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Evaluation of the antibacterial efficacy of bamboo charcoal/silver biological protective material

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ABSTRACT

Bamboo charcoal supporting silver (BC/Ag) was prepared by activation and chemical reduction. The BC/Ag composites were characterized by silver particle size and distribution, silver ion (Ag^+) release and antibacterial properties. Scanning and transmission electron microscopy (SEM and TEM) showed that the Ag particles were distributed uniformly on the BC matrix. The Ag particle size was found to be less than 150 nm based on TEM. The Ag content and surface morphology of the BC/Ag composites depended on the initial concentration of $AgNO_3$, and the higher the Ag content, the smaller the specific surface area obtained on the BC. The antibacterial effects of the BC/Ag composite powders were assessed from the minimum inhibitory concentrations (MICs) and by the plate-counting method, and an excellent antibacterial performance was discovered.

1. Introduction

Bamboo is a renewable resource that can provide a viable source of energy when the cycle of its plantation and usage is properly scheduled. Pyrolysis of bamboo in the absence of air provides bamboo tar and bamboo charcoal (BC), the latter of which is used as an effective adsorbent for removal of humidity and odors and as a convenient solid fuel. Bamboo charcoal has a number of beneficial characteristics, including high electric conductivity and self-lubricity, and can be used as a friction material and an electromagnetic shield material [1]. On the other hand, an increasing number of bioresources have also been utilized in the preparation of adsorbents, and some studies have shown that certain bioresources have high potential for use as adsorbents. Among these, bamboo is recognized as one of the most popular bioresources, and its adsorption characteristics have been the subject of many studies [2,3].

Silver ions have long been known to have strong inhibitory and bactericidal effects, as well as a broad spectrum of antibacterial activities [4,5], and silver has therefore been used commercially, taking advantage of these antibacterial properties [6]. Silver-based antibacterial composites have to release Ag^+ to a pathogenic environment in order to be effective. The oxidation of metallic silver to the active species Ag^+ is possible through interaction of the silver with water molecules. If silver is immobilized on porous hosts, the release time of silver ions can be delayed, so that silver-supporting materials will have great potential for use in this application [7]. At present, antibacterial agents are mainly based on organic materials [8,9], which are often not usable under conditions where chemical durability is required. However, the use of silver-supporting inorganic materials can overcome this disadvantage well, and materials such as zeolites, calcium phosphate, silica and carbon fiber have all been developed as inorganic supports for antibacterial silver-containing materials [10–13].

BC/Ag composite materials are of particular interest because of their unusual antibacterial properties, and are used as water purifiers or biological protective material. The aims of the present work are to prepare antibacterial BC supporting silver (BC/Ag) using a chemical reduction method and to examine the surface structures and chemistry before and after the addition of the silver. Moreover, studies of biomaterials for use in fighting Ciprofloxacin-resistant and Methicillin-resistant bacteria are very limited; therefore, in order to explore the antibacterial effects of BC/Ag composites, zone of inhibition testing, minimum inhibitory concentrations (MICs) and the plate-counting method were used in this study to examine the antibacterial activity of the BC/Ag composites against Gram-negative *Pseudomonas aeruginosa* (*P. aeruginosa*), Ciprofloxacin-resistant *P. aeruginosa* (CRPA), *Escherichia coli* (*E. coli*) and *E. coli* JM109 (pUK18), and Gram-positive *Staphylococcus aureus*

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(*S. aureus*), Methicillin-resistant *S. aureus* (MRSA) and *Bacillus subtilis* (*B. subtilis*).

