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The antibacterial activity and mechanism of mussel shell waste derived material

Mei Li [a], Zhitong T. Yao [b,*], Tao Chen [a], Zhanghua H. Lou [a,*], Meisheng Xia [a]

[a] Ocean College, Zhejiang University, Hangzhou 310058, China
[b] College of Materials Science and Environmental Engineering, Hangzhou Dianzi University, Hangzhou 310018, China

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ABSTRACT

The preparation, characterization and antibacterial activity of mussel shell waste doped with silver are reported. The characterizations were performed by X-ray diffraction (XRD), scanning electron microscopy equipped with an energy dispersive spectrometer (SEM-EDS), X-ray photoelectronic spectroscopy (XPS), and Fourier transformed infrared spectroscopy (FT-IR). The antibacterial activity was evaluated against typical Gram-negative bacteria *Escherichia coli* and Gram-positive bacteria *Staphylococcus aureus*. XPS measurement and EDS analysis confirmed the incorporation of silver in the mussel shell. The antibacterial test demonstrated that the prepared material had good antibacterial property, which was mainly attributed to the silver present and slightly higher pH value.

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1. Introduction

The development of materials with the ability to inhibit bacterial growth has gained great interest over the past few years, due to their potential uses in coating, paint, textile, etc. As compared with the organic ones, inorganic antibacterial materials have advantages in terms of the durability, safety, chemical stability and thermal resistance [1–4]. These materials are generally based in metallic ions, such as Ag^{+}, Cu^{2+} and Zn^{2+}, among which silver is well known to have a great and broad spectrum antibacterial activity and relatively high safety [5–9]. Recently, various antibacterial materials containing silver, such as Ag–montmorillonite, Ag–zeolite, Ag–SiO_2 and Ag–polymer fibers have been prepared [10–16]. For practical applications, the recyclable antimicrobial materials are necessary. Dong et al. [17] prepared Ag–$CuFe_2O_4$ magnetic hollow fibers and studied their antibacterial efficacy against four bacteria. Typical ferromagnetism behavior exhibited from the material enabled their feasible recyclability.

Shellfish cultivation is an expanding economic activity worldwide. However, intensive shellfish production generates a large amount of waste. Recycling shell waste can be a good alternative to disposal, in terms of eliminating the environmental problem and yielding economic benefit. Calcium carbonate ($CaCO_3$) in the shell waste can be converted into CaO using heat treatment (calcination or pyrolysis), which triggers antibacterial activity for the treated shells. Oikawa et al. [18] compared the antibacterial activities of four calcined shells using total aerobic counts and *Escherichia coli*. It was found that the calcined surf clam shell had the highest activity. Bae et al. [19] investigated the bactericidal effects of scallop shell on food-borne pathogenic bacteria. The results indicated that CaO, as a substitute for synthetic chemical substances, has potential uses in the disinfection and sanitization of foods. Choi et al. [20] also reported that adding shell powder could enhance the shelf life and quality of kimchi. In this work, we attempt to prepare an antibacterial material using mussel shell waste as a starting material. The raw material and prepared antibacterial material were characterized and analyzed by means of XRD, SEM-EDS, XPS and FT-IR. The antibacterial activity was evaluated through growth inhibition of *Staphylococcus aureus* and *E. coli*. The antibacterial mechanism was discussed as well.

2. Materials and methods

2.1. Materials

The raw mussel shell waste was supplied by Zhoushan Seafood Processing Factory, East China. It was firstly washed removing the residual meat and attachments, and then coarsely ground. The ground sample was immersed in NaOH solution to remove the stratum corneum. Thereafter, it was dried overnight at 100 °C and finely ground using a ball mill. Sodium hydroxide and silver nitrate were of analytical grade from Sinopharm Chemical Reagent Co. Ltd.

* Corresponding authors. Tel./fax: +86 571 86919158.
E-mail addresses: sxyzt@126.com (Z.T. Yao), iwr@zju.edu.cn (Z.H. Lou).

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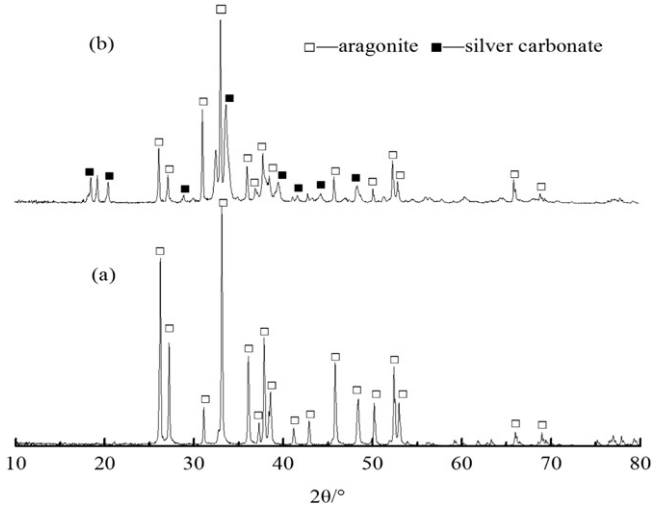

Fig. 1. XRD patterns of mussel shell (a) and antibacterial material (b).

