INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact Factor: 6.078
(Volume 7, Issue 4 - V7I4-1648)
Available online at: https://www.ijariit.com

Antibacterial activity of filter material based on garnet and silver vein graphite composite

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ABSTRACT

Disinfection of microbially contaminated water is an important part of water purification, aiming to inactivate pathogenic microorganisms in water. Silver nanoparticles have attracted tremendous interest for water disinfection because of their antibacterial efficiency. This study focuses on the use of garnet sand coated with silver-graphite composite in deactivating waterborne microbes. Graphite was purified by acid leaching, followed by surface modification with conc. HNO_3. The silver-graphite composite material (SVG) was synthesized using $AgNO_3$ as a precursor and trisodium citrate as a reducing agent. SVG was coated around garnet sand with the assistance of three types of clays namely montmorillonite, red clay and kaolinite. Garnet sand was directly coated with silver nanoparticles to compare the durability and antibacterial efficiency. Garnet sand coated with SVG using red clay (75%) and montmorillonite (75%) showed a higher percentage coating than that used with kaolinite (25%). The antibacterial activity of coated garnet was tested using Escherichia coli. The results revealed that all coated garnet sand samples were able to decrease the concentration of E. coli from water, and no effect was observed from raw garnet sand. The antibacterial efficiencies were (13-70%), (15–57%), (38-67%) for kaolinite, red clay and montmorillonite coated garnet sand, respectively. The garnet sand coated with silver nanoparticles showed the highest efficiency (96-100%) with the lowest percentage of coating. According to the results obtained, garnet sand coated with SVG using montmorillonite showed the highest antibacterial activity and the highest percentage of the coating. Therefore, this study, suggests that the filter system with the garnet sand coated with SVG using clays can be used as a potential cost-effective alternative filtration medium for the disinfection of water.

Keywords: *Graphite, antibacterial activity, kaolinite, red clay, montmorillonite*

1. INTRODUCTION

Microbial water pollution is highly pathogenic and therefore affects human health. Thus, disinfection treatments are critical to prevent waterborne infectious diseases. Several techniques have been applied in the disinfection of pathogenic microorganisms including chemical and physical processes such as chlorination [1], ozonation [2], ultraviolet light [3] and photocatalytic process [4]. However, these processes raise economic, environmental and health issues. For instance, chlorination can produce toxic by-products (DBPs) many of which are carcinogenic (trihalomethanes (THMs), haloacetic acids (HAAs) [5,6]. In addition to chlorination, other chemical agents such as ozone also produce DBPs (bromate, iodate and chlorate) [7], and require the use of complicated equipment. Furthermore, pathogenic microorganisms increasingly develop resistance towards common chemical disinfectants. Therefore, the demand for effective and superior antibacterial agents has become a timely requirement to kill waterborne pathogenic bacteria.

Silver nanoparticles (AgNPs) can be used as effective antibacterial materials against various microorganisms including *Pseudomonas aeruginosa* [8], *Escherichia coli* and S*taphylococcus aureus* [9]. However, silver nanoparticles tend to aggregate to minimize their surface energy during synthesis which diminishes its efficiency over time [10]. Therefore, to overcome these problems, AgNPs have been immobilized on different support materials such as zeolite [11], clay [12] and graphene oxide [13] for a wide range of applications including water treatment.

Among these materials, graphene oxide has gained considerable attention due to larger surface areas and the functionalities attached on the basal plane including hydroxyl, epoxide, carbonyl, and carboxyl groups. These functional groups allow strong interaction of silver nanoparticles and graphene oxide sheets [10,14]. But the synthesis of graphene oxide is a complex and costly process that needs expensive hazardous chemicals.

