Preparation of Bacterial Cellulose/Inorganic Gel of Bentonite Composite by In Situ Modification

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Abstract To evaluate the possibility of Bacterial cellulose/Inorganic Gel of Bentonite (BC/IGB) composite production using in situ method, the BC/IGB composite was successfully produced by in situ modification of BC in both HS medium and corn cob hydrolysate. The results showed that the BC/IGB composite obtained in HS medium (one classical medium for BC production) had a higher water holding capacity, but the water retention capacity of the BC/IGB composite obtained in corn cob hydrolysate was better. The performance of BC/IGB composite depended on the environment of in situ modification. Using different media showed significant influence on the sugar utilization and BC yield. In addition, BC/IGB composite produced by in situ method was compared with that produced by ex situ method, and the results shows that water holding capacity of BC/IGB composite obtained through in situ method was better. XRD results showed the crystallinity of BC/IGB composite related little to its performance as water adsorbent. Overall, in situ modification is appropriate for further production of BC composite and other clay materials.

Keywords Bacterial cellulose · Inorganic gel of bentonite · In situ modification · Water holding capacity

Introduction

As a biodegradable polymer, bacterial cellulose (BC) has many advantages such as hyperfine structure, high crystallinity, high hydrophilicity, high Young's modulus, controllable synthesis, etc., thus BC could be used in many fields such as papermaking, food, medicine, membrane applications, acoustic material [1, 2]. Recently, to solve the problem of substrate cost for BC production, various low-cost substrates have been applied for BC production [3, 4] and lignocellulosic hydrolysate seems to be one potential alternative for industrial production of BC due to its low-cost and great availability in nature [5–8].

To be one excellent material for industrial application, BC especially showed great potential as water adsorbent for its great capacity for water absorption [9, 10]. However, because of its poor dissolution properties and processing difficulties, BC's application as water adsorbent for water conservation is still limited [9, 11–17]. Usually, BC modification could be done with some physical or chemical methods to enhance its performance [18–21]. But to our knowledge, few work focused on the modification of BC as water adsorbent.

In recent years, BC modification can be carried out using in situ method by fermentation, which has many advantages such as simple operation, high efficiency, avoiding use of hazardous substances, etc. [22, 23]. Thus, in situ modification by adding exogenous substrate to the culture medium is a potential method to improve the physical properties of BC. In fact, many BC composites including BC-starch, BC-hydroxyapatite, etc. [24, 25] have been produced by in situ modification. But little work focused on the BC composite production as water adsorbent by IGB addition. Undoubtedly, to modify BC as water

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adsorbent, finding suitable substrate for in situ modification is important.

Bentonite is a clay mineral mainly composed of montmorillonite, and it has been investigated for many years [26]. Inorganic gel prepared from IGB has a series of unique properties and this kind of IGB could have excellent water holding capacity [27, 28]. More specially, the inorganic gel generated from IGB is nontoxic, can be compatible with aqueous fermentation environment, and has little influence on fermentation, thus it is suitable for fermentation and can be used for in situ modification of BC to produce composite as water adsorbent [29]. Because of its low-cost, IGB is also one potential alternative substrate for industrial production of BC.

In this study, to evaluate the possibility of BC/IGB composite production using in situ method, BC/IGB modified composite was produced by fermentation on both HS medium and corncob hydrolysate for the first time. Besides, to evaluate the potential of BC/IGB composite as water adsorbent, the water holding capacity and water retention capacity of BC/IGB composite were measured.

Materials and Methods

Feedstock

Detoxified corncob hydrolysate was kindly offered by Zhong-Ke New Energy Co., Ltd. (Ying-Kou, China). By HPLC analysis, corncob acid hydrolysate composition was glucose 2.9 g/L, xylose 37.9 g/L, and arabinose 4.9 g/L. Inorganic gel of bentonite (IGB) was obtained from Material Technology Co., Ltd. (Jiangsu JiuChuan Nami, China).

Microorganism, Medium and Fermentation Condition

Gluconacetobacter xylinus CH001 (preserved in the Laboratory of Energy and Biochemical Engineering, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences) was used for BC production.

The pre-culture was performed on pre-cultivation medium (g/L, mannitol, peptone 3, yeast extract 5, initial pH 6.0) at 28 °C and 150 rpm for 48 h. Then, 8 % (v/v) seed culture was translated into HS medium (g/L, glucose 20, yeast 5, peptone 5, Na₂HPO₄ 2.7, citric acid 1.15, pH 6.0) or corncob acid hydrolysate (pH 6.0) with and without IGB. And the static fermentation was carried out at 28 °C for 14 days.

