Metal incorporated Philippine Abaca fiber (Manila hemp) as a potential novel filter for water disinfection

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Abstract. The contamination of water sources by pathogenic bacteria poses a threat both in the environment and in human health. The incorporation of metal nanoparticles in polymer matrix which is abundantly available in a country can be improved to enhance its antimicrobial property. This study was focused on the development of a novel antibacterial water filter from synthesized silver-copper nanoparticles (Ag-CuNPs) incorporated into Philippine abaca fiber (Manila Hemp). Pre-treatment method and determination of adsorption capacity of abaca fiber towards metal ions, Ag⁺ and Cu²⁺ were done prior to synthesis of Ag, Cu and Ag-Cu nanocomposites. Alkali treatment of the fiber confirms OH, C-O and C=O of a cellulose, pectin and lignin in the FTIR analysis. These groups effectively altered the nature of the abaca fiber to hydrophobic, thus, increasing its adsorption capacity up to 80%. Morphological and structural properties of the formed nanoparticles were confirmed using scanning electron microscopy (SEM) revealing polymeric matrices of the fiber in the particle size of 80 nm – 100nm. UV-Vis spectra revealed a broadening of the absorption spectra of a bimetallic Ag-Cu nanoparticles at 410 nm. The antimicrobial assay results revealed promising synergism of the combined silver and copper nanoparticles against both Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) bacterial strains in synthetically prepared water. Also, a relatively proximate bactericidal efficiency was attained between the CuNPs and Ag-CuNPs abaca fiber composites. The developed Ag-CuNPs abaca fiber composite can act as novel antibacterial water filter for water disinfection.

1. Introduction

Nowadays, most of the bodies of water in the Philippines have been contaminated with various types of pollutants. Even the tap water can still contain numerous microorganisms. Natural plant fibers have been used as water purification aid [1-2]. In 2018, Umuokpong and Edward, two teenagers who demonstrated the effectiveness of a mixture of powdered coconut husk fibers and fibers from barks, stems and leaves of Costus afer as water filter [1]. The fibers were able to separate the pollutants from the water through ion exchange. Otterhall et al. used softwood pulp fibers and coffee filter papers to eliminate faecal bacteria [2]. In addition to nanofibers, metal nanoparticles incorporation, such as gold, silver, copper, titanium and zinc, are sighted to have various beneficial applications because of their catalytic activity, magnetic activity, optical and electronic property and antimicrobial activity [3]. Nanofibers provide high surface-area-to-volume ratio, microporosity, and ability for drug loading, and this makes them essential in the field of nanobiotechnology. Cellulose is one of the most abundant
natural biopolymers which exhibit critical importance. It is reported that cellulosic matrix can be a viable polymeric matrix for the incorporation and synthesis of silver and copper nanoparticles. The effectiveness of silver, Ag, nanoparticles to disrupt the microbes is depended on the metal size, fixation and shape [4]. Copper, Cu, on the other hand, also demonstrated antimicrobial activity when incorporated into fibers but have less absorptivity compared to Ag [5].

Abaca fiber or *Manila Hemp* is one excellent source as cellulose that can be a matrix for the impregnation of metal nanoparticles. The tensile strength of abaca fiber contributes for it to be an efficient matrix and reducing agent for the synthesis of silver and copper nanoparticles. Thus, this study aims to develop an antibacterial water filter using Ag-Cu nanoparticle incorporated in local abaca fiber (Manila Hemp). The synthesized nanocomposite was exploited as antibacterial water filter against a wide range of bacterial communities such as *Staphylococcus aureus* and *Escherichia coli*, usually found in contaminated water. The study involved the determination of adsorption capacity of abaca fiber for both Ag$^+$ and Cu$^{2+}$ ions; in-situ synthesis of Ag-Cu nanocomposite, Ag nanocomposite and Cu nanocomposite; and evaluation of the synergistic effect of Ag-Cu nanocomposite against *Staphylococcus aureus* and *Escherichia coli*.

