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Highly effective antibacterial activity by the synergistic effect of three dimensional ordered mesoporous carbon-lysozyme composite

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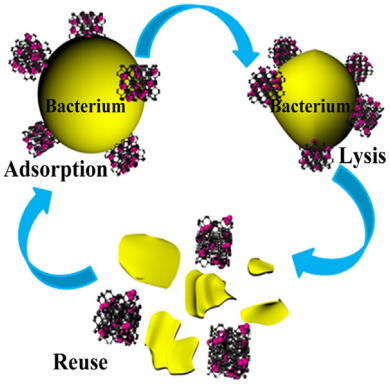
GRAPHICAL ABSTRACT



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ABSTRACT

Aiming at developing a safe and efficient alternative to traditional drinking water disinfection, this work successfully synthesized a novel antibacterial material with high surface area, ultra large pore size and tunable loading of immobilized lysozyme. The immobilized enzymes exhibit high antibacterial efficacy without forming carcinogenic disinfection byproducts. Critical immobilization parameters were optimized to keep the activity of the immobilized enzyme at a high level. The immobilization of lysozymes on 3DOm COOH could be confirmed by the characterizations of transmission electron microscopy, X-ray diffraction and Zeta-Potential. In addition, the structural stability of lysozymes on 3DOm COOH were studied by Fourier transform infrared spectroscopy. The antibacterial performance of 3DOm COOH-Lyz were specifically investigated based on the disinfection efficacy of *Staphylococcus aureus* in water. The results revealed that the immobilization capacity and relative activity of immobilized lysozyme were 814 mg/g carrier and 80%, respectively, under the optimal immobilization conditions. And the antibacterial material with the initial mass ratio of lysozyme and 3DOm COOH as 3:1 exhibited maximum bacteria removal efficiency (98.1%) at pH 5. Moreover, the reusability test indicated that 3DOm COOH-Lyz has

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certain operational stability, and remains 82% bacterial removal efficiency even in the fifth cycle, which provides a promising application for safe and efficient drinking water disinfection in small-scale and emergency water treatment.

1. Introduction

Water, one of the most precious natural resources on earth, is being contaminated by various pollutions created by human activities. Among them, microbial contamination of water causes a major menace to public health and aquatic species. Especially pathogenic bacteria are responsible for waterborne diseases and have detrimental effect on human health [1]. Access to clean, safe and sustainable drinking water is a persistent need in the present century. Therefore, disinfection methods such as chlorination and ozonation, and UV treatment are widely applied to the disposal of the microbial contaminations, which can efficiently inhibit some pathogenic microorganisms. Nevertheless, chlorination and ozonation will cause the formation of carcinogenic and mutagenic disinfection byproducts (DBPs) and lead to secondary pollution due to the presence of natural organic matter (NOM), bromide and iodide in source waters [2,3]. Recently, considerable interest has arisen in the study of "green" disinfection technologies that are not only free of producing DBPs, but also portable, nontoxic, and require low energy input. As "green" antibacterial agents, functional nanomaterials, such as Ag nanoparticles [4–7], TiO_2 nanoparticles [8–10], Graphere oxide [11,12] and ZnO nanoparticles [13–15] and, etc. or some natural organic compounds, such as chitosan [16,17], have been used for small-scale water treatment or for emergency response in disaster relief.

Lysozyme (EC 3.2.1.17) as a common natural and "green" antibacterial agent is often used to kill microorganisms [18,19]. The antibacterial activity of lysozyme is primarily attributed to the damage of bacterial cell walls by catalyzing hydrolysis of β-1,4 glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine in peptidoglycans [18]. However, lysozyme is unstable in water, can easily run off with water flow, and exhibits poor sterilizing effect in drinking water because it is not easy to be anchored on the surface of bacteria and viruses at low concentration. To solve these problems, the immobilization of enzyme on the appropriate supports has been widely investigated. This strategy can enhance its operation stability and thermostability, also improve its efficacy due to the increased local concentration, and provide easy separation and recovery [20–22]. The studies of enzyme behaviors on a variety of supports, such as mesoporous carbon [23], mesoporous zirconia thin films [24], porous zirconia microtubes [18,25], mesoporous silica materials [26], magnetic nanoparticles [27], single-walled carbon nanotubes [28], and, etc. have elucidated that the catalytic activity and stability of immobilized enzymes depend on the pore size, surface characteristics and morphology of the supporting materials, and the immobilized methods and parameters. Matching the pore size of the supports with enzyme is essential for remaining the enzymatic activity [29].

