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PREPARATION OF MAGNETIC ANTIBACTERIAL COMPOSITE BEADS Fe_3O_4/ALGINATE/Ag

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ABSTRACT

In recent years, the synthesis of magnetic polymer beads has much attractive attention from many technological fields such as biomedical and environmental. In addition of stabilizing effect for magnetic particles core, the polymer shells can also be further functionalized, thus enlarging the spectrum of possible applications of magnetite nanoparticles. In this study, the novel magnetic composite beads of Fe_3O_4/alginate/Ag were synthesized and characterized for point-of-use water disinfection. Magnetite nanoparticles were prepared from waste spent pickling liquors and then were encapsulated by natural alginate to form the spherical beads of the size about 1-2 mm. Silver nanoparticles were loaded on the beads with the large content (24.62 wt.%). The as-prepared beads have a high saturation magnetization value, Ms = 59 emu/g, and very good antibacterial activity against both gram (-) and gram (+) bacteria. With the concentration 35 µg mL^{-1} the magnetic beads can kill 100 % *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, whereas this value found 70 µg mL^{-1} for *Lactobacillus fermentum*, 105 µg mL^{-1} for *Salmonella enterica*, and 140 µg mL^{-1} for *Bacillus subtilis*.

Keywords: magnetic antibacterial beads, sodium alginate, silver nanoparticles, water disinfection.

1. INTRODUCTION

Magnetic nanoparticles have received recently a considerable amount of attention due to their wide applications in various fields, which ranges from biomedical to industrial. Especially, in the field of water and wastewater treatment where the challenges associated with separation and recovery of the small size of adsorbent materials from contaminated water are becoming increasingly important [1]. However, the practical use of magnetic nanoparticles raises a series of problems concerning their intrinsic instability [2, 3]. Hence, they should be chemically stabilized by grafting or coating with organic or inorganic substances. Synthesis of magnetic polymer beads constitutes a new topic of research rapidly developing last decade. In addition of stabilizing effect, the polymer shells can also be further functionalized, thus enlarging the spectrum of possible applications of magnetite nanoparticles [4, 5].

Alginate is a natural polysaccharide extracted from brown seaweed, it has many advantages such as low cost and availability, biocompatibility, biodegradability, and ease of gelation [6-8]. It has found numerous applications in diverse fields such as pharmaceutical, biomedical, agriculture, and environmental. There are some studies reported recently on the encapsulation of magnetic nanoparticles in the alginate matrix for different applications: drug delivery [6], heavy metal adsorption [7] or dye removal [8].

In the field of water purification, chlorination is the most widely used method for disinfecting water because of their high efficiency and low cost. But chlorine may react with natural organic matter to form the toxic by-products, some of which have been identified as carcinogenic [9]. In recent years, silver nanoparticles have been a well-known antibacterial agent due to its higher antimicrobial activities against gram-positive and gram-negative bacteria. Ag nanoparticles were also studied recently to immobilized onto various inorganic or organic substrates, to improve their safe and effective use in practice [10,11].

In this study, we synthesize the magnetic antibacterial composite beads Fe_3O_4/alginate/Ag for point-of-use water disinfection. The waste spent pickling liquors was used for magnetic nanoparticles synthesis, they have been encapsulated by the natural alginate and further immobilized with Ag nanoparticles. The as-prepared beads were characterized by FE-SEM, EDX methods, the magnetic and antibacterial properties were also examined.

2. EXPERIMENTAL

2.1. Chemicals

The Fe_3O_4 nanoparticles were synthesized from the spent pickling liquors of Hoa Phat Steel Co. according to our recent work [12]. Sodium alginate is an extracted product from natural brown algae of Nha Trang Institute of Technology Research and Application. $Ca(NO_3)_2$ of reagent grade was supplied from Xilong Scientific Co. (China). $AgNO_3$, $NaBH_4$ of analytical grade were purchased by Merck Co. (Germany).

