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Research paper

Preparation and antibacterial activity of chitosan-based nanocomposites containing bentonite-supported silver and zinc oxide nanoparticles for water disinfection

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ABSTRACT

This study was conducted to develop novel chitosan nanocomposites and to optimize the minimum amount and contact time required to achieve complete inactivation of bacteria in water. Gram-negative *Escherichia coli* and Gram-positive *Enterococcus faecalis* bacteria were used to test the antibacterial activity of chitosan cross-linked with glutaraldehyde and chitosan nanocomposites in water. The silver and zinc oxide nanoparticles supported on bentonite were synthesized using microwave-assisted synthesis method. The resulting bentonite-supported silver and zinc oxide nanoparticles were dispersed in a chitosan biopolymer to prepare bentonite chitosan nanocomposites. The obtained bentonite chitosan nanocomposites were characterized with BET surface area measurements, FTIR, XRD, ICP-AES and SEM. When using cross-linked chitosan, it was demonstrated that factors such as pH, particle size and surface area influenced the inactivation of bacteria. For instance, the antibacterial activity of cross-linked chitosan was illustrated to increase with an increase in contact time. Meanwhile, an improvement in the inactivation activity was indicated with the introduction of silver and zinc oxide nanoparticles containing bentonite into the chitosan matrix. Although both silver and zinc oxide containing bentonite chitosan nanocomposites exhibited good antibacterial activity against bacteria, with removal efficiencies of at least 51%, the best antibacterial activity was demonstrated for silver–zinc oxide bentonite chitosan nanocomposite, with a removal efficiency of at least 78%. Furthermore, the antibacterial activity of bentonite chitosan nanocomposites was identified to be influenced by the amount of material, contact time and bacterial concentration. Finally, leaching tests demonstrated that bentonite chitosan nanocomposites were stable and, consequently, could be effectively used as antibacterial materials for water disinfection.

1. Introduction

The World Health Organization (WHO) estimates that more than 3.4 million people, many of them children, die each year from water related diseases. Globally, waterborne diseases are the second leading cause of death in children below the age of five years. It is estimated that 10% of diseases worldwide can be prevented by improving the water supply, sanitation, hygiene and management of water resources (Prüss-Üstün et al., 2008; WHO, 2013). The gravity of water shortages, including quality issues and their effects on the health of consumers, makes it necessary to direct considerable and focused efforts toward research and development programs in the drinking water sector (Savage and Diallo, 2005). WHO defines safe drinking water as water whose microbial, physical and chemical characteristics comply with their standards and national standards (WHO, 2006a). The greatest threat posed to drinking water resources arises from bacterial contamination. In addition to affecting the quality of water, bacterial contamination of water is a concern as they cause diseases that could be life-threatening upon ingestion or exposure. WHO recommends that any water intended for drinking purposes should contain fecal and total coliform counts of 0 in a 100 ml sample (WHO, 2006b). Given these concerns, many traditional treatment methods, both chemical (chlorine, ozone, iodine) and physical (ultraviolet radiation) (Boorman et al., 1999; Woo et al., 2002; Tiwari et al., 2008), have been applied to inactivate bacteria in water supplies. Although these methods can effectively reduce and control pathogenic bacteria to the desired levels, in recent years, research has revealed that such methods can lead to the formation of harmful disinfection byproducts (DBP) (Richardson, 2003a; Krasner et al., 2006).

Chemical disinfectants such as chlorine and ozone can react with various constituents of water to form DBP that are carcinogenic. However, one of the most complex and important challenges in water treatment

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is that some of these bacteria have become increasingly resistant to the available disinfectants and now require extremely high disinfectant doses, leading to the formation of greater abundance of DBP. Therefore, there is an urgent need to re-evaluate conventional disinfection methods and to consider innovative approaches that can offer enhanced reliability and robustness of disinfection while avoiding DBP formation (Richardson, 2003b, 2004; Li et al., 2008). Apart from DBP formation, the above technologies are often costly and time-consuming.

In the past two decades, advances in nanoscience and nanotechnology have expanded the possibilities for the development of high-performance nanomaterials targeted at solving the current problems related to water quality. There are four classes of nanoscale materials that are being evaluated for use as functional materials for water treatment: (1) metal/oxide nanoparticles, (2) carbonaceous nanomaterials, (3) zeolites and (4) dendrimers. These materials possess a broad range of physico-chemical properties that make them attractive for use as separation and reactive media for water treatment (Savage and Diallo, 2005; Tiwari et al., 2008). They can also be functionalized with various chemical groups to increase their affinity toward a given compound. They can be prepared as nanosorbents, nanocatalysts and reactive membranes and, therefore, exhibit promising and enhanced properties of selective bacteria inactivation and removal (Savage and Diallo, 2005; Tiwari et al., 2008; Ray et al., 2012). Moreover, given the concerns regarding the treatment resistance of pathogenic bacteria in water, the search for new disinfection agents has become a critical issue.

