ARTICLE DOI: 10.5504/BBEQ.2011.0010 A&EB

PREPARATION AND APPLICATION OF BACTERIAL CELLULOSE SPHERE: A NOVEL BIOMATERIAL

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ABSTRACT

This paper explores preparation of spherical bacterial cellulose (BC), a novel biomaterial, and its adsorption capacity is charaterized. The effect of shaking speed and culture duration on fermentation production of BC spheres is analyzed; BC spheres are produced after 72 h fermention at 30ºC with a shaking speed of 160 rpm. The spheres have a diameter range of 3-5 mm. The scanning electron micrograph photograph shows that BC spheres have a loose and porous structure. Repetition using tests on adsorption of Bovine serum albumin (BSA) and Pb^{2+} had been carried out. The results indicated that BC spheres can be recovered from BSA-BC complex and Pb-BC complex by eluting with NaOH and sodium citrate solution, respectively. So BC spheres have a vast potential for application in the fields of biomaterial bioseparation and sewage treatment.

Biotechnol. & Biotechnol. Eq. 2011, **25**(1), 2233-2236

Keywords: bacterial cellulose spheres, biomaterial, bioseparation, sewage treatment

Introduction

Natural cellulose spheres are often applied in bioseparation, immobilized reaction, cell suspension culture, and as an adsorbent for sewage treatment. Liu Minghua et al. and Liang et al. adopted natural fiber as raw material and prepared cellulose spheres as an adsorbent by alkalizing, aging, carbonizing, dissolving, inverse suspension, and other steps (7, 9, 10, 11, 12). The process suffers from complicated procedures and emission of harmful substances, such as waste alkali and acids, organic solvents, and other byproducts.

Bacterial cellulose (BC) is a glucose polymer produced through bacterial fermentation (16). This macromolecular polymer features the same molecular formula and properties of natural cellulose. A fiber bundle of 40 to 60 nm thick is formed by micro-fibers with a diameter range of 3 to 4 nm. These bundles aggregate randomly to produce a developed, superfine structure, thus forming a typical type of nano-biomaterial (3).

Fermentation production of BC is completed in two ways, static and dynamic. In the static method, membranous BC will form on the gas-liquid interface; whereas in the dynamic method, threadlike, flocculent, or conglomerate BC will be produced after fermentation during shaking or mechanical stirring. In suitable conditions, spherical BC could be produced (1, 4, 17).

The membranous BC produced from or by static method has compact texture, high tensile strength, high elastic modulus, high water permeability, high water-binding capacity, strong ability to maintain its shape(6), and good tearing resistance. Moreover, the material is characterized by good biocompatibility and biodegradability, so it could be widely applied to the medical, biological, and chemical industries (15).

Spherical BC produced from dynamic method is translucent, loose, porous, and has a hydrophilic network structure. Its specific surface area increases with decreasing spherical diameter, so it could be used as a carrier to adsorb or crosslink various kinds of substances (e.g., enzyme, cell, protein, nucleic acid, and other compounds) (13). Spherical BC may be applied in bioseparation, immobilized reaction, cell suspension culture, and as an adsorbent for sewage treatment. Compared with natural spherical cellulose, the fermentation production of BC spheres is simple, controllable and environment friendly. Moreover, BC sphere can be used repeatedly, expanding their potential applications.

This experiment explores the optimum conditions for producing BC globules by fermentation from *Gluconacetobacter xylinum* CGMCC No.2955. This paper also measures the adsorptive capacity of BC spheres for proteins and heavy metals, as well as the feasibility of such as BSA and Pb^{2+}, and the recovery of BC spheres.

Materials and Methods

Strains

Gluconacetobacter xylinum CGMCC No.2955 was screened by the Key Laboratory of Industrial Microbiology of the Ministry of Education and stored by China General Microbiological Culture Collection Center.

Culture medium

The medium used for BC spheres production and seed culture consisted of the following (v/v): carbon source 2.5%, peptone

0.75%, yeast extract 1%, disodium phosphate 1%, acetate acid 1%, initial pH value 6.0 (5, 14).

