Water disinfection using zinc phosphide nanowires under visible light conditions

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ABSTRACT

Common methods of bacteria disinfection after water treatment include chlorination and ultraviolet irradiation. Although very effective, those methods require continual investment of energy or materials. Previous studies have shown that photocatalysis can enhance water disinfection. This study addressed the potential use of zinc phosphide nanowires to promote water disinfection under visible light conditions as a potentially economical alternative. Disinfection studies were conducted using boron nitride decorated zinc phosphide nanowires, unfunctionalized zinc phosphide nanowires, 1,4-benzenedithiol functionalized zinc phosphide nanowires, and bare zinc foil. Bioreactors inoculated with *Escherichia coli* isolates were exposed to the photocatalysts under visible light. *E. coli* was enumerated at five-min intervals during the disinfection period. At least a 5-log reduction in microbial load was achieved for all materials, with the unfunctionalized zinc phosphide nanowires consistently producing the highest log reduction. For each material, more than a 4-log reduction was observed after only 5 min of exposure. Minimal photoreactivation or dark repair (less than a 1.48-log increase) was observed. Disinfection efficacy did not differ significantly ($p < 0.05$) between the three isolates. Although the specific photocatalytic mechanism is not yet known, this study indicates that zinc phosphide nanowires can enhance disinfection of water using only visible light.

1. Introduction

In the United States, bacteria from fecal material are a leading cause of water impairment [1]. Fecal contamination from humans or animals is a potential source of enteric bacteria. To mitigate health risk, water is disinfected to eliminate fecal-derived pathogens remaining after drinking water or wastewater treatment. Widely used and effective disinfection treatments include chlorination and ultraviolet (UV) irradiation [2–4]. Chlorination is very effective and has the added benefit of residual disinfection, but it may form carcinogenic by-products [5,6]. UV treatment requires neither special handling nor chemicals, but it is moderately expensive and bacterial reactivation often occurs [3], especially with environmental isolates [7]. The ability of photocatalysts to degrade bacteria has been observed since the mid-1980s [8]. For instance, bacterial inactivation by titanium dioxide (TiO_2) under UV light has been studied extensively [8–12]. In most studies, nanomaterials such as ZnO and TiO_2 have tended to be used in conjunction with UV light because the UV radiation spectrum matches the excitation energies of these photocatalysts [10,12–14]. However, ZnO is unstable in water, and bacterial inactivation using TiO_2 under visible light is

slow [15,16].

The photocatalytic properties of zinc phosphide (Zn_3P_2) make it attractive for use in water disinfection in the visible light range. Zinc phosphide, an inorganic compound, is useful in photovoltaic applications [17]. Because its components are inexpensive and are available abundantly in the earth's crust [14,18], zinc phosphide can be mass produced in nanowire forms [18]. Also, Zn_3P_2 nanowires have high specific surface areas, which is essential for their use as catalysts [19]. With a bandgap of 1.5 eV (in the infrared regime), these nanowires can be activated by wavelengths in either the visible or the ultraviolet regimes [19].

However, similar to ZnO, zinc phosphide is not stable in water [18,19]. Because Zn_3P_2 is highly toxic, stable forms are needed for use in aqueous environments. In a process developed by Vasiraju, et al. [19], zinc phosphide has been stabilized by non-conformal decoration with boron nitride (BN). Further, functionalization of Zn_3P_2 with organic molecules may enhance stability, as shown by Brockway, et al. [17,18,20] and Ramos-Sanchez, et al. [14]. Stabilization of these nanowires, using either BN decoration or organic molecule functionalization, enhances their suitability for use in aqueous media [17,19].

Enhancing the stabilities of materials that have high specific surface areas, such as Zn_3P_2 nanowires, makes them suitable for disinfecting water using only sunlight or inexpensive sources of visible light. Also, because these stabilized nanowires are easily activated and made from inexpensive materials, they are potentially useful as photocatalysts for disinfecting water. To our knowledge, the use of Zn_3P_2 nanowires to promote photocatalytic degradation of bacteria in water has not been studied yet.

This study addresses the potential use of various forms of zinc phosphide nanowires to promote the disinfection of *E. coli* photocatalytically under visible light conditions. To evaluate whether different isolates vary in responses or repair mechanism with this treatment, environmental *E. coli* isolates from three sources were used. Disinfection studies were performed, and then photoreactivation and dark repair were evaluated after treatment. Stability studies were conducted to assess any degradation of the treatment materials in water. These preliminary studies show the potential suitability of this class of nanomaterials to disinfect water.