Silver (Ag) nanoparticles have drawn considerable interest for water disinfection because of their antibacterial activity, and they have attracted application in various consumer products (Lin et al., 2012). Ag nanoparticles are a well-known disinfectant that is effective for a wide spectrum of bacteria and viruses. It is thought to be more effective and is more widely used for Gram-negative bacteria. Drinking water often contains a broad range of both types of bacteria (Gram-negative and Gram-positive). Therefore, the treatment of water in which both types are likely to be present with a disinfectant that has been reported to be more effective against Gram-negative bacteria makes it difficult to achieve complete inactivation of bacteria (Shahverdi et al., 2007; Theivasanthi and Algar, 2011; dos Santos et al., 2012). Consequently, this approach will compromise the effectiveness of the disinfectant and increase the amount required. To compensate for this shortcoming and achieve thorough inactivation for a wide spectrum of bacteria, inorganic metal/oxide nanoparticles are often combined to form nanoparticle hybrids. Among the inorganic metal oxide nanoparticles that have been tested extensively for their antibacterial activity are zinc oxide (ZnO) nanoparticles. ZnO nanoparticles have been studied extensively using various pathogenic and non-pathogenic bacteria. They have also been reported to possess strong antibacterial activity against a broad range of bacteria (Reddy et al., 2007; Jones et al., 2008; Li et al., 2008; Azam et al., 2012; Motshekga et al., 2013). Therefore, it is highly probable that a combination of Ag and ZnO nanoparticles will be effective against both Gram-negative and Gram-positive bacteria that are typically found in water. Both Ag and ZnO nanoparticles possess larger surface areas for interaction and higher reactivity than the corresponding bulk materials and therefore produce stronger antibacterial effects (Emami-Karvani and Chehrazi, 2011; Azam et al., 2012). These unique properties make nanoparticles appealing compared to their bulk counterparts. However, the disadvantage of nanoparticles is that when used as individual components of functional materials, they tend to agglomerate, which reduces their effectiveness. Another disadvantage is that the release of nanoparticles into the environment during the treatment process poses a health risk, as the toxicity effect to the end user is not well known. For nanoparticles to be applied effectively for water disinfection, they typically must be supported on substrates such as carbon nanotubes, clays or polymers (Savage and Diallo, 2005; Li et al, 2008; Tiwari et al., 2008).

Clays and clay minerals are an excellent material for this purpose given the various relevant concerns. Clays such as bentonite and clay minerals such as montmorillonite, kaolinite, palygorskite and halloysites have been used as supporting substrates for nanoparticles in various water purification systems (Yavuz et al., 2003; Meteš et al., 2004; Bhattacharyya and Gupta, 2006; Karapinar and Donat, 2009). They can be used as individual components or as substrates for composite materials. Bentonite (Bent), which consists of more than 70% montmorillonite, has attracted considerable interest because it is easily available in bulk quantities, economically attractive and environmentally friendly and because it possess excellent swelling and adsorption properties. Bent has been used as a support to disperse and stabilize nanoparticles in various applications (Ayari et al., 2005; Hashemian, 2010; Zamparas et al., 2012).

Although there are numerous studies regarding the antibacterial activity of clay-supported metal/oxide nanoparticles, most have used the disk diffusion method to test their antibacterial effect, and no reports of further water treatment applications of these materials are available in the literature (Magaña et al., 2008; Santos et al., 2011; Shameli et al., 2011a; Hrenovic et al., 2012; Bagchi et al., 2013). To limit the leaching of nanoparticles into the water, clays that contain metal/oxide nanoparticles are often imbedded in various polymer matrices. Therefore, the robustness of applying these metal or metal oxide nanoparticles decorated clays in water disinfection is established when they are incorporated within a polymer. In this work, chitosan (Cts) biopolymer was used as a matrix. Cts is the second most plentiful natural biopolymer. It was chosen because it is non-toxic and possesses inherent antimicrobial properties. However, the antimicrobial activity of Cts is affected by a number of factors, including its molecular mass, the species and concentration of the bacteria, and the type and pH of the solution. Cts has also been widely used as an adsorbent for transition-metal ions and organic species because the amino ($—NH_2$) and hydroxyl ($—OH$) groups on Cts chains can serve as coordination and reaction sites (Zheng and Zhu, 2003; Chang and Juang, 2004; Li et al., 2008; Raafat and Sahl, 2009; Kittinaovarat et al., 2010; Guibal et al., 2013).

To date, few studies have been performed concerning the antibacterial activities of clay polymer nanocomposites, although such studies are necessary and significant. In this study, Bent was used as a supporting substrate for Ag, ZnO and Ag–ZnO nanoparticles. A facile microwave-assisted synthesis method was employed for the impregnation of the nanoparticles on the clay, while solvent-casting method was used to disperse nanoparticles containing clay in the Cts matrix. The obtained Bent Cts nanocomposites were therefore expected to demonstrate effective antibacterial activity against *Escherichia coli* (*E. coli*) and *Enterococcus faecalis* (*E. faecalis*) bacteria, which served as representatives of Gram-negative and Gram-positive bacteria, respectively.

2. Materials and methods

2.1. Materials

Pristine Bent, which was used as the solid support for Ag, ZnO and Ag–ZnO nanoparticles, was obtained from Ecca Holdings (Pty) Ltd, South Africa. Cts was purchased from Sigma Aldrich (South Africa) as a flake material. Glutaraldehyde (GLA, 50 wt.% in H_2O); phosphate buffered saline (PBS, pH 7.4); sulfuric acid (H_2SO_4, 98%); acetic acid ($C_2H_4O_2$, 99%); sodium chloride (NaCl); sodium hydroxide pellets (NaOH); silver nitrate ($AgNO_3$, 99.98%), which was used as the Ag precursor; and ZnO nanoparticles dispersed in ethanol were purchased from Sigma Aldrich, South Africa. All aqueous solutions were prepared using distilled water. Sodium thiosulfate ($Na_2S_2O_3$), nutrient broth and nutrient agar were purchased from Merck, South Africa. The bacterial strains used to provide the antibacterial activity were Gram-negative *E. coli* (ATCC 11775) and Gram-positive *E. faecalis* (ATCC 14506) from the American Type Culture Collection.