2. Materials and methods

2.1. Preparation of BC/Ag composites

BC powders (particle size <10 μm, Taiwan Paiho) were activated with surfactant sodium alginate under stirring for 1 h. The activated BC powders (2 g) were then immersed in 100 mL of biamminesilver nitrate ($[Ag(NH_3)_2]NO_3$) solution, which was formed by adding 25 wt.% aqueous ammonia to $AgNO_3$ solution at room temperature. This will favor an increased negative surface charge of BC in the basic $[Ag(NH_3)_2]NO_3$ solution and interaction of the NH_3 ligands of $[Ag(NH_3)_2]^+$ complexes with the surface of BC via hydrogen bonds [7]. The weight ratios of BC:$AgNO_3$ prepared were approximately equal to 1:0.25, 1:0.5, 1:1, 1:2 and 1:3. After stirring for 1 h, dilute aqueous solution of hydrazine monohydrate was introduced to the BC–$[Ag(NH_3)_2]NO_3$ solution in appropriate quantities (molar ratio 1:1 with respect to silver nitrate) using a syringe. Stirring was continued under an inert atmosphere at room temperature for another 4 h. The BC/Ag particles were separated and washed with deionized water and ethanol, then dried in a vacuum at 60 °C overnight. The samples were designated, for example, BC/Ag-0.25, which denotes BC reacted with $AgNO_3$ in the weight ratio 1:0.25.

2.2. Characterization

Phase identification of composites was performed using X-ray diffraction (XRD; Siemens D5000) with Cu Kα radiation, and the average grain sizes (D) of Ag were determined from the XRD peaks using Scherrer's formula. The morphology of the composites was observed using a scanning electron microscope (SEM, Hitachi S-800) and a transmission electron microscope (TEM, Philips CM-200) equipped with an energy-dispersive X-ray (EDX, Hitachi S-300) microanalysis system. The Brunauer–Emmett–Teller (BET) specific surface areas (S_{BET}) of the BC/Ag composites were determined by a NOVA 1000e automatic physical absorber using highly purified nitrogen gas at 77 K. The concentration of silver ions released from the BC/Ag composites to the aqueous medium was measured using an atomic absorption spectrophotometer (Pantech, GBC-932AA) [14].

2.3. Test of antibacterial properties

P. aeruginosa (ATCC 27853), CRPA, *E. coli* (ATCC 25922), *E. coli* JM109, *S. aureus* (ATCC 25923), MRSA and *B. subtilis* were obtained from the Food Industry Research and Development Institute, Taiwan, and were used as the reference strains in antibacterial testing. The antibacterial spectrum of the BC/Ag composites was evaluated by zone of inhibition testing. A standard inoculum of the test organism with 10^7 colony-forming units (CFU) mL^{-1} was swabbed onto the surface of a Muller–Hinton (MH) agar plate, and then discs of filter paper impregnated with antibacterial agents (6 mg mL^{-1}) were placed on the agar. The plates were incubated overnight at 37 °C, and the clear zones around the disc were measured.

The antibacterial effects of the composites were evaluated by the determination of MICs using the broth dilution method. Tubes containing 3 mL MH broth with 10-fold dilutions of the BC/Ag composites ranging from 0.3 mg L^{-1} to 0.3 g L^{-1} were inoculated with 10^7 CFU mL^{-1} of bacteria. The inoculated tubes were then incubated at 37 °C for 18 h. After incubation, tubes were examined without shaking for visible turbidity; the MIC was determined as the lowest dilution of composites that produced no visible turbidity [15]. The test was performed three times for each strain, and results in agreement on two or more occasions were adopted as the MIC of the strain.

To further investigate the antibacterial effects of the composites, the plate-counting method was used [7]. Approximately 10^5 CFU of *S. aureus* were cultured on MH agar plates supplemented with BC/Ag-1 particles in amounts of 10–50 mg. BC/Ag-free MH plates cultured under the same conditions were used as a control. The plates were incubated at 37 °C for 24 h and the numbers of colonies were counted.