2. Materials and methods

2.1. Microbial strains and culture media

Disinfection experiments were conducted on environmental *E. coli* isolates from three sources: wastewater treatment plant (WWTP), cattle, and feral hog. The WWTP isolate was prepared from the secondary effluent of a domestic wastewater treatment plant in Texas. The cattle and feral hog fecal samples were collected from the Attoyac Bayou (Texas) watershed; *E.coli* identification and isolation were conducted by the Soil and Aquatic Microbiology Laboratory at Texas A&M University. The harvested stock specimens were stored in a $-20\,°C$ freezer until use. Isolation confirmation was performed on a modified mTEC (membrane Thermotolerant *E. coli*) medium. For each isolate, a loop full of stock culture was aseptically transferred and then enriched in Luria-Bertani (LB) Broth for 4 to 6 h at $42\,°C$ under aerobic conditions and continuous agitation. After enrichment yielded at least $10^8\,CFU/mL$, 5 mL of each culture was transferred into 100 mL of sterile water in a separate 200 mL beaker. All glassware and media were aseptically handled before and after autoclave sterilization ($121\,°C$ for 20 min).

2.2. Synthesis of zinc phosphide nanowires

Three nanowire samples and bare zinc foil were used in this study. Boron nitride (BN) decorated zinc phosphide nanowires, unfunctionalized zinc phosphide nanowires, 1,4-benzenedithiol (BDT) functionalized zinc phosphide nanowires, and zinc foil were designated materials A, B, C, and D, respectively. All nanowires were synthesized on top of zinc foils using chemical vapor deposition (CVD) as outlined by Vaddiraju, et al. [14,17–20]. As previously mentioned, to enhance nanowire stabilities in water, a method involving the non-conformal decoration of Zn_3P_2 nanowires with BN ceramic [19] was used. Brief descriptions of these processes follow. Zinc phosphide nanowires were formed when phosphorous vapor was transported onto zinc foils maintained at 380–400 $°C$. A red phosphorus powder source maintained at 480 $°C$ allowed for this phosphorus vapor transport, and a 20 sccm hydrogen flow aided this transport. All nanowire synthesis was performed at a pressure of 400–500 mTorr. It is thought that nanowire formation results from self-catalysis via zinc droplets [14,17,18,20,21]. After synthesis and before removal from the vacuum chamber, the nanowires were exposed to either 1,4-benzendithiol (BDT) or decomposed tribromoborazine to obtain BDT functionalized [14,17,18,20] and BN decorated Zn_3P_2 nanowires, respectively [19]. For the latter, the amount of tribromoborazine was carefully controlled to ensure a non-conformal BN coating was obtained on top of the nanowires [19].

2.3. Disinfection trials

To assess disinfection, Materials A, B, C, and D were each immersed and suspended in the respective aerobic reactors at $25\,°C$ for the duration of the experiment. The control reactor did not contain any photocatalyst. In the preliminary study, performed on the WWTP isolate, the control was exposed to a low-pressure UV-C germicidal lamp (Bryant Energy, Indianapolis, USA). The average irradiance was approximately $3.26\,mW/cm^2$. However, each reactor containing a nanomaterial was exposed only to visible light.

Other than in the preliminary study, all disinfection trials were conducted in the presence of visible light. Reactors were exposed to three fluorescent lamps, each with an approximate intensity of 30,000 Lux. For all trials and the control, enumeration of *E. coli* was conducted immediately after inoculation (0 min) and thereafter at 5-min intervals for the duration of the experiment. During the disinfection period, which lasted 20 to 30 min, reactors were continuously mixed using magnetic stirrers under aerobic conditions at $25\,°C$.

2.4. Reactivation studies

After the disinfection experiments, each culture was split into two reactors. In order to assess dark repair, one reactor was completely covered in foil. The other reactor remained exposed to light in order to determine photoreactivation. The reactors were continuously mixed by magnetic stirrers under aerobic conditions. All reactivation studies were conducted at ambient room temperature. *E. coli* enumeration was conducted after 24 h. Reactivation was assessed as log repair following treatment.