To circumvent this, we have developed a novel composite from silver nanoparticles and abundant and low cost vein graphite. The composite showed higher antibacterial efficiency against *Escherichia coli* and *Staphylococcus aureus* [15]. However, this material is in powdered form and is difficult to handle in practical water treatment. Garnet granules are used as a medium in a multi-media filters. The southern and eastern coasts of Sri Lanka are characterized by red-colored beaches due to the presence of garnet-rich sands [16,17]. Garnet granules can reduce bed expansion and particle abrasion in multi-media filters during backwashing due to their high specific gravity and high hardness. Coating garnet sand with silver-vein graphite composite provides physical support and increases the accessibility of binding sites.

Clays and clay minerals are excellent fillers for metal composites. Kaolinite, montmorillonite and bentonite have been used as support materials for silver nanoparticles in water disinfection [18,19,20]. Clays are relatively abundant in nature, low-cost, and have a high surface area leading to excellent sorption capacities [21] and are useful as binding agents.

Preliminary studies have shown that silver-vein graphite composite exhibits potential for water disinfection [15]. In this study, to alleviate the problem of handling powdered silver-vein graphite composite, garnet sand was coated with this composite along with the assistance of three different clays (kaolinite, red clay, montmorillonite) separately. Moreover, the efficiency of removing the waterborne pathogen *Escherichia coli* is tested. Consequently, this study aims to compare the antibacterial efficiency of developed filter materials with silver nanoparticle-coated garnet sand.

2. MATERIALS AND METHODS

2.1. Materials

Vein graphite was collected from a commercial graphite mine, whereas garnet sand samples were collected from Hambanthota, Sri Lanka. All the reagents nitric acid, hydrochloric acid, sulfuric acid, silver nitrate, trisodium citrate, eosin methylene blue agar and nutrient broth used for this study were purchased from Sigma-Aldrich. The bacterial strain used to investigate the antibacterial activity was Gram-negative *Escherichia coli* obtained from the Medical Research Institute, Sri Lanka.

2.2 Synthesis of silver-vein graphite composite

Silver vein graphite composite was synthesized as reported in a previous study [15] and used without any further modification.

2.3 Synthesis of garnet sand coated with silver nanoparticles

$AgNO_3$ solution was heated to boil on a magnetic stirring hot plate. Then, 5 mg of garnet sand was added to the solution. Afterward, 20 mL of 1 % trisodium citrate was added dropwise. The mixture was vigorously stirred for 15 minutes and cooled down to room temperature. Then, silver nanoparticles coated garnet sand was separated by filtration and washed with distilled water. Finally, the samples were dried in an oven at 90°C for 24 hours.

2.4 Coating of silver vein graphite composite on garnet sand with the assistance of clay

To obtain clay dispersion, purified clay (kaolinite, montmorillonite, red clay) silver-vein graphite composite, garnet sand and distilled water were added into the conical flask. It was shaken for 3 using a shaker table. Coated material was air-dried for 1 hour followed by drying in an oven at 100 °C for 1 hour to remove moisture. The prepared material was heat-treated in a muffle furnace at 450 °C for 1 hour. The same procedure was repeated using kaolinite, montmorillonite and red clay.

2.5 Characterization

The crystalline phases of the samples were determined by X-ray diffraction (Ultima IV X-Ray Diffractometer) using Cu-Kα radiation with a step size of 0.02°. The percentage of the coating was identified using a gemological microscope (Stereo zoom).

2.6 Antibacterial testing

Gram-negative *Escherichia coli* was used to evaluate the antibacterial activity of five types of samples. The growth of bacterial species was determined by the plate colony-counting method after dilution. Briefly, 250 mL of broth cultures of *E. coli* was prepared and 1 mL of the 24 hours incubated cultures were mixed with 50 mL of sterilized saline. A 50 mg of different garnet sand samples were added to the flasks one at a time, and the contents were stirred on a rotary shaker (VRN- 480) at 37 °C temperature and shaken at 120 rpm.