Preparation of BC spheres

One or two loops slant seeds of *G. xylinum* CGMCC No.2955 were inoculated into a 500 ml conical flask containing 100 ml liquid seed culture medium and incubated for 24 h at 30ºC and 160 r/min. After incubation, 2 ml cellulase (10 000 U/ml) was added for enzymolysis at 30ºC for 2 h. Bacterial cells were centrifugally washed with deionized water for three times and then were suspended in 100 ml fermentation culture medium. Five milliliters of the suspension was taken and inoculated into the 500 ml conical flask containing 100 ml liquid culture medium. The flasks were shaken to spread the cells at 0, 100, 130, 160, 190, and 220 r/min, respectively. Spherical BC was produced by agitating the culture at 30ºC from 24 to 120 h. After fermentation, BC was washed with flowing water to remove the residual culture medium and bacteria. FESEM was adopted for SEM analysis (1530VP, LEO/Germany).

Suspension test of BC spheres

BC spheres were suspended in BSA solutions of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/ml, respectively, in seven 100 ml graduated cylinders. Suspension of BC spheres was observed.

Adsorption and elution tests of BSA

A total of 25 mg BC spheres (dry weigh) were placed in test tubes with different amounts of the BSA solution (three test tubes for each concentration). Water was added to reach 10 ml and final BSA concentrations were: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/ml, respectively. Adsorption test took place at 37ºC for 100 rpm and 2 hours. The test tubes were heated in a 37ºC bath at 100 r/min for 2 h to enable full adsorption of BSA in the BC spheres. Residual concentration of BSA after adsorption was measured by Bradford Method. Briefly, the supernatant in conical tubes was taken and diluted properly; then CBB G-250 reagent was added; after 2 minutes the adsorbance at 595 nm was checked. Therefore, the amount of adsorbed BSA=initial BSA-residual BSA, and the adsorpted ratio (%)=the amount of adsorbed BSA/initial BSA*100%.

Taking 0.1 mol/L NaOH as eluent, BC spheres were eluted until no BSA was detected in the supernatant. Eluate was collected and the content of BSA was assayed. BSA recovery ratio was then calculated as follows: BSA recovery ratio (%)=eluted BSA/adsorbed BSA*100%.

Adsorption and elution tests of Pb^{2+}

A total of 25 mg BC spheres (dry weigh) were placed in test tubes with different amounts of the Pb^{2+} solution (three test tubes for each concentration). Water was added to reach 10ml and final Pb^{2+} concentrations were: 0, 20, 40, 60, 80, 100, 150, and 200 mg/L, respectively. Adsorption test took place at room temperature for 100 rpm and 2 hours. Residual concentration of Pb^{2+} after adsorption was measured by an atomic adsorption spectrophotometer (AA-6800, Shimadzu, Japan).

Taking 0.1 mol/L sodium citrate as eluent, BC spheres were eluted. Eluate was collected and the content of Pb^{2+} was assayed by an atomic adsorption spectrophotometer (AA-6800, Shimadzu, Japan).

As described above formula, the amount of adsorbed Pb^{2+}=initial Pb^{2+}-residual Pb^{2+}; the adsorpted tatio of Pb^{2+} (%)=the amount of adsorbed Pb^{2+}/initial Pb^{2+}*100%; and Pb^{2+} recovery ratio (%)=eluted Pb^{2+}/adsorbed Pb^{2+}*100%.

Results and Discussion

Different shapes of BC spheres were produced by changing shaking speeds. Static culture (zero shaking speed) resulted in membranous and elastic BC (**Fig. 1a**); tadpole-like BC formed at shaking speed of 130 rpm and 48 hours of culture, as shown in **Fig. 1b**; BC spheres appeared at 160 and 190 rpm, seen from **Fig. 1c**; for shaking at 100 and 220 rpm, BC particles agglomerate and have a shape of snowflake, shown in **Fig. 1d**.