BC Modification

Both in situ and ex situ modification of BC were carried out and the BC/IGB modified composite materials were successfully prepared. For in situ modification, different concentration (g/L, 0.5, 1, 2, 5) of IGB into both media (HS medium and corncob hydrolysate) respectively before fermentation, and then the initial pH of all mixed media was adjusted to 6.0 and sterilized at 121 °C for 20 min. After cooling of the media, 8 % (v/v) seed culture of *Gluconacetobacter xylinus* was inoculated into the medium and the culture was statically incubated in incubator for 14 days in 250 mL flasks. After fermentation, BC dry weight, BC/IGB composite water holding capacity, BC/IGB composite water release rate [30], macroscopic and microscopic morphology and conversion rate of sugar were evaluated.

Besides in situ modification, ex situ modification was also carried out by adding different concentrations (g/L, 0.5, 1, 2, 5) of IGB into 250 mL flasks containing BC products (obtained from the static fermentation in corncob hydrolysate) and then shaken at 28 °C and 180 rpm for 48 h.

BC Yield, SEM, and XRD Analysis

After fermentation, the fermentation broth and BC were isolated by vacuum filtration. BC macroscopic morphology was observed directly. Then, BC was treated with 1.5 % (w/v) NaOH at 80 °C for 2 h. After that, BC was repeatedly washed with distilled water. Finally, BC was dried at 105 °C to a constant weight and weighed. BC yield (g/L) was defined as the BC dry weight (g) in per liter.

Microscopic morphology of vacuum-dried BC samples were observed by a Hitachi S-4800 high-resolution field emission scanning electron microscope (FE-SEM; Hitachi, Tokyo, Japan) operated at 2.0 kV and 10 μ A.

X-ray diffraction spectra of BC samples were recorded from 2 θ of 10° to 60° with a PANalytical Rigaku D/max-250 diffracted equipped using Cu K α radiation at λ of 0.154 nm (10 kV, 100 mA).

Analysis of BC Water Holding Capacity and Water Release Rate

Determination of Water Holding Capacity

Water on the surface of wet BC was absorbed carefully and the weight of BC was recorded as M_0 . After drying at 105 °C, the weight of dried BC was recorded as M_1 . The water holding capacity was defined as M_0/M_1 .

Determination of Water Release Rate

The BC sample was placed at room temperature, and then its weight was stated at different time intervals.

Results and Discussion

Effect of IGB Addition on Macroscopic Morphology of BC Samples

To evaluate the effect of IGB addition on macro-morphology of BC samples, BC fermentation was carried out on four different kinds of media (HS medium, HS medium with IGB, corn cob hydrolysate and corn cob hydrolysate with IGB). After fermentation, four types of BC products were obtained and their macro-morphologies are shown in Fig. 1.

During BC fermentation, when the growing BC is in contact with the wall of the box or beaker, it will moves downwards into the nutrient broth, and the growth rate would be limited, thus the BC morphology especially the

thickness would be changed. This phenomenon is so-call “wall effect” [31]. In this study, the macro-morphologies of four BC products were all thick and round, suggesting that the BC synthesis “wall effect” was not influenced by IGB addition.

Two different media were applied in this work, one was classical medium for BC production (HS medium), and the other was the potential low-cost substrate for BC production (corn cob acid hydrolysate) [8, 32]. For HS medium, although it is suitable for BC production its cost is one problem for industrialization. For corn cob acid hydrolysate, its cost is suitable for industrialization, but how to improve BC yield in it was another problem. Therefore, it is necessary to compare the effect of IGB addition in both media.

On one hand, IGB was suspended in the HS medium after adding into HS medium. As the fermentation went on, the IGB particles were gradually miscible with the HS medium, and during this process, the IGB particles entered into the BC slowly. Finally, with the continuous synthesis of BC, part of the IGB particles was “wrapped” into the BC film successfully by entering the pore existing in the

Fig. 1 The macro-appearances (the actual pictures for BC products) of four different products. **a** BC (obtained on HS medium); **b** BC (obtained on HS medium) + IGB; **c** BC (obtained on corn cob hydrolysate); **d** BC (obtained on corn cob hydrolysate) + IGB

BC samples. After fermentation in HS medium, Sample a (obtained in HS medium with no IGB addition) was brighter, more uniform, and smoother than Sample b (obtained in HS medium with 0.5 g/L IGB adding).

On the other hand, the fermentation on corn cob hydrolysate was different: the color of Sample c (obtained in corn cob hydrolysate with no IGB addition) was similar to Sample d (obtained in corn cob hydrolysate with 0.5 g/L IGB addition), but Sample c was smoother than Sample d. It is possible that the HS medium is better than corn cob hydrolysate for BC synthesis thus the effect of IGB was more obvious in the HS medium than in the corn cob hydrolysate.