To evaluate the influence of contact time, 1 mL of each sample was drawn after 1 h, 2 h, 3 h, 4 h, 5 h and 6 h from the flask, diluted at a 10^4 dilution and was plated with a spread plate method on eosin methylene blue agar. The plates were incubated at 37°C for 24 h. Raw garnet sand was used as a negative control. A commercially available antibiotic (Ofloxacin) was used as a positive control. After the incubation period, colonies on each plate were counted using a colony counter (Galaxy 230). The treatments were prepared in triplicate and repeated at least for three separate occasions. The antibacterial efficiency was recorded using the following equation.

$$\% \text{ Reduction of bacterial count} = ((\text{Viable count at time}_0 - \text{Viable count at time}_x) / \text{Viable count at time}_0) \times 100$$

3. RESULTS AND DISCUSSION

Raw garnet sand, garnet sand coated with AgNPs and garnet sand coated with SVG using clay were subjected to XRD analysis for phase identification. Figure 1 shows the XRD pattern of raw garnet sand, garnet sand coated with AgNPs, and garnet sand coated with SVG using different clays (kaolinite, montmorillonite, red clay).

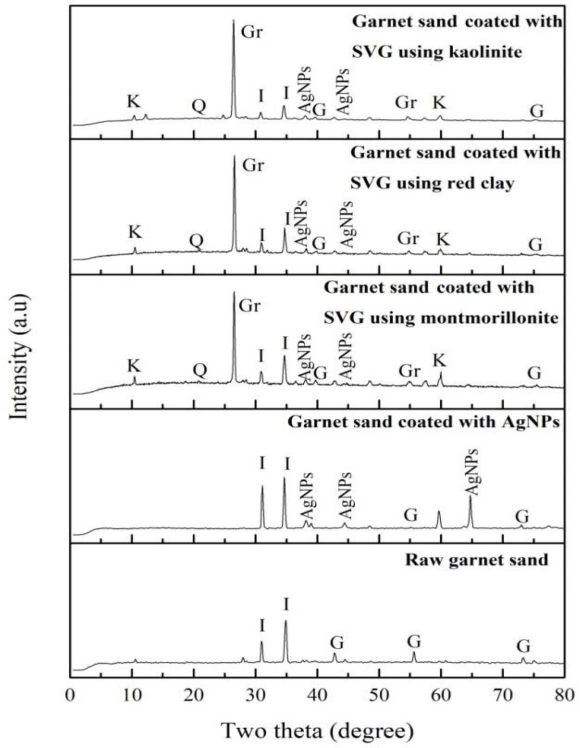


Figure 1. XRD spectra of the raw garnet sand, garnet sand coated with silver nanoparticles (AgNPs), garnet sand coated with SVG using red clay, garnet sand coated with SVG using montmorillonite and garnet sand coated with SVG using kaolinite

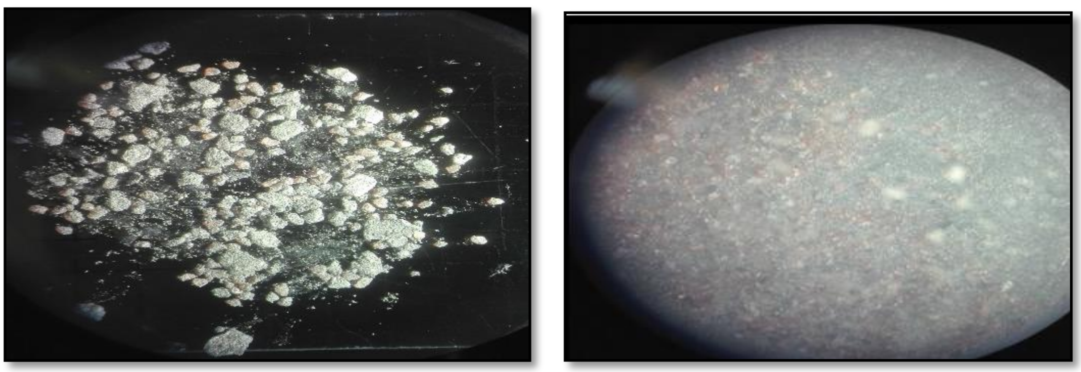
Q - Quartz, Gr - Graphite, I - Ilmenite, G - Garnet, K – Kaolinite