2.2. Antibacterial material preparation

The pretreated mussel shell was dispersed in 0.1 M $AgNO_3$ solution with a w/v ratio of 1:10. The pH value of the mixture was adjusted to 6.5 under continuous stirring for 4 h. The antibacterial material was obtained by filtration, washed with deionized water and dried in the oven.

2.3. Characterizations and tests

XRD analysis was employed to determine the crystalline phases present in raw mussel shell and prepared antibacterial material. A Rigaku D-max/IIB X-ray power diffractometer was operated at 40 kV and 34 mA using CuKα radiation. Scanning electron microscopy (SEM) investigation was conducted in a Hitachi S-4800 field emission scanning electron microscope to observe the microstructure of samples. The antibacterial material was mounted on specimen stub, sputter-coated with platinum prior to the observation. N_2 adsorption measurement was carried out to evaluate the specific surface area of materials. N_2 adsorption–desorption isotherms were recorded at 77 K using a Micromeritics model ASAP 2020 adsorption analyzer. XPS measurement was carried out on a VG ESCALAB Mark II spectrometer equipped with a non-monochromatic Mg Kα X-ray source at a pass energy of 50 eV.

2.4. Ag^+ release and pH test

The antibacterial material was dispersed in the sterile phosphate buffer saline (PBS, pH 7.2) with a w/v ratio of 1:200 and shaken for different times (2, 6, 12, 20 and 24 h). Ag^+ concentrations in the filtrate were measured.

The pH values of solutions dispersed with the antibacterial material were tested as follows. The powder was dispersed in distilled water with a w/v ratio of 1:20 to make slurries. After 1 h, the pH value of the filtrate was measured.

2.5. Antibacterial test

The antibacterial activities of the prepared material against *E. coli* and *S. aureus* were determined by the agar dilution method [21,22]. The minimum inhibitory concentration (MIC) values were examined at concentrations of 0–1000 ppm for two stains and determined by the lowest concentration agent that completely inhibited visible growth on the plate [23]. The dried materials were weighted (0, 1.5, 3, 6, 9, 12, 15 mg) and exposed to UV radiation to ensure sterilization. After that, they were added into 15 ml agar suspension at 50 °C, transferred to a dish and slowly cooled until forming a gel. The initial concentrations of *E. coli* and *S. aureus* were adjusted to 10^4 CFU by dilution. A sterile inoculating loop was dipped into the bacterial suspension and streaked in a pattern on the agar plate. Agar plates were incubated at 37 °C for 24 h before being observed.

3. Results and discussion

3.1. XRD analysis

The XRD patterns of mussel shell and the prepared antibacterial material are displayed in Fig. 1(a) and (b), respectively. It can be seen

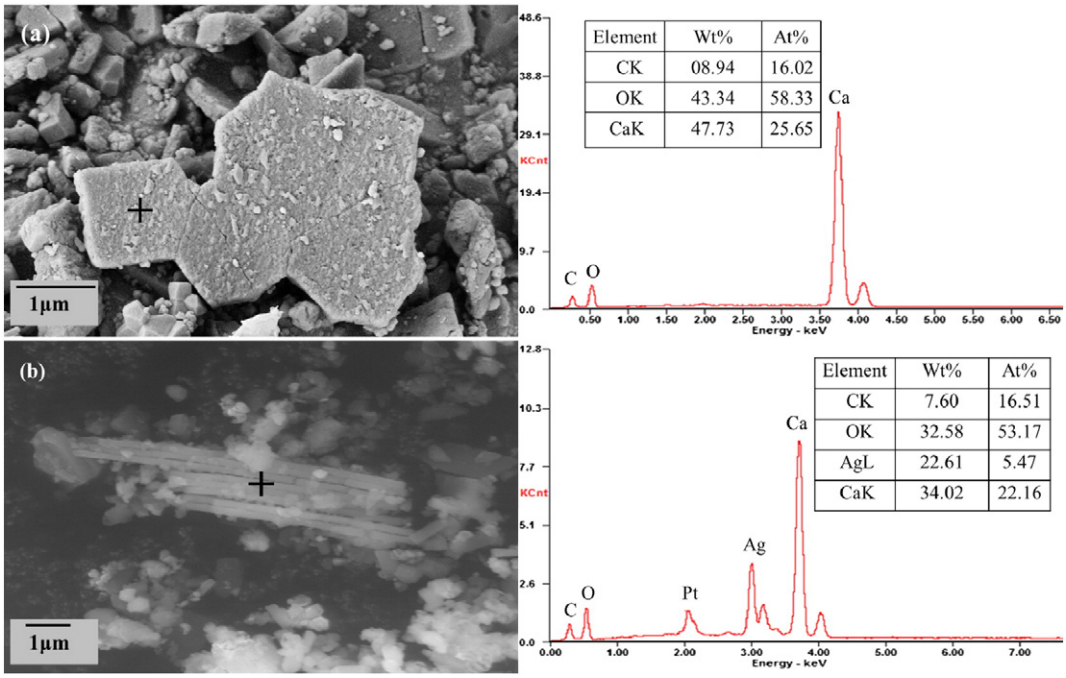

Fig. 2. SEM images of mussel shell (a) and antibacterial material (b).

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