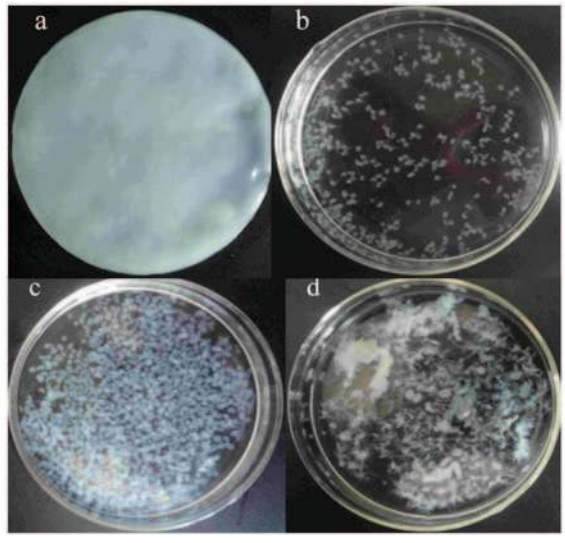

Fig. 1. Comparison of BC shapes at different shaking speeds
a: BC membrane; **b**: tadpole-like BC; **c**: spherical BC; **d**: snowflake BC

BC spheres of different diameters may be obtained at conditions of shaking speed 160 and 190 rpm, and also fermentation length 24, 48, 72, 96, and 120 hours (**Table 1**). The BC spheres used for the follow-up tests are products of 72 h fermentation at 160 r/min and 30ºC.

TABLE 1

Relationship between fermentation length and diameters of BC spheres

Culture time (h)	BC sphere diameter (mm)	
	160 r/min	190 r/min
24	0.5-1	0.5-1
48	2-3	1-2.5
72	3-5	3-4
96	3-6	3-4
120	BC spheres adhere to each other	BC spheres adhere to each other

Macro and micro morphology of BC spheres

The BC spheres produced from 72 h fermentation at 160 r/min and 30ºC are shown in **Fig. 2a**. The BC spheres are white, translucent, and have a round or elliptical shape. The spheres have a loose structure and a diameter between 3 to 5 mm. The SEM graph of BC spheres shown in **Fig. 2b** proves their loose and porous morphology. This result is similar with that reported by Cheng et al. (2).

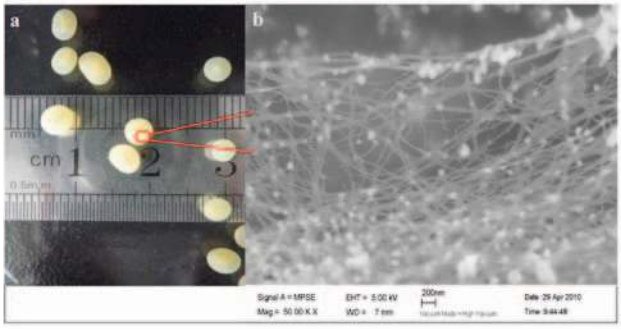

Fig. 2. Macro and micro morphology of BC spheres

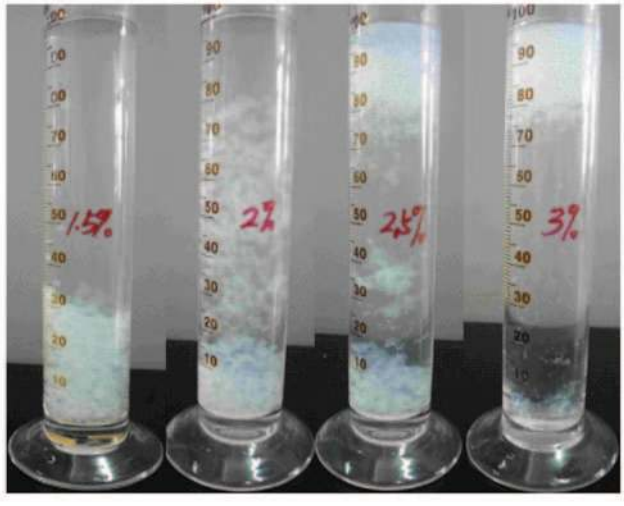

Fig. 3. BC spheres suspension test in BSA solution
a: 1.5% BSA; **b**: 2.0% BSA; **c**: 3.0% BSA