The peaks relevant to garnet can be seen in all the samples. SVG coated garnet sand samples show peaks relevant to garnet, graphite and silver nanoparticles (AgNPs). The major crystallographic peaks of graphite, corresponding to (002) and (004) appear prominently in this diffractogram (JCPDS # 75-1621). The prominent peaks of AgNPs, as well as silver-vein graphite composite, were observed at 2θ values of 38.1°, 44.0°, and 64.5°. These peaks can be assigned to (111), (200), and (220) planes of face-centered AgNPs (JCPDS # 87-0597).

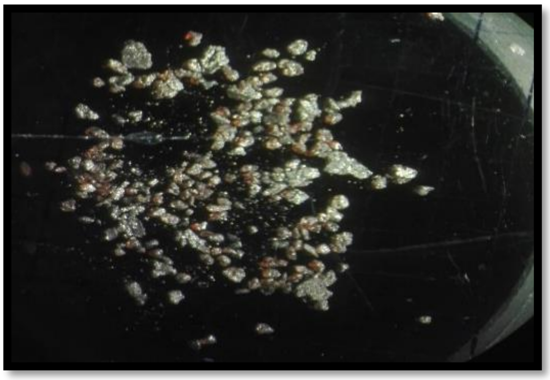
These results are also consistent with previous studies [22]. The peaks relevant to AgNPs are prominent in garnet sand coated with silver nanoparticles compared to those coated with SVG using clays. Furthermore, kaolinite and ilmenite peaks can also be seen in all the samples.

Durability of coating

Each fabricated material was observed under a gemological microscope in order to determine the durability of coatings (Figure 2). The coating of prepared material should be strong and durable for the use in water filtration applications. Coated material using kaolinite (25%) showed less durability. Interestingly, the material coated using red clay (75%) and montmorillonite (75%) showed higher durability over that coated using kaolinite. Garnet sand coated with silver nanoparticles showed almost no durability of the coating. Table 1 shows the percentage coatings of all the samples. However, chemical and physical properties of clay minerals may affect the durability of the coatings.



(a) **(b)**



(c) (d)

(e) (f)

Figure 2. Gemological microscope Images for garnet sand coated with silver-vein graphite composite using (kaolinite: (a) before dissolving in water (b) after dissolving in water; montmorillonite: (c) before dissolving in water (d) after dissolving in water; red clay: (e) before dissolving in water (f) after dissolving in water).

Table 1. The Results of the percentage of the coating

Sample name	Percentage of coating
Garnet sand coated with silver nanoparticles	0
Garnet sand coated with SVG using kaolinite	25%
Garnet sand coated with SVG using montmorillonite	75%
Garnet sand coated with SVG using red clay	75%

Antibacterial activity

Antibacterial testing was carried out using gram-negative *E. coli.* The remaining bacterial colonies were carefully analyzed after 24 hours. Figure 3 shows the removal efficiency of *E. coli* with time.