2.5. Enumeration of microorganisms

E. coli was enumerated by spread plating in triplicate on MacConkey agar plates; serial dilution was done when necessary. Plates were inoculated with volumes ranging from 0.1 mL to 1 mL and then incubated for 24 h at $30\,°C$. After incubation under aerobic conditions, colony-forming units (CFUs) were counted and then multiplied by the dilution factor. The number of cells (N) was reported as the average of the triplicate plate counts. All enumeration values (N_t) at time t were normalized using the initial enumeration (N_o) of *E. coli* prior to treatment. Additionally, a single factor analysis of variance (ANOVA) was performed for each time interval on the normalized replicated plate counts.

2.6. Assessment of nanowire degradation

The synthesized nanowires were characterized using scanning electron microscopy (SEM). A detailed discussion of the X-ray diffraction (XRD) and transmission electron microscopy analyses appears in previous publications by Vaddiraju and coworkers [17–20]. After synthesis, the physical dimensions (diameters and lengths) of the as-obtained nanowires were measured using SEM analysis. Additionally, to determine nanowire photostability, scanning electron micrographs were taken before and after the disinfection trials.

3. Results and discussion

3.1. Preliminary disinfection study

A preliminary proof-of-concept disinfection trial was conducted on only the WWTP isolate (Fig. 1). Because treated wastewater typically undergoes UV disinfection, the control was subjected to UV light during this preliminary study. At least a 5-log reduction in the surviving culturable fraction of *E. coli* was achieved for all materials. The disinfection extent during the initial 20-min exposure provided the basis for the subsequent studies. This preliminary study demonstrated not only that a 20 min exposure time was sufficient to achieve greater than a 7-log
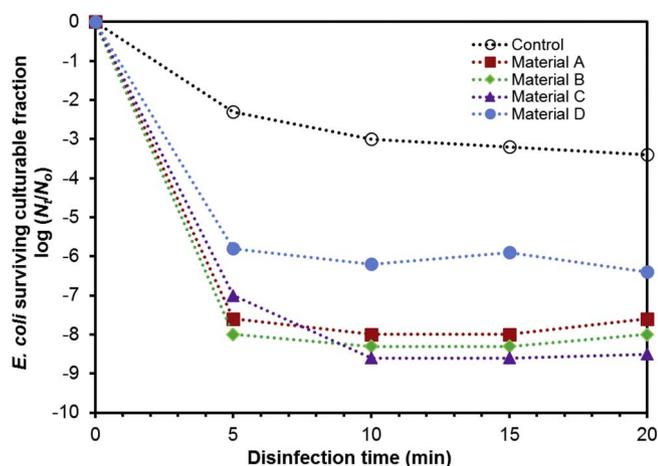
Fig. 1. Preliminary disinfection study: average log of surviving culturable fraction of WWTP *E. coli* isolate, n = 3 repeated measurements. Only the control was exposed to UV. Materials A, B, and C were zinc phosphide nanowires: boron nitride decorated, unfunctionalized, and 1,4-benzenedithiol functionalized, respectively. Material D was bare zinc foil.

reduction using the nanowires, but also that all reactors containing a photocatalyst outperformed the UV control during the same duration.

3.2. Disinfection studies

The unfunctionalized zinc phosphide nanowires (Material B) consistently produced the highest log reduction during the treatment period for each isolate (Fig. 2). Previously in the preliminary study, all materials including bare zinc foil, which was originally included as an exemplar of an inert material, achieved at least a 5-log reduction in microbial load. However, in subsequent studies, zinc foil (Material D) performed as well as BDT functionalized zinc phosphide nanowires (Material C). Although Material C promoted adequate disinfection (greater than a 4-log reduction), it was the least effective nanomaterial used in this study.

For each of the three isolates, increased disinfection was observed for each reactor treated with Material A, B, C, or D. Whereas after 20 min the control reactor never achieved greater than a 2.8-log reduction, each treated reactor exceeded a 7-log reduction for every isolate during the same period.

For each of the three isolates, the reactor treated with Material B produced the highest log reduction of *E. coli* after 5 min of exposure. This reduction was equal to or exceeded that achieved for all other materials after 20 min. The reactors treated with Material C or D were the least effective, but still achieved more than a 4-log reduction after only 5 min of exposure.

For each material, disinfection was enhanced to a similar degree for all isolates (Fig. 3).

To compare disinfection efficacies of the respective materials on the individual isolates, one-way ANOVA was conducted for each time interval, using values normalized by the initial enumeration of *E. coli*. With few exceptions (Material C at 5 and 10 min and Material D at 5 min), disinfection efficacies did not differ significantly ($p < 0.05$) between the three isolates for any of the materials. Therefore, we assume that all materials are equally effective for environmental *E. coli*, and potentially for other waterborne microorganisms.

