroxycinnamic acids and minor free polyphenols.[45] Using polyphenol content as an index, metals reduced with coffee would be expected to give the highest disinfection percentage.[46] One possible benchmark in assessing the effectiveness of natural products as reducing agents is to measure their total polyphenol content, often expressed as gallic acid equivalents (GAEs). The natural products used as reductants are summarized in Table 1 and Supplemental Table 3.

Table 1. Efficacy Composition of Natural Products by Composition of Antioxidant

natural product	GAEs (mg/mL)	primary antioxidant photochemical	efficacy
orange juice	0.38[a,b,c]	vitamin C	3
jasmine tea	0.12	catechins and phenolic acids	2
coffee	0.05[d]	bound phenolic acids	1
prune juice	0.41	(pro)anthocyanidins, catechins, flavonoids	4

[a]Lower value. [b]mg of ferulic acid equivalents/mL. [c]Orange juice has a large amount of ascorbic acid and shown here for comparison purposes only (an index of 1 indicates best disinfection with large ZoI value, 4 is worst disinfection with smaller ZoI value). [d]mg/g.

Total polyphenols have been shown to be radial ion scavengers and/or antioxidants,[47] so they would be expected to have a protective effect, and therefore a proportional correlation may be expected between total phenol content and antioxidant activity. This correlation has been confirmed in a number of studies.[48,49] In addition to the antioxidant properties of the natural product polyphenols[50] are the counter-trend of ROS catalyzed by the nanoparticles. The greater the antioxidant effect, the lesser the generation of ROS and the lower ROS-induced cell damage and morbidity. Using GAEs as a benchmark for total polyphenol content, a ranking order can be deduced. The reported GAEs for the natural products as well as orange juice (for ascorbic acid, used in our study) are listed in Table 1. The GAE ranking obtained from Table 1 is P > orange juice (O) > J > C, in terms of GAE. In the same study, the GAE content varied considerably on how the coffee was prepared or whether the coffee was from instant or ground coffee beans, varying from 0.05 to 0.15 (mg/g);[51] therefore the coffee which was utilized by us could have had a higher GAE content than tabulated, suggesting that a quasi inverse relationship between GAE content and effectiveness of the reduced NPs.[52−54]

To explain the observed results, two biological properties of the natural products were considered: reducing potential and antioxidant index. Survey of the literature suggested that most if not all natural products including the ones which were evaluated (coffee, ascorbic acid, jasmine tea, and prune juice) were capable of reducing silver, since their ORP were less than 0.8 V.[55] LAI can be measured in a number of ways, but from analysis of published literature[56] results prune juice had the greatest index value, while generally coffee had the lowest index value and was in line with our hypothesis that agents with a high antioxidant index would counter NPs[57] generated ROS,[58] thus rendering that system less efficient than a system that had a low antioxidant index. Comparison of literature values would suggest that apple juice would be a good natural agent (due to its low antioxidant index), whereas prune or pomegranate juice[59] would be a poor agent due to its high antioxidant index, even though both would successfully reduce silver.[60] From the above discussion, it can be seen that nanodisinfection can be achieved if the natural product has an ORP of at least 0.8 V[61] and low GAE (<0.1 mg) content.

To conclude, disinfection of microbes in activated sludge was achieved (0.01%/6 h) with Ag/TiO_2, higher than other similar published works. It was shown that natural products that have lower ORP (< +0.8 V) and low antioxidant quotient (<0.1 mg/g GAEs) would be excellent candidates as reducing agents. Our data indicated that light roasted coffee reduced Ag^+ to Ag metals, which was found to be highly potent disinfectants. Conversely, natural products that had high GAE content, such as prune juice, would also be effective reducing agents, but metals reduced in this manner would exhibit poor disinfection.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The formulations of the Ag/TiO_2 NPs, chemical composition in natural fruit juices and beverages, and the different parameters to examine the strength of a reduction and/or antioxidation parameters are listed as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

Synthesis and formulation were carried out by J.D. under J.L.L.'s supervision. The biological evaluation was also completed by J.D. under supervision by S.B. Data acquisition and analysis were undertaken by J.L.L. The experimental design, selected data analysis, first draft construction, and final manuscript were undertaken by S.B. He also supervised the submission of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

NPs, nanoparticles; *I*, efficacy; LAI, low antioxidant index; ORP, oxygen reduction potential; $O_2^{\bullet-}$, superoxide radical; $^{\bullet}OH$, hydroxyl radical; ORAC, oxygen radical absorbance capacity assay; ROS, reactive oxygen species; STEM, scanning transmission electron microscopy; TEAC, Trolox equivalent antioxidant capacity assay; TMO, transition metal oxides; UV, ultraviolet; VB, valence band; ZoI, zone of inhibition; CB, conductance band; CFU, colony forming units/per milliliter; DPPH, 2,2-diphenyl-1-picrylhydrazyl assay; E^0, standard reduction potential; EDS, X-ray energy dispersive spectroscopy; EELS, electron energy loss spectroscopy; GAEs, gallic acid equivalents

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