2.2. Preparations and characterization of Fe_3O_4 /alginate/Ag beads

The alginate bead formation was investigated at first, using Ca^{2+} solution: an aqueous solution of sodium alginate with various concentrations, from 0.2 to 1.5 wt%, was slowly injected into 0.3 M $Ca(NO_3)_2$ solution. After 24 hours for aging, the resulting beads were washed with distilled water three times. The shape of obtained beads was observed by the ZEIZZ optical microscope.

For the composite beads preparation, Fe_3O_4 nanoparticles were dispersed in the sodium alginate solution, the mass ratio of Fe_3O_4:alginate was 2:1. Injecting this solution to 0.3 M $Ca(NO_3)_2$ solution for composite granulation. After aging for 24 hours, the Fe_3O_4/alginate beads were collected by a magnet, rinsed three times with distilled water. The resulting Fe_3O_4/alginate beads were further dispersed in the $AgNO_3$ solution, with Ag^+ concentration varied from 5 to 20 mM, stirring gently for 12 hours, then adding $NaBH_4$ to reduce Ag^+ to Ag^o, molar ratio $NaBH_4$:$AgNO_3$ was 1:1. Collect the product with a magnet, rinse it several times with distilled water.

The obtained composite beads were characterized by scanning electron microscope (S-4800, HITACHI, Japan), and energy dispersive X-ray spectroscopy (JSM-6510LV, Jeol, Japan). Magnetic properties were determined on the vibration sampling system (VSM).

2.3. Antibacterial test

Antibacterial activity of magnetic composite beads was tested by using of standard microdilution method which anables to determine the MIC (minimum inhibitor concentration) or MBC (minimum bactericidal concentration). Firstly, a serial dilution of composite is added in a 96-wells plate from high concentration, for example 128 μg mL^{-1}, to zero, as blank sample. Then a bacterial suspension with 5×10^5 CFU/mL is added in every well. After 24 h at 37 °C incubation the wells are visually evaluated for turbidity. For determining the MBC, 100 μL last three transparent suspensions are chosen to be spread on new agar plates. After another 24 h incubation at 37 °C colony formation shows up and the lowest concentration with biocidal activity is taken as MBC. Different bacterials of both gram (+) and gram (-) were used for the tests: *Staphylococcus aureus (Sa), Bacillus subtilis (Bs), Lactobacillus fermentum (Lf), Salmonella enterica (Se), Escherichia coli (E.coli), Pseudomonas aeruginosa (Pa).*

3. RESULTS AND DISCUSSION

3.1. Formation of Fe_3O_4 /alginate bead and magnetic property

The bead formation is occurred immediatly upon the contact of the alginate and Ca^{2+}, the mechanism of this gelation process involves guluronic residues with the specific chelation of Ca^{2+} forming the so-called “*egg-box*” structure [13]. The size and shape of the beads are highly dependent on the concentration of sodium alginate salt. Figure 1 presents the images of alginate beads observed on the optical microscope, the beads were prepared with diferent alginate concentrations. It is clear that with the low concentrations (less than 1 wt%), no uniform and round grain was produced. In the case of higher concentrations, from 1-1.5 %, the uniform spherical beads of diameter 1-2 mm were well formed.

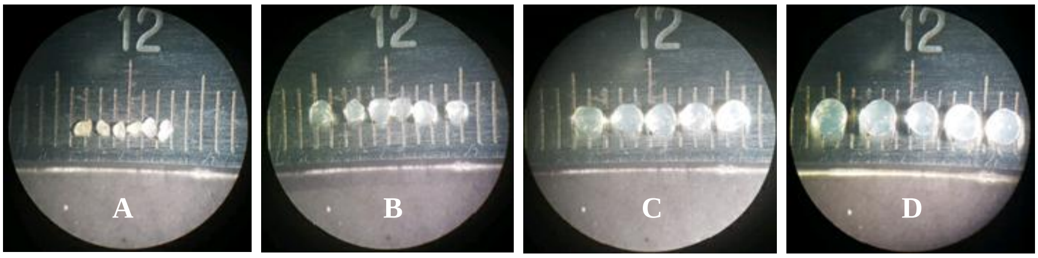

Figure 1. Optical images of alginate bead at differents concentrations:

(A) 0.2 %; (B) 0.5 %; (C) 1 %; (D) 1.5 %.