Adsorption and elusion tests of BSA

BC spheres settled in solution of BSA concentration lower than 2%; spheres float in BSA solution of higher concentrations, such as 2.5% and 3%, as shown in **Fig. 3**. Densities of solutions increase with the content of solute, which is the reason of this phenomenon. However, BSA unceasingly spreads into and is adsorbed by BC spheres. BC spheres in 2.5% and 3% solutions began sinking after 20 minutes; 60 minutes later, they settled at the bottom. Adsorption tests were carried out at 100 rpm to avoid the influence of aggregation in BSA solutions.

The adsorption quantity had an increasing trend with increasing BSA concentration, while the adsorption ratio had a descending trend; as shown in **Fig. 4**, there was a plateau from 0 to 1.0 mg/ml, and then adsorption ratio decreased. Adsorbed BSA molecules increased on the surface of BC spheres with the rise of BSA concentration. When BSA on the surface of BC spheres reached the saturation point, the BC spheres were unable to adsorb continuously even as BSA concentration rises (8).

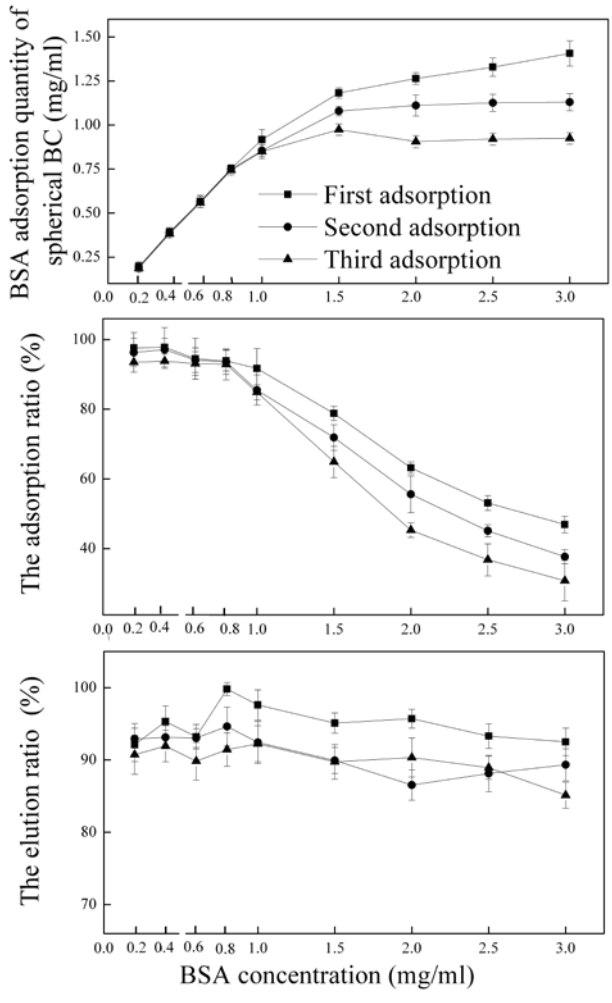

Fig. 4. Effect of BSA concentration on adsorption ratio and elution ratio

BSA-adsorbed BC spheres were eluted by 0.1 mol/l NaOH, the first recovery ratio was more than 92.1%, indicating that BC spheres could be reused after elution. Then regenerated BC spheres were used repeatedly. The adsorption quantity, adsorption ratio, and elution ratio were slightly decreased, as shown in **Fig 4**. So BC spheres could be applied repeatedly to bioseparation and immobilized reactions.