2. Methods

2.1. Materials and chemicals

Sample of local abaca fiber (*Manila Hemp*) was obtained from the province of Albay, Bicol, Philippines. Standard solution of 0.01 M silver nitrate (AgNO$_3$) and murrexide indicator were received from Scientia Tech. Corporation. Analytical grade reagents and solutions of 0.1 M sodium hydroxide (NaOH), 0.01 M sodium chloride (NaCl), 5% potassium dichromate (K$_2$Cr$_2$O$_7$), 1.0 M sucrose (C$_{12}$H$_{22}$O$_{11}$), 0.01 M ethylenediaminetetraacetic acid (EDTA), and 0.01 M copper sulfate pentahydrate (CuSO$_4$$\cdot$5H$_2$O) were prepared at the Polytechnic University of the Philippines College of Science Laboratory.

2.2. Alkali treatment of abaca fiber

The fibers were cut into approximately not more than 1 cm and washed with distilled water to remove impurities. After washing, these fibers were placed in an oven at 115 °C until dry. About 15.0 g of the dried fibers was immersed in a 0.1 M NaOH solution for 1 hour at 40°C - 60°C with continuous spinning. Then the fibers were washed with distilled water until neutral pH followed by oven drying.

2.3. Adsorption capacity of abaca fiber

The adsorption capacity of abaca fiber with Ag$^+$ ions were performed by immersing 10 g of dried fibers in 80 mL 0.01 M AgNO$_3$ at 40°C - 50°C with continuous spinning for 40 minutes. Every 5 min, 5 mL aliquot of the solution was subjected to titration for the determination of the effects of varying time in the adsorption capacity of the fiber. Similar procedure was performed for the adsorption capacity of the fiber with Cu$^{2+}$ ions whereas, dried fibers were immersed in 0.01 M CuSO$_4$ solution under 40°C - 50°C and continuous spinning for 60 min wherein after every minute, 5 mL aliquot was subjected for titrimetric analysis.

2.4. In-situ synthesis of nanoparticles in abaca fiber

For the synthesis of abaca fiber Cu nanocomposites, CuNPs, 10 g of alkali treated fiber was immersed into 80 mL 0.01 M CuSO$_4$ solution and heated at temperature range of 50°C - 60°C with spinning. Addition of 30 mL 0.1 M NaOH was done after 25 min of heating with 0.01 M CuSO$_4$ solution. A 25 min heating with the said solution would allow the abaca fiber to adsorb Cu ions. Then, at the same temperature, 10 mL of 1 M sucrose was added dropwise after 30 min and every 15 min thereafter up to a total 60 mL. The sucrose serves as the reducing agent for the synthesis of the Cu nanoparticles. The in-situ synthesis for CuNPs lasted for 5 hours in total. The CuNPs synthesized abaca fiber was then subjected into oven drying. For the synthesis of Ag nanocomposite, 10 g of alkali treated abaca
fiber was immersed into 80 mL AgNO$_3$ solution for 20 min. This allowed the fiber to adsorb Ag ions. Then, 30 mL of 0.1 M NaOH was added and heated at a temperature range of 50°C - 60°C with spinning. The sample was then added with 5 mL of 1 M sucrose for reduction. Then, 5 mL aliquot was collected after 5 minutes prior to the addition of reducing agent. The reduction and synthesis process were continued up to 30 min until an approximately 30 mL of sucrose was added. Aliquots were collected at 5 min interval and then subjected in UV-Vis spectrophotometer.

The synthesis of bimetallic Ag-cu nanoparticles was done by immersion of 10 g of alkali treated abaca fiber in mixed 80 mL of AgNO$_3$ and CuSO$_4$$\cdot$H$_2$O solution. The mixture was heated with temperature ranging from 50°C - 60°C with continuous spinning for 25 minutes. Then 30 mL of 0.1 M NaOH was added and the mixture was continuously stirred for another 30 minutes before adding 60 mL of 1 M sucrose dropwise.