Obviously, IGB addition showed certain influence on the macroscopic morphology of BC samples obtained in both HS medium and corn cob acid hydrolysate, but the deeper influence of IGB addition should be got from the measurement of microscopic morphology of BC samples.

Effect of IGB Addition on Microscopic Morphology of BC Samples

Because IGB addition had a more obvious influence on the macroscopic morphology of BC samples in HS medium than in corn cob acid hydrolysate, the effect of IGB addition on microscopic morphology of BC sample obtained in HS medium was further analyzed by SEM (Fig. 2). As shown in Fig. 2a, without IGB addition, BC had an obvious three-dimensional network structure with various superfine micro fibers. The micro fibers have many mutual windings with high density. Apparently, a number of pores with diverse sizes existed among these micro fibers and these pore structures can absorb the solution or various suspending substances, thus the in situ modification of BC can be fulfilled.

When IGB was added into HS medium, the microscopic morphology of BC sample was changed: Fig. 2b shows that IGB has attached to the BC surface and entered into the hole among micro fibers, and finally the BC surface and

holes were filled. There were lots of hydroxyl groups on the surface of BC and IGB, thus there was weak hydrogen bonding between them [33, 34], which can enhance the BC physical–mechanical properties [35]. Also, the modification of the BC media (for example the addition of carboxy metil cellulose) has been reported to change the diameter of the BC fibers. But in this study, the in situ modification showed little influence on the dimensions of the BC fibers. Overall, the microscopic morphology of BC samples proved the possibility of in situ modification of BC by IGB adding.

Effect of IGB Addition on BC Production in HS Medium

Although the possibility of in situ modification of BC by IGB adding was proven, its effect on BC production still needs to be learned. The effect of different IGB concentrations on BC production in HS medium by *G. xylinus* and its water holding capacity were shown in Fig. 3. As it depicted, when IGB concentrations were 0.5 and 1 g/L, the BC dry weight obtained were higher than that in the medium without IGB addition. But when IGB concentration was higher, the BC dry weight increased little when compared with that in the medium without IGB addition. Interestingly, when IGB concentration was higher than 2 g/L, its influence on BC production was smaller. Undoubtedly, using IGB as substrate for BC production can partly reduce the cost of fermentation medium and increase the final BC yield as well, but the IGB concentration should be controlled in a suitable range.

The effect of IGB addition on residual sugar concentration was different from its effect on BC production (Fig. 3). When IGB concentration was higher than 1 g/L, it was beneficial for sugar utilization of *G. xylinus*. In contrast, when the IGB concentration was lower than 1 g/L (0.5 g/L), it showed a negative effect on sugar utilization of *G. xylinus*. It is possible that the IGB particles could be absorbed by BC easily when the IGB concentration was

Fig. 2 SEM photographs of BC samples collected from HS medium. **a** BC (obtained on HS medium); **b** BC (obtained on HS medium + IGB)

Fig. 3 The influence of IGB addition on BC (obtained on HS medium) dry weight, the residual sugar concentration and water holding capacity

low (<1 g/L), and thus the BC dry weight increased, but the existence of IGB in medium might affect the nutrient utilization of *G. xylinus* and thus the sugar consumption was lower. It is also possible that the medium viscosity increased as the IGB concentration to be higher, and thus limit the diffusion of IGB particles, leading to a reduction of the number of IGB particles which could enter into BC. Therefore, the BC dry weight would be lower but the sugar utilization increased instead as the IGB concentration to be higher.

Compared with the effect of IGB addition on BC production and sugar utilization of *G. xylinus*, the effect of IGB addition on BC water holding capacity was more complicated. As shown in Fig. 3, the water holding capacity of BC decreased obviously as the IGB addition to be higher when the IGB concentration was lower than 1 g/L. Surprisingly, when the IGB concentration was 2 g/L, the water holding capacity of BC increased significantly. The water absorption and swelling properties of IGB might explain this phenomenon [35]. However, when IGB concentration increased to 5 g/L, the water holding capacity of BC changed insignificantly. It is possible that the pores of BC were filled of IGB particles thus its water holding capacity was restricted. Based on this point, it is necessary to control the IGB concentration when using IGB for in situ modification of BC to produce BC/IGB composite as water absorbent.

Effect of IGB Addition on BC Production in Corncob Hydrolysate

Although the effect of IGB addition was more obvious in HS medium than in corncob acid hydrolysate, it is still necessary to learn the effect of IGB addition on BC production in corncob hydrolysate because lignocellulosic

hydrolysates have more potential of industrialization for BC production [5–8]. Not only the effect of IGB addition on BC yield but also its effect on BC composite performance as water holding material should be learned.