All disinfection trials were conducted in the visible light range. Within 20 min, *E. coli* load was reduced by at least 7-log for all isolates (Fig. 3), a result comparable to those of UV disinfection studies by Guo [22] and Quek [23]. Moreover, this level of disinfection compares well to those found in other studies in which photocatalysts were used under visible light conditions. For example, using a Ag/BiOI nanocomposite under visible light, Zhu [8] achieved a 4-log reduction of *E. coli* within
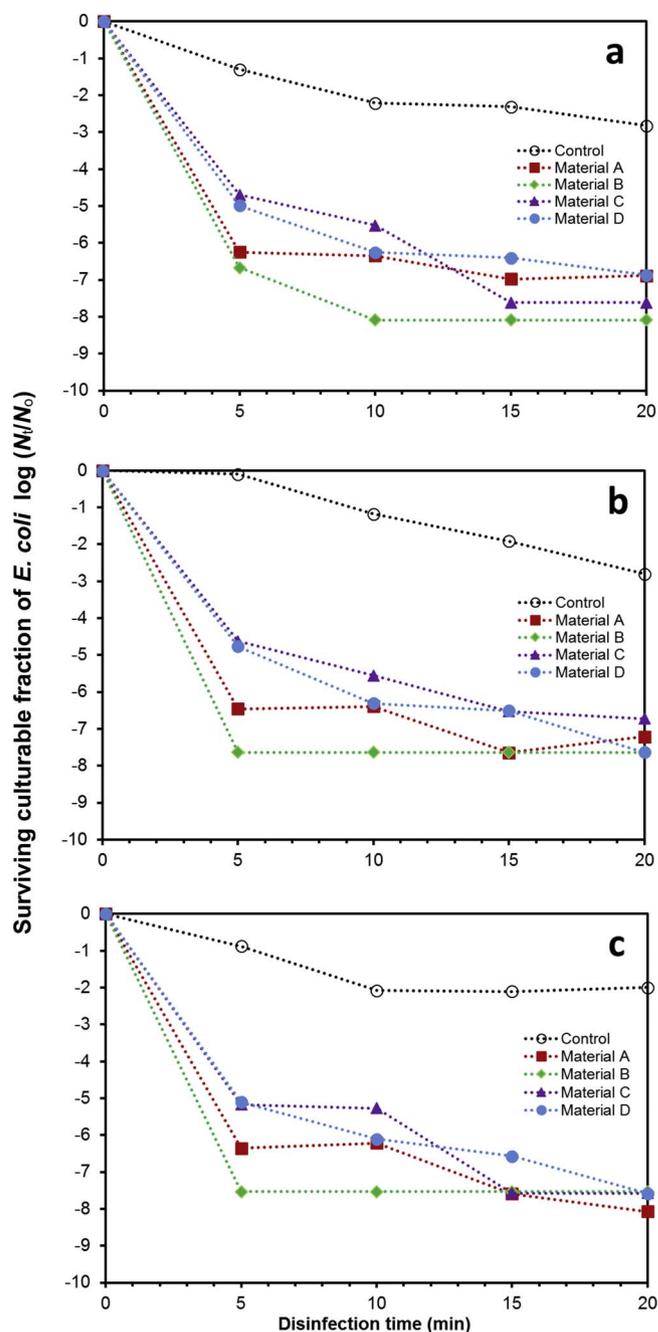

Fig. 2. Average log of surviving culturable fraction of a) WWTP, b) cattle, and c) feral hog *E. coli* isolate, n = 3 repeated measurements. All reactors exposed only to visible light. Materials A, B, and C were zinc phosphide nanowires: boron nitride decorated, unfunctionalized, and 1,4-benzenedithiol functionalized, respectively. Material D was bare zinc foil.

10 min of treatment.

A possible question is whether UV light affected disinfection in the current study. Although fluorescent lamps do emit some UV light, that portion is small (less than 4%). Therefore, it is unlikely that UV activation of zinc phosphide was the primary contributor to the photocatalytic degradation. Nevertheless, whether these nanomaterials would be further activated by UV light, thus increasing disinfection, merits additional research.