Three dimensional ordered mesoporous carbon (3DOm C) with tunable pore size from 10 to 40 nm has been synthesized in the previous study [30]. The ultralarge mesoporous structures make it promising for the immobilization of numerous enzymes whose sizes fall in this range. The large surface area, ordered mesoporous structure, and thermal and mechanical stability could also provide a favorable microenvironment for the immobilization of enzymes. Compared with other mesoporous structures [31–39], the three dimensionally ordered mesoporous structure offers interconnected mesoporous structures which can increase the accessible adsorption sites, promote the mass transport of enzymes in the pore channel to achieve high adsorption capacity in a short time, and avoid pore blockage. Also, this structure can help the transport of substrates leading to the improved enzymes activity. For the first time, the application of this ultralarge mesoporous 3DOm C was applied for the impregnation of enzymes.

Among various enzyme immobilization methods, adsorption is usually considered as the most attractive and effective choice because of its simple operation and high effectiveness, which mainly depends on the electrostatic attraction, hydrogen bond and Van der Waals forces between enzymes and the surface of carriers [20]. However, this immobilization method cannot avoid the aggregation of enzymes on the support, which could cause a considerable loss of the enzyme activity and severe leakage of enzyme in practical applications [40]. Covalent cross linking can immobilize enzymes on the supports by cross-linking reagents [41,42], which can provide stable interactions between enzymes and carrier, resulting in higher capacity and the stability of immobilized lysozyme compared to unspecific immobilization [18,25].

The key to the development of "green"antibacterial agent based on lysozyme is the choice of immobilization carriers and methods. This study firstly used this carrier material, 3DOm C, which possesses interconnected ultralarge pores suitable for mass transport and adsorption of enzyme. 3DOm C is very special in high capacity of lysozyme, remaining the activity of each anchored lysozyme molecules and facilitating stable interactions among the enzymes. In addition, high affinity of 3DOm C for cells greatly assists enzyme hydrolysis for bacteria.

In this study, carboxylated-3DOm C (3DOm COOH) immobilized with lysozyme by covalent cross-linking (3DOm COOH-Lyz) was used to purify the drinking water contaminated with pathogenic bacteria. This approach combines the strong adsorption affinity between 3DOm C and bacteria in the water with the outstanding antibacterial activity of the immobilized lysozyme inside the 3D pore network of 3DOm COOH. Its antibacterial efficacy and reusability were evaluated using the aqueous suspensions of Gram positive bacterium *Staphylococcus aureus*. A plausible mechanism for the bacterial removal pathway was also proposed. 3DOm COOH-Lyz with high efficacy, easy operation and low DBPs, has potential application in potable water disinfection in small-scale and emergent water treatment.

2. Experimental part

2.1. Materials

Chicken egg white lysozyme (E.C. 3.2.1.17), N-hydroxysuccinimide (NHS) (98%) and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (USA) and used without further purification. *Staphylococcus aureus* (bacterium, ATCC6538) was purchased from Agricultural Culture Collection of China. And *Micrococcus lysodeikticus* cell and mannitol salt agar medium plates were obtained from Sangon (Shanghai, China). All other chemicals were of analytical grade and ultrapure water (18.25 MΩ/cm) was used for preparing the solutions and rinsing the glassware. And all ultrapure water and glassware were sterilized at 121 °C for 15 min with autoclave before each antibacterial experiment.