The effect of IGB concentration on BC production by *G. xylinus* in corncob hydrolysate was shown in Fig. 4. Obviously, with more IGB particle entering into the BC during the fermentation, the BC dry weight obtained was higher. Interestingly, BC dry weight increased almost linearly as the IGB concentration to be higher. In contrast, the pH value of the fermentation broth varied little in the medium with different IGB concentrations, demonstrating that IGB addition had certain buffering capacity for BC fermentation on corncob acid hydrolysate.

The effect of IGB concentration on sugar conversion rate (BC yield on sugar consumption, % w/w) was also shown in Fig. 4. When the IGB concentration was lower than 1 g/L, the sugar conversion rate decreased slightly as IGB concentration to be higher. Interestingly, when the IGB concentration increased to 2 and 5 g/L, the sugar conversion rate was higher for 25.8 and 20.6 %, respectively. This indicates that IGB addition can change the fermentation environment of corncob hydrolysate, and thus affects the sugar consumption and BC production.

In addition to the effect of IGB concentration on BC yield and sugar conversion rate of in situ fermentation in corncob hydrolysate, its effect on the water holding capacity of BC/IGB composite was further analyzed to evaluate its capacity as a water holding material. As shown in Table 1, the water holding capacity of BC/IGB composite decreased gradually as the IGB concentration to be higher, which was the opposite of in situ modification in HS medium. Thus, for BC water holding material production by in situ modification in corncob acid hydrolysate, it is necessary to control the IGB concentration. Obviously,

Fig. 4 The influence of IGB concentration on BC (obtained on corncob hydrolysate) pH, dry weight and sugar conversion rate

Table 1 Water holding capacity of in situ and ex situ modified BC/IGB samples synthesized with various concentrations of IGB

Sample number	Addition of IGB (g/L)	Water holding capacity (g water/g sample)	
		In situ modification	Ex situ modification
A	0.00	112.16 ± 0.25	95.94 ± 1.28
B	0.50	72.35 ± 6.19	52.24 ± 0.59
C	1.00	46.81 ± 3.06	30.46 ± 3.20
D	2.00	34.53 ± 5.14	34.21 ± 1.05
E	5.00	31.88 ± 2.70	29.02 ± 2.94

the water holding capacity was related to the specific surface area and pore size of BC/IGB composite. As mentioned above, the in situ modification by IGB addition might be influenced by a different fermentation environment, and thus the water holding capacity varied with different media.

Comparison of Water Holding Capacity of BC/IGB Composite Prepared by In Situ and Ex Situ Method

Besides in situ modification, BC/IGB composite could also be prepared by ex situ modification, namely make the composite after BC fermentation (see part of Methods and Materials). To compare the effects of in situ modification and ex situ modification on water holding capacity, the ex situ modification by IGB addition was also carried out. Similarly, IGB addition showed a negative effect on water holding capacity but when IGB concentration was higher than 1 g/L, the water holding capacity of BC/IGB composite changed little. This phenomenon showed that too many IGB particles may completely cover the surface of the BC sample, resulting in deterioration of water absorption; and different modification conditions might also showed various influences on the original structure of the BC. As a result, their effects on water holding capacity might be very different. Although BC/IGB composite was successfully prepared by in situ and ex situ method, in situ method seems a better one when the BC/IGB composite is used as water absorbent.

Water Release Rate of BC/IGB Composite

Besides water holding capacity, water release rate was another important parameter to evaluate the water holding capacity of materials. As shown in Fig. 5, the effect of IGB addition on the water release rate was different from the effect on water holding capacity. Overall, the water release rate of Sample A was obviously higher than that of Sample B, and a little higher than Sample C. During the initial stage of water evaporation (0–4 h), the water release rates of three samples were close and their water content decreased fastest in this period. 4 h later, the water release rate of Sample A was still fast but the rate of Samples B

Fig. 5 Water release rate for BC/IGB samples (obtained on corncob hydrolysate) **a** BC; **b** BC + 0.5 g/L IGB; **c** BC + 1 g/L IGB

and C were slower. It is possible that the interspaces of the BC had been occupied with IGB particles, and the combination of BC, IGB particles and water were tighter, thus the water release rate of Samples B and C was slower when compared with Sample A. Interestingly, the water release rate of sample A became slower after 20 h and after 30 h, the water release rate of three samples were maintained at lowest rate. After 50 h, most water in the three samples had been evaporated. Overall, the water retention performance order of three samples was Sample C > Sample B > Sample A. Thus, although IGB addition showed no obvious effect of water holding capacity of BC/IGB composite obtained on corncob hydrolysate, it could control its water release rate. In other word, BC/IGB composite obtained on corncob hydrolysate prepared by in situ method still show potential as water absorbent.

XRD Analysis

It was found that the performance of BC/IGB composite as water absorbent was complex and possibly related to fermentation substrate and IGB concentration from the results above. To explore