While the unfunctionalized zinc phosphide nanowires (Material B) consistently achieved the largest log reduction (Figs. 2 and 3), particularly after only 5 min of disinfection, the formation of phosphine (PH_3) may have aided in disinfection. However, all treatments achieved
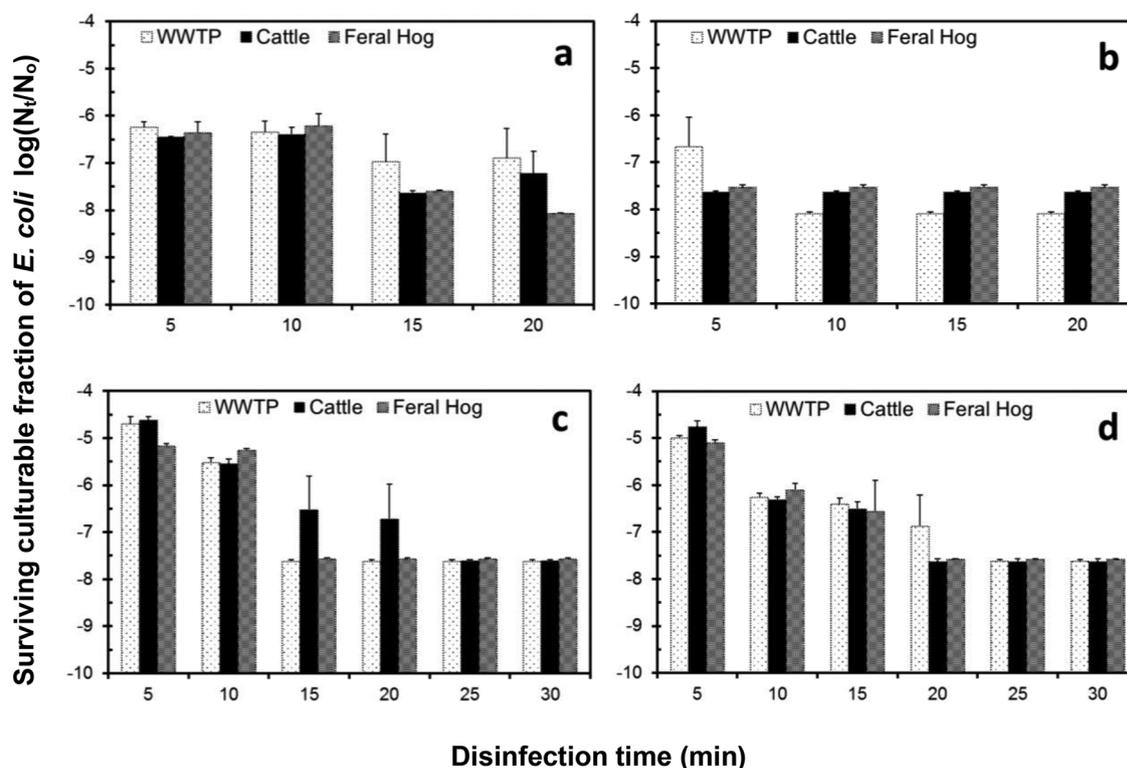
Fig. 3. Log of surviving culturable fraction of *E. coli* in reactors containing a) boron nitride decorated zinc phosphide nanowires (Material A); b) unfunctionalized zinc phosphide nanowires (Material B); c) 1,4-benzenedithiol functionalized zinc phosphide nanowires (Material C); and d) zinc foil (Material D). Error bars represent standard deviation of n = 3 repeated measurements.

greater than a 4-log reduction over the same duration. Only minimal additional disinfection was achieved by extending the exposure time with this material. Thus, shorter treatment may suffice to achieve adequate disinfection. Shorter residence time could potentially reduce disinfection costs. Therefore, depending of the level of disinfection required, a less expensive material could serve the desired purpose.

While Material B may have achieved the highest reduction in microbial load, it is not the most suitable material for water disinfection due to instability in aqueous solutions. As further discussed in a subsequent section, previous studies [17,18] indicate the necessity of stabilizing Zn_3P_2 nanowires by either decorating them with BN (Material A) or functionalizing their surfaces with organic molecules (Material C). Otherwise, the zinc phosphide may react in water to form phosphine, which is toxic [24].