2.5. Characterization
UV-vis spectrophotometer was used for the determination of silver nanoparticles. Relative to surface plasmon resonance absorption property of the said metal nanoparticle, the absorption spectra were analysed in the range of 300 nm to 700 nm. Characterization of morphology and particle size was done through the use of Scanning Electron microscopy (SEM). Different sample composites were analysed at the De La Salle University, Science and Technology Research Centre. Analysis of the structural modification through alkali treatment of the abaca fiber was done through fourier transform infrared spectroscopy (FTIR) analysis.

2.6. Antimicrobial Assay
The antibacterial property of the synthesized nanoparticle composites was tested against *Escherichia coli* and *Staphylococcus aureus*. Bacterial inoculum subcultures were grown overnight in the incubator at 37 °C. A microbial suspension of *Escherichia coli* and *Staphylococcus aureus* were prepared by transferring a loop of bacterial colony in 10 ml sterile water. 1 mL aliquot was then measured and transferred into another sterile water for serial dilutions of 102 for each filter. All of the apparatus used and the nanoparticle composites were autoclaved. The 7 g nanoparticle composites were inserted in a 60 mL sterile syringe tube which served as the water disinfection column. Each synthetic water samples containing *Escherichia coli* and *Staphylococcus aureus* were then allowed to flow in different columns which contain different nanoparticle composite filters. The treated water samples were collected in a vial and a 2 mL aliquot were collected and transferred into specific petri dishes. Petri dishes were properly labelled with details and specifications. A triplicate of 2 mL aliquot of treated samples was subjected into pour plating and labelled properly. All of the plates were then transferred in an incubator for 24 hours. Each plate is then read and the number of colonies forming units are determined and recorded. The bacterial concentrations of the bacterial growth in each plate were also determined.

3. Result and discussion

3.1. Effect of alkali treatment to abaca fiber
The major components of the *Manila Hemp* fiber were cellulose, hemicellulose, lignin and pectin which was confirmed by FTIR. Alkali treatment of the fiber subsequently removed the binding materials leaving mostly cellulose. The reduced of absorption band around 3400 cm$^{-1}$ was attributed alteration of the associated hydroxyl (O-H) group present in the fiber and peak around 1650 cm$^{-1}$ to 1250 cm$^{-1}$ associated to C-O and C=C stretching groups was no longer observed due to the treatment. These functional groups are very essential to the binding of metal nanoparticles. Different functional groups such as hydroxyl, and carboxylic group present into the fiber can promote efficient binding of the metal ions, and anchoring of the formed nanoparticles onto the surface and matrix of the fiber. Moreover, the abaca fiber itself serves as the medium for the in-situ synthesis of both metal
nanoparticles. Polymeric matrices in Manila hemp can significantly influence the mean particle size of the formed nanoparticles.

3.2. Adsorption of metals into abaca fiber
The adsorption capacity of the polymeric matrix of Manila Hemp fiber towards Cu and Ag ions were investigated. At the beginning of the contact time, a drastic increase in the concentration of the adsorbed copper ions was exhibited. An optimum amount of 1.07 mg Cu per gram of the Manila Hemp fiber was adsorbed at the first 5 minutes of mixing. Similarly, highest amount of Ag adsorbed per gram of the Manila Hemp fiber was found at the first 5 minutes of the adsorption process, with the value of 0.91 mg/g. It is found that the initial concentration of the adsorbate, amount of the used adsorbent, and contact time are some of the important key factors that significantly influence the adsorption of metal ion. It was observed that the adsorbed amount of copper ions increased with increasing temperature. Increasing the concentration of the copper ions showed a slow rise in the rate of adsorption attributed to the number of active adsorption site, the -CH$_2$OH group act as the major active site for binding Cu ions, at the end of adsorption process – significantly limiting the adsorption of the Cu ions. The colour changes observed indicate presence of AgNP and Ag-CuNPs in the samples. However, comparing the experimental absorption spectra of Ag nanoparticles alone and bimetallic Ag-Cu nanoparticles, as seen in figure 1 that the same broadening in the absorption spectra of the bimetallic Ag-Cu nanoparticles was attained at the characteristic wavelength of absorption of AgNP at 390-470 nm. Unfortunately, CuNP peak expected around 575 nm was not observed. This is a clear indication of fast oxidation of CuNP to CuO even after embedded into the Manila Hemp fibers. Additional treatment should be done to avoid oxidation.