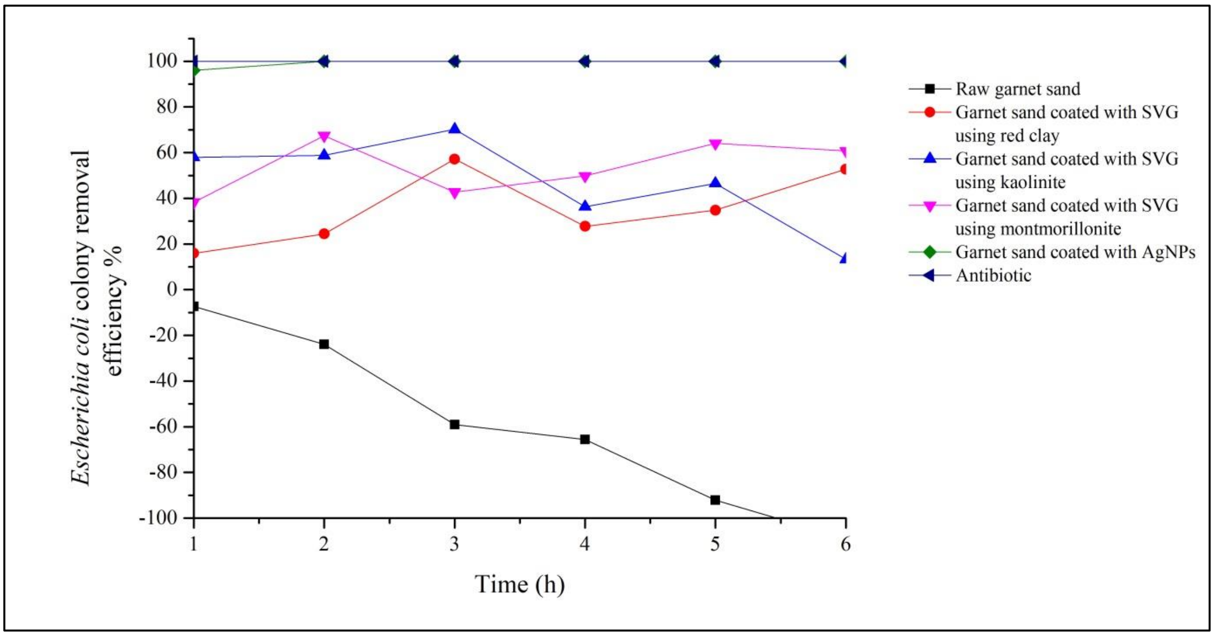


Figure 3. Percentage *Escherichia coli* colony removal efficiency with time.

Positive control (antibiotic) showed the highest antibacterial efficiency (100%) and no effect was observed from raw garnet sand. The reduction of the number of colonies showed that the garnet sand coated with SVG using montmorillonite has the highest antibacterial activity followed by those coated with red clay and kaolinite. The antibacterial efficiencies lied between 13-70% for kaolinite, 15–57% for red clay and 38-67% for montmorillonite. Most of the bacterial inactivation occurred during the first hour of incubation. Garnet sand coated with silver nanoparticles showed the highest efficiency among all the coated garnet samples with a percentage efficiency of 96-100%. However, the coating was not much durable.

Silver nanoparticles have higher antibacterial activity due to high surface-area-to-volume ratio. This results in higher surface exposure towards bacteria [23]. Many suggestions have been put forward for possible antibacterial mechanisms of silver nanoparticles. The most possible mechanism is the destruction of cells due to Ag^+ released from AgNPs, which strongly binds to thiol groups (SH) found in enzymes and proteins on the cellular surface. In addition, the AgNPs can interfere cell division which leads to bacterial cell death [24]. Another suggestion is that oxidative damage of AgNPs, produces reactive oxygen species (ROS), which attack enzymes and proteins resulting in irreversible damage to DNA replication [25].

4. CONCLUSIONS

The antibacterial activity and durability of coatings of raw garnet sand, garnet sand coated with silver nanoparticles and garnet sand coated with SVG using clays (kaolinite, red clay, montmorillonite) were prepared and compared with each other. The antibacterial efficiencies were 13-70% for kaolinite, 15–57% for red clay, and 38-67% for montmorillonite. Garnet sand coated with silver-vein graphite using red clay and montmorillonite showed higher durability than that coated using kaolinite (25%). The garnet sand coated with silver-vein graphite composite using montmorillonite showed the highest antibacterial efficiency and the highest durability of the coating. Therefore, garnet sand coated with silver-vein graphite composite using clays would be a promising filtration medium that could be applied in water disinfection.

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