The specific photocatalytic mechanism was not determined in this study. It would be fair to suggest that a reactive oxygen species (ROS), such as the hydroxyl radical, was created when the zinc phosphide nanowires were activated by visible light. More study is needed, though, to understand the reaction mechanism that is responsible for the disinfection. Still, using only a visible light source, or even sunlight, is certainly less expensive than the maintenance costs associated with UV irradiation. However, assuming the creation of an ROS, it is unlikely that Zn_3P_2 catalyzed disinfection would provide any residual effect, unlike chlorination. Also, it has not been determined if any harmful disinfection by-products would be created.

Nevertheless, the initial results are promising: high efficiency of disinfection on several types of environmental *E. coli*, with easily activated photocatalysts created from inexpensive materials. Future study should address not only the specific photocatalytic mechanism, but also the efficacy of Zn_3P_2 nanowires under a variety of strictly controlled conditions, such as pH, total dissolved solids, dissolved oxygen, and bacterial loading. Experimental conditions should simulate those typically observed after water or wastewater treatment, when UV irradiation or chlorination is generally conducted.

To optimize a treatment system, further study is required to determine the kinetic rates of bacterial inactivation for each material, particularly in the 0 to 5 min period, where the greatest reduction occurred. No difference in disinfection efficacy was observed between the WWTP, cattle, and feral hog *E. coli* isolates. Thus, these materials seem equally effective on different sources of environmental *E. coli*.

3.3. Reactivation assessments

Reactivation was assessed as log repair after treatment (Table 1). For all materials, and for both light and dark post-treatment conditions, minimal reactivation was observed. Log increases in the average cell number for any given material or isolate did not exceed 1.48 for dark repair or 1.43 for photoreactivation. In both cases, the remaining level of *E. coli* disinfection 24 h after treatment was less than the USEPA's recreational water quality criterion of 126 CFU per 100 mL.

The extent of photoreactivation and dark repair using the Zn_3P_2 nanowires are comparable to that observed in studies using UV disinfection [22,25]; for instance, Martin [25] observed up to 1.2-log increase due to photoreactivation. Similar to use of UV [4,6,22], the use of photocatalysts would not provide residual disinfection. However, there are no potentially carcinogenic by-products or safe-handling

Table 1
Log increase of *E. coli* after 24 hr of reactivation.

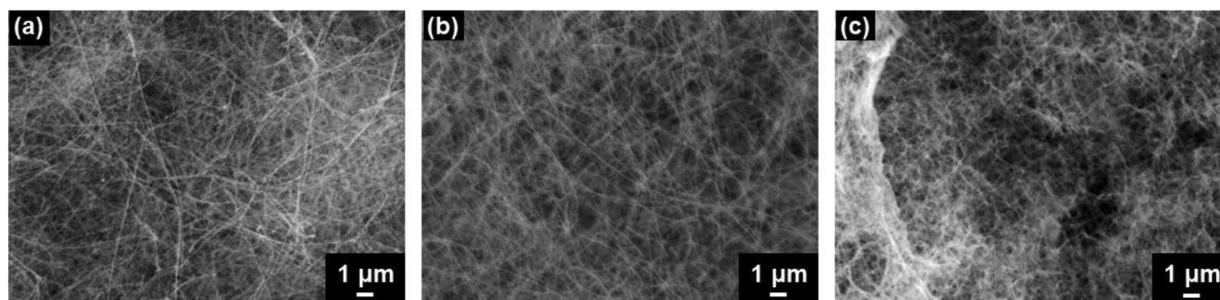|	WWTP		Cattle		Feral Hog	
	Dark Repair	Photo-reactivation	Dark Repair	Photo-reactivation	Dark Repair	Photo-reactivation
Material A	0.42	0.34	0.80	0.57	1.48	1.30
Material B	1.30	1.60	0.82	1.37	0.52	1.01
Material C	0.60	1.43	0.82	1.43	1.00	1.22
Material D	0.56	1.43	0.56	1.43	1.00	1.37

Fig. 4. Scanning electron micrographs of Zn_3P_2 nanowires (a) as-obtained, (b) exposed to deionized (DI) water for 10 min, and (c) exposed to DI water for 20 min.

issues, as may occur with chlorination [5].

3.4. Photostability of zinc phosphide nanowires

Degradation of the nanowires, such as changes in dimensions or morphologies (i.e., appearance of pores on the surface), were not observed in this study. Scanning electron micrographs were taken of the as-obtained unfunctionalized Zn_3P_2 nanowires (Fig. 4). The diameters of the nanowires were 20–40 nm and the lengths were tens of microns. No significant changes to the morphologies or the dimensions were observed after the nanowires were exposed to deionized (DI) water for 10 and 20 min (Figs. 4b and c, respectively).