![Absorption Spectra of Ag NPs & Ag-Cu NPs](image)

Figure 1. The absorption spectra of the AgNPs (below) compared to Ag-CuNPs (above) were fairly similar showing peak at the characteristic AgNP absorption peak indicating possible oxidation of CuNPs to CuO.

Figure 1 shows a broad peak at the Ag-CuNPs peak (upper) compared to AgNPs (lower). Pure CuNPs absorption would normally appear at around 590 nm while CuO will appear at 220-250 nm. Oxidation and weak adsorption of CuNPs onto fibers have been reported previously [5]. Nevertheless, UV Vis may not the best method to confirm presence of CuNPs as it does not absorbed well.
Furthermore, the SEM images showed that CuNPs are adsorbed onto the Manila Hemp as well as AgCuNPs. Tiny speckles onto the surface of the CuNPs treated Manila hemp fiber indicated that the NP metal was adsorbed as shown in figure 2. From the measurement of the small particles, an average size of $107 \pm 20$ nm CuNPs and $80 \pm 10$ nm Ag-CuNPs were adsorbed onto the Manila hemp. Also, using EDTA titration, it was confirmed that Cu and Ag was adsorbed by the Manila Hemp fibers. With the significant influence of the amount of adsorbent, initial concentration of the adsorbate, and contact time, an average of $0.89 \pm 0.10$ mg of Cu and $0.079 \pm 0.06$ mg of Ag was adsorbed per gram of Manila Hemp fiber.

![SEM images of adsorption of CuNPs and Ag-CuNPs onto Manila Hemp fibers confirmed nanoparticle size of the metal.](image1)

**Figure 2.** SEM images of adsorption of CuNPs and Ag-CuNPs onto Manila Hemp fibers confirmed nanoparticle size of the metal.

### 3.3. Bacterial activity of metal particles against bacteria

Each slide of the three replicate samples was then observed under the microscope for colony forming units (CFU). The percentage (%) of inhibition was determined as $\frac{[(C_c-C_t)/C_c] \times 100}{C_c}$, where $C_c$ is an average of three replicates of CFU in the negative control, and $C_t$ is an average of three replicates of CFU in the treated sets [6]. The metalNPs unto the Manila hemp successfully eradicated growth of bacteria in water. After bacterial suspensions of gram-positive bacteria, *Staphylococcus aureus* and gram-negative bacteria, *Escherichia coli*, were allowed to flow in a column containing a 7 g of different Manila hemp treated nanocomposites. As shown in table 1, almost all of the metalNPs treated Manila hemp have similar inhibition with the CuNPs-containing Manila Hemp fiber has a relatively higher % bacterial reduction relative to other treated bacterial suspension. This indicates that Cu and Ag nanoparticles both have significant contribution in the eradication of the bacteria. However, either one of them may be just enough for this purpose.