The Fe_3O_4/alginate composite beads were prepared by dispersion of Fe_3O_4 nanoparticles in 1 % sodium alginate solution, then the mixture was injected in Ca^{2+} solution. The prepared magnietic composite beads have a similar shape to a sole alginate beads (Figure 2A) and they can be collected by a magnet (Figure 2B).

The mass ratio of Fe_3O_4:alginate was chosen 2:1. In principe the magnetic property of composite beads will be increased with increasing the Fe_3O_4 content, but it is difficult to granulate the beads in the case of higher Fe_3O_4 content. Figure 2B presents the magnetization hysteresis curves of sole Fe_3O_4 nanoparticles and Fe_3O_4/alginate beads, measured by the VSM magnetometry, under a magnetic field ranging from -10 to 10 kOe at room temperature. The results showed the high saturation magnetization for Fe_3O_4 nanoparticles, Ms = 79 emu/g. This value decreased to 59 emu/g for the Fe_3O_4/alginate composite beads.

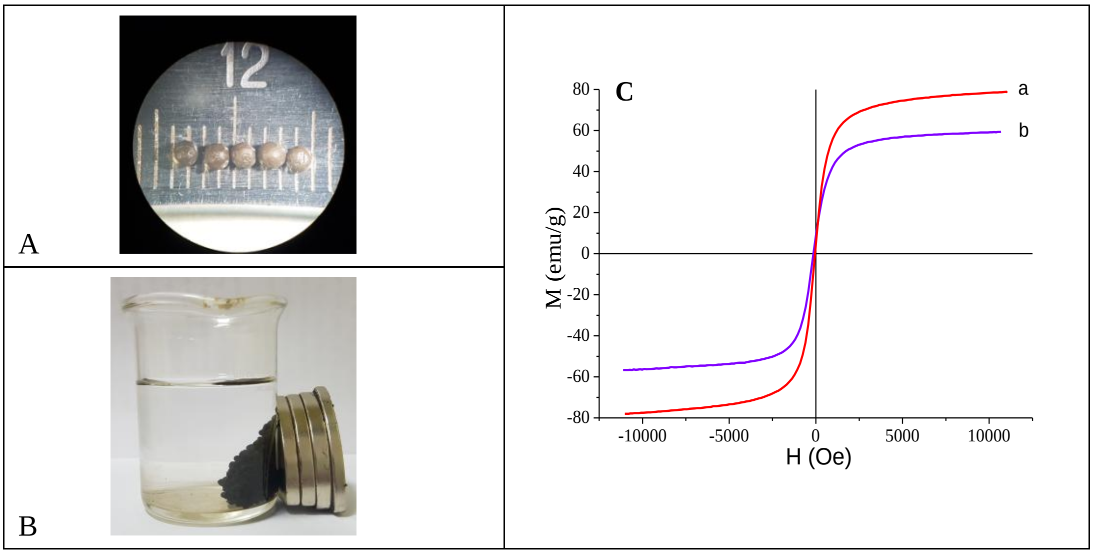

Figure 2. Optical image (A) and photo of Fe_3O_4/alginate beads collection with a magnet (B); C: magnetization curve of Fe_3O_4 (a) and Fe_3O_4/alginate (b).

3.2. Synthesis of Fe_3O_4/alginate/Ag composite beads

Fe_3O_4/alginate/Ag composite beads were synthesized by loading Ag^+ ion from $AgNO_3$ solution on the surface of Fe_3O_4/alginate beads, then Ag^+ ions were reduced by $NaBH_4$ to metallic silver. The presence of silver in the composite beads can be examined by EDX analysis.