Similarly, micrographs were taken of the BN decorated Zn_3P_2 nanowires before exposure to DI water and after for 10 and 20 min (Fig. 5). Like the as-obtained Zn_3P_2 nanowires, the BN decorated nanowires did not degrade and no changes in morphologies or dimensions were observed. From a previous study, the BDT functionalized Zn_3P_2 nanowires also were expected to remain stable in water [17].

The syntheses of unfunctionalized and BDT functionalized Zn_3P_2 nanowires have been reported by Vaddiraju [14,17,18,20] aimed at understanding their stabilities and their use in the fabrication of thermoelectrics and as photocatalysts for splitting water in obtaining hydrogen fuel. In the current disinfection studies, the scanning electron micrographs did not show degradation of the nanowires. Moreover, in a previous study, nanowires decorated with organic molecules (as in Material C) did not degrade even after 120 days exposure to tetrahydrofuran [17]. Therefore, we conclude that these materials are stable for the treatment times currently used.

It is important to note that in the case of Material B, bare Zn_3P_2 may degrade to release phosphine. Therefore, the unfunctionalized Zn_3P_2 nanowires may not be stable in aqueous media for long durations, and thus unsuitable for water disinfection. However, BN decorated and BDT functionalized zinc phosphide nanowires (Materials A and C, respectively) should remain stable in water over extended periods. Still, longer bench testing is required to ensure that these nanowires would not degrade during prolonged use.

4. Conclusion

Under visible light conditions, several types of zinc phosphide nanowire photocatalysts were successful in disinfecting water contaminated with *E. coli*. These studies show microbial degradation with at least a 4-log reduction within 5 min of treatment, with minimal reactivation observed 24 h after treatment. The best disinfection results, however, were obtained for the unfunctionalized nanowires, which may not be suitable for long-term use in water. Conversely, for the treatment times used in this study, BN decorated and BDT functionalized nanowires achieved comparable results and are not expected to degrade in water. From our previous studies, it is also expected that BN decorated nanowires will not easily foul or corrode over extended periods of time. Because zinc phosphide is toxic, further studies are needed ensure that zinc or phosphorus contamination does not occur upon exposure to the nanowires. A significant advantage of these photocatalysts is that they do not require activation with UV light, which may prove to be an economical alternative to current water disinfection processes. In addition to the stability of the nanowires, the specific photocatalytic mechanism(s) and the kinetic disinfection rates deserve further investigation. Potential fouling of the photocatalysts, if any, and the creation of potentially harmful disinfection by-products should also be addressed

Water disinfection using zinc phosphide nanowires merits further study to characterize the disinfection mechanism(s) and to assess the broader effectiveness against other waterborne microorganisms. The advantages of using photocatalysis for water disinfection include: 1) high disinfection efficiencies, 2) shorter treatment times, 3) potential applicability to various types of environmental microorganisms, and 4) inexpensive and simple to use.
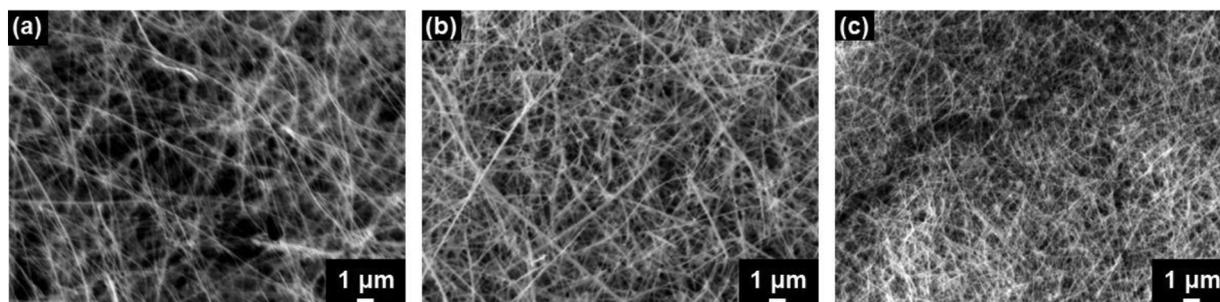
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Fig. 5. Scanning electron micrographs of boron nitride decorated Zn_3P_2 nanowires (a) as-obtained, (b) exposed to DI water for 10 min, and (c) exposed to DI water for 20 min.

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