Adsorption and elusion tests of Pb^{2+}

The Pb^{2+} quantity adsorbed by BC spheres increase with increasing Pb^{2+} concentration (**Fig. 5**). When Pb^{2+} concentration is around 100 mg/L, the first adsorption quantity has an obvious increasing trend with an adsorption ratio higher than 84.2%. When the Pb^{2+} concentration continuously rises, the increasing trend slows down gradually.

Having adsorbed Pb^{2+}, BC spheres were eluted by 0.1 mol/l sodium citrate; the first recovery (elution) ratio was

75.22% to 55.71%, which suggests that recovery of BC spheres is available. Then regenerated BC sphere were also used repeatedly. However, the adsorption quantity, adsorption ratio, and elution ratio of Pb^{2+} were decreased, as shown in **Fig 5**. Further experimental studies shall be made on elution requirements to boost elution efficiency. So BC spheres could be applied to sewage treatment and adsorbance reaction of heavy metals.

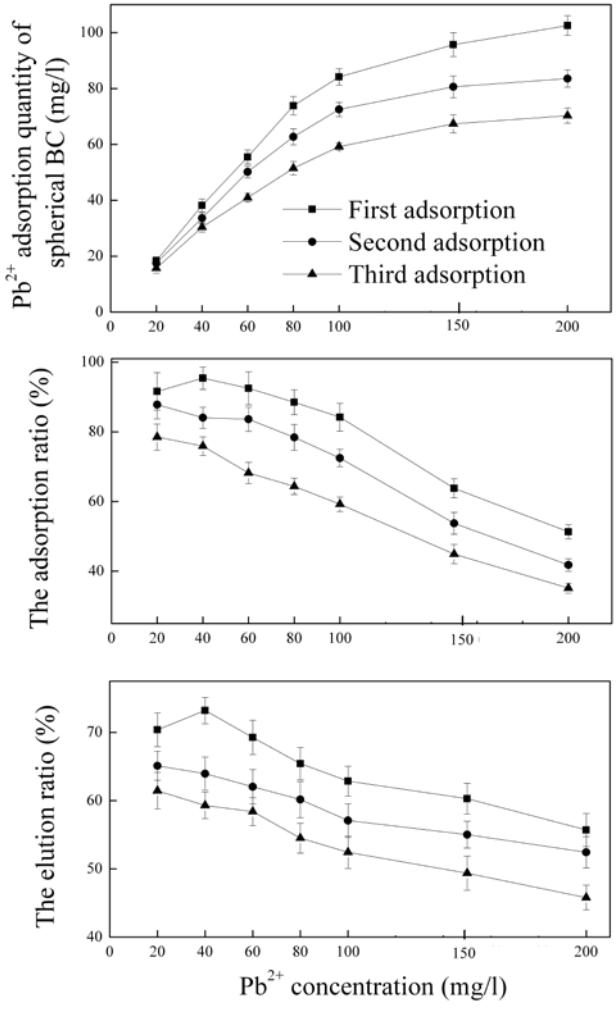

Fig. 5. Effect of Pb^{2+} concentration on adsorption ratio and elution ratio

Conclusions

This paper explored the effect of revolution speed and culture length on the fermentation production of BC spheres; spheres with a diameter of 3 to 5 mm will be produced through fermentation for 72 hours at 160 rpm and 30°C. BC spheres have a loose and porous structure, observed in SEM graph. The results of BSA and Pb^{2+} adsorption and elution tests show that the BC spheres could be reused after BSA or Pb^{2+} is eluted; though the recovery ratio of Pb^{2+} is lower than that of BSA. BC spheres could be applied to bioseparation, immobilized reaction, or sewage treatment; reusage is the advantage of BC spheres, which satisfies the requirement of sustainable development. Thus, BC spheres have a vast potential for future application.

Acknowledgments

This work was funded by the National Natural Science Foundation of China under No.20976133 and the Foundation for Excellent Doctoral Dissertations of TianJin University of Science and Technology under No. B201002.

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