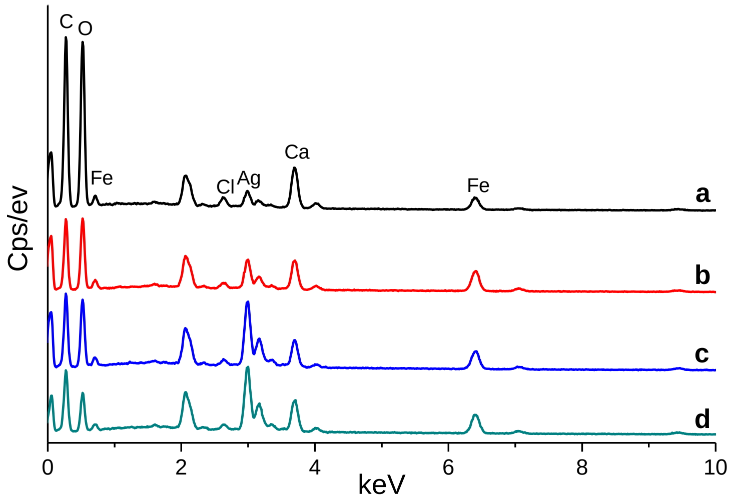

Figure 3. EDX spectra of Fe_3O_4/alginate/Ag composite beads synthesized with different $AgNO_3$ concentrations: 5 mM (a), 10 mM (b), 15 mM (c), 20 mM (d).

The obtained EDX results demonstraed in Fig. 3 indicate clearly the chemical composition of the as-prepared magnetic beads that includes Fe, C, O, Ca, Cl and Ag. Intensity of the Ag peak at 3 keV was increased with increasing the concentration of $AgNO_3$ solution from 5 to 20 mM.

The chemical composition of Fe_3O_4/alginate/Ag beads has been also quantitativly determined and presented in Table 1. The Ag amount in the composite beads increased obviously when $AgNO_3$ concentration increased from 5 to 15 mM, in the case of 20 mM, the silver content increased a little, so the value 15 mM was selected for the synthesis of Fe_3O_4/alginate/Ag composite beads, with the incorporated Ag amount 23.8 wt% .

Table 1. Elements of Fe_3O_4/alginate/Ag nanocompositebeads synthesized with different concentrations of $AgNO_3$ solution.

Element	**$AgNO_3$ concentration**							
	5 mM		10 mM		15 mM		20 mM	
	Weight (%)	Atomic (%)	Weight (%)	Atomic (%)	Weight (%)	Atomic (%)	Weight (%)	Atomic (%)
Fe	11.71	3.49	10.67	3.33	10.87	4.05	11.96	4.56
O	44.54	46.31	45.76	49.79	37.79	49.08	34.13	45.43
C	34.05	47.16	29.94	43.39	23.11	39.98	23.82	42.23
Ca	5.34	2.22	4.16	1.81	4.07	2.11	5.10	2.71
Cl	0.47	0.22	0.48	0.23	0.33	0.20	0.36	0.22
Ag	3.89	0.60	8.98	1.45	23.81	4.59	24.62	4.86
Total	100.00		100.00		100.00		100.00	

The structural morphology of Fe_3O_4/alginate/Ag composite beads (synthesized with 15 mM $AgNO_3$ solution) was characterized by field emission scanning electron microscope (FE-SEM), Fe_3O_4 nanoparticles and Fe_3O_4/alginate beads were also analyzed for comparison, the obtained results are shown in Figure 4.