To determine the degree of the antibacterial effect in the presence of BC/Ag composites, the number of remaining bacteria was examined. The test process was described as follows: 20 mg of the BC/Ag-0.25 and BC/Ag-1 powders were added into 3 mL of MH broth containing 3.5×10^6 CFU mL^{-1} bacteria. The mixture was aerobically incubated at 37 °C under vibration for 24 h. 30 μL of the above suspension was cultured on an agar plate and incubated at 37 °C for 18 h. In this study, the degree of the antibacterial effect is presented as the reduction ratio of the bacteria. The equation for quantitative antibacterial evaluation is given by $R(\%) = (A-B)/A \times 100\%$, where R is the percentage reduction ratio, A the number of bacterial colonies from the untreated bacteria suspension (without BC/Ag powders) and B is the number of bacterial colonies from the bacteria suspension treated with BC/Ag powders for 18 h.

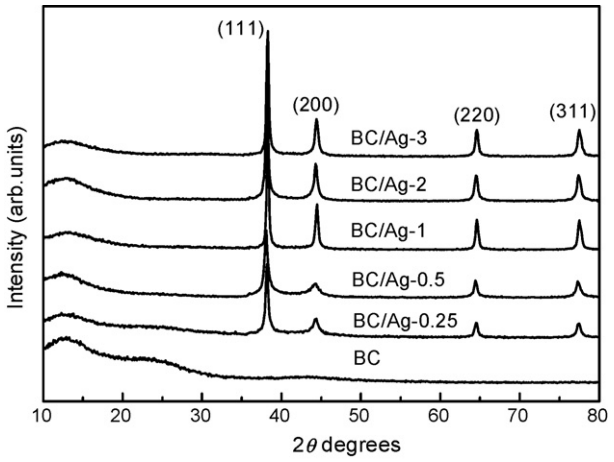

Fig. 1. XRD spectra of BC and BC/Ag composites.

3. Results and discussion

3.1. Structure characterization

Fig. 1 shows the XRD patterns of the BC/Ag composites. The weak diffraction peaks near $2\theta = 12°$ and 25° were due to the amorphous phase of BC, their intensity distinctly decreased with increasing Ag content. The XRD spectra of the BC/Ag composites were in good agreement with the values for silver nanoparticles in the literature [16]. The prominent peaks at 2θ values of about 38, 44, 65 and 77 represent the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) Bragg's reflections of face-centered cubic crystalline silver. Furthermore, increasing the amount of $AgNO_3$ in the BC induced the obvious enhancement of the characteristic peaks of silver, implying the development of larger and highly crystalline silver nanoparticles. We can estimate the size of the nano-Ag grains ($2\theta = 38°$) using Scherrer's formula, $D = 0.9\lambda/\beta\cos\theta$, where D is the crystallite size in nm, λ is the radiation wavelength (0.154056 nm for Cu Kα), β is the bandwidth at half-height and θ is the diffraction peak angle [17]. The calculated crystallite sizes were in the range of 35–100 nm.

SEM and TEM images were used to evaluate the surface morphology and size distribution of the silver deposited on the BC surface. As shown in Fig. 2, ultra-fine and disaggregated silver particles were homogeneously distributed on the surface of the BC. Pure BC has a porous surface. The silver particles are granular in nature and seem to be nanosized, typically in the range of <100 nm, which was in agreement with the XRD observations. EDX analysis confirmed the existence of Ag in the BC matrix and quantitatively revealed the Ag nanoparticles content (Fig. 2E). Table 1 gives the Ag content and S_{BET} of each BC/Ag composite, and shows that Ag content increased with increasing initial concentration of $AgNO_3$ solution, and S_{BET} of the BC/Ag composites decreased markedly with increasing Ag content, due to the direct blockage of pores by Ag particles. Ag content, particle size and S_{BET} can be controlled

Table 1
Preparation conditions, silver content and S_{BET} of the BC/Ag composites

Sample	Preparation conditions		W_{Ag} (%)	S_{BET} ($m^2\ g^{-1}$)
	BC (g)	$AgNO_3$ (g)		
BC	2	0	0	317
BC/Ag-0.25	2	0.5	6.52	253
BC/Ag-0.5	2	1	11.98	225
BC/Ag-1	2	2	26.09	171
BC/Ag-2	2	4	28.79	156
BC/Ag-3	2	6	33.78	97