Cellulose fibers have been tested for its antimicrobial tendency but adding metal nanoparticles will greatly improve this [7]. Previous studies conducted reported that increased load of Ag and Cu nanoparticles in cellulose matrix enhances the antibacterial effect of the nanoparticles [3,7]. The relatively thick peptidoglycan layer in the cell wall of gram-positive bacteria acts as a protective layer and hindrance for the electrostatic interaction and the entrance of metal nanoparticles into the inside of...
bacterial cell. It was described that metal nanoparticles can promote release of ions upon contact with bacterial cell. A higher concentration of metal nanoparticles being introduced with bacterial suspensions allows a higher release of ionic species and formation of reactive oxygen species. Thus, it can be inferred that increasing the loaded concentration of metal nanoparticles in cellulotic matrix promotes a higher antibacterial property. A similar amount of metalNPs as depicted in this study resulted to similar % inhibition. The relative amount of loaded metal nanoparticles greatly affects its bacterial activity. It was elaborated that increasing the amount of metal nanoparticles made contact with bacterial suspension results a more effective antibacterial activity [3].

Table 1. Average % bacterial inhibition of metalNP Manila hemp filter (n=3).

| Manila hemp filter treatment | S. Aureus (%) | E. Coli (%) |
|-----------------------------|---------------|-------------|
| AgNPs                       | 51.18         | 82.61       |
| CuNPs                       | 54.60         | 86.02       |
| Ag-CuNPs                    | 50.47         | 82.69       |
| AgNPs and CuNPs             | 47.88         | 81.93       |

Pinto et al. [3] observed the antibacterial property of CuNPs against the bacterial suspension of *Staphylococcus aureus*. The log reduction of bacterial growth in their experiment observed that a gram-positive bacterium was less inhibited comparable with a gram-negative strain of bacteria. Similar to this study, a strain of *Staphylococcus aureus* has a relatively higher resistance compared with strains of a gram-negative bacteria. The cell wall of gram-negative bacteria is more susceptible into interaction with metal nanoparticles because of its relatively thin layer of peptidoglycan which ranges from 2 to 3 nm. In this regard, a gram-positive bacterium has a relatively thicker peptidoglycan layer than ranges from 30 nm and above. Thus, in this regard it can be inferred that antibacterial activity of metal nanoparticles against gram-positive bacteria are not pronounced compared with gram-negative bacteria. On the other hand, *Escherichia coli* as a gram-negative bacteria have a higher electrostatic interaction between metal ions and the negatively charged cell wall. Ruparelia et.al [8] reported that release of metal ions occurs upon introduction of metal nanoparticles into a solvent or a solution. These metal ions are then attracted into the surface of the negatively charged cell wall of bacteria and the interaction of metal ions causes rupture in the cell membrane leads to protein denaturation and cell death.

Furthermore, the results reported by Sondi and Salopek-Sondi [9] for metalNPs showed approximate 70-90% bacterial reduction against *Escherichia coli* with Cu nanoparticles exhibiting highest inhibition which was relative with the data gathered in this study. It was reported that the bacterial activity of metal nanoparticles is attributed to the relative increase in permeability of the bacterial cell wall upon contact with metal nanoparticles. The increase in permeability affects the passage and transport mechanism of bacterial cell which leads to bacterial death. Moreover, CuNPs have the higher effectivity against gram-negative bacteria such as *Escherichia coli* compared to AgNPs.

4. Conclusion

Metal nanoparticle treated Philippine *Manila hemp* fibers were tested for its antibacterial property and was efficiently inhibited at least 50% of water-borne bacteria. The treated *Manila hemp* fibers showed a relative difference in the antibacterial activity against gram-positive *Staphylococcus aureus* and *Escherichia coli* gram-negative bacteria. Amount of loaded AgNPs and CuNPs treated *Manila hemp* fibers can relatively enhance the antibacterial activity of the metal nanoparticles. The developed Ag-CuNPs Abaca fiber composite act as an efficient water filter for water disinfection is comparable with reported data. However, it was found out that the CuNPs-Abaca fiber composite have the highest antibacterial effect against the two bacterial strain. Although more tests should be done, preliminary data reveals that Philippine *Manila hemp* fibers incorporated with Ag-CuNPs can be a potential novel water purification filter.
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