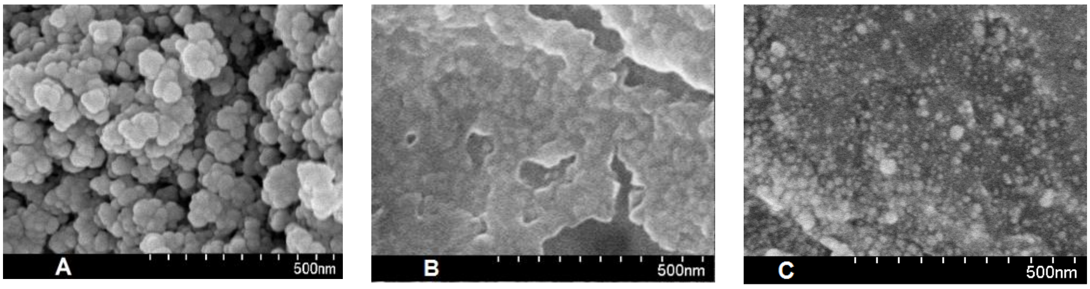

Figure 4. FE-SEM images of Fe_3O_4 particles (A), Fe_3O_4/alginate (B) and Fe_3O_4/alginate/Ag (C).

FE-SEM image of Fe_3O_4 showed the spherical particles with an average size of about 20 nm (Fig. 4A). Figure 4B shown the FE-SEM image of Fe_3O_4/alginate beads, the magnetic nanoparticles homogenously dispersed within the polymer matrix. After the Ag incorporation onto magnetic beads, by Ag^+ adsorption (in $AgNO_3$ solution) and then reduction by $NaBH_4$, the silver nanoparticles of about several nm were appeared in the bead surface as seen in Figure 4C.

3.4. Antibacterial tests

The antimicrobial activity of Fe_3O_4/alginate/Ag composite beads against different bacteria, *Staphylococcus aureus (Sa), Bacillus subtilis (Bs), Lactobacillus fermentum (Lf); and Gram (-): Salmonella enterica (Se), Escherichia coli (E.coli), Pseudomonas aeruginosa (Pa),* was evaluated, by using standard microdilution method which anables to determine the MBC (minimum bactericidal concentration) value. The obtained results were shown in Table 2.

Table 2. Antibacterial activity of Fe_3O_4/alginate/Ag composite beads.

***Fe₃O₄/alginate/Ag* content (µg /ml)**	**Percentage of inhibition of micro-organism (%)**					
	Gram (+) bacteria			Gram (-) bacteria		
	Sa	***Bs***	***Lf***	***Se***	***E.coli***	***Pa***
140	100	100	100	100	100	100
105	100	0	100	100	100	100
70	100	0	100	20	100	100
35	100	0	40	0	100	100

As can be seen in Table 2, the magnetic beads Fe_3O_4/alginate/Ag present an excellent antibacterial activity for both gram (+) and gram (-) microorganisms. They can strongly inhibit *Sa, E.coli and Pa*, with low value MBC = 35 µg mL^{-1}, this value was 70 µg mL^{-1} for *Lf*, 105 µg mL^{-1} for *Se*, and 140 µg mL^{-1} for *Bs*. Sole Fe_3O_4 and Fe_3O_4/alginate beads were also tested under the similar conditions for a comparison, they both exhibited no antibacterial activity, even with concentration of 140 µg mL^{-1}. Furthemore, Fe_3O_4/alginate/Ag composite beads are easily separated and recycled due to their good magnetic property. These results are very promising and we beleave that the magnetic Fe_3O_4/alginate/Ag beads could be developed into the novel effective disinfection material.

4. CONCLUSION

In this work, we synthesized and characterized the magnetic composite beads Fe_3O_4/alginate/Ag of the size about 1-2 mm, with the large Ag content (24.62 wt.%). The as-prepared material has a high saturation magnetization value, Ms = 59 emu/g, and very good antibacterial activity against both gram (-) and gram (+) bacteria. With the concentration 35 µg mL^{-1} the magnetic beads can kill 100 % *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa,* whereas this value found 70 µg mL^{-1} for *Lactobacillus fermentum*, 105 µg mL^{-1} for *Salmonella enterica*, and 140 µg mL^{-1} for *Bacillus subtilis*. The results are well promising and we beleave that this novel material has potential to be used in water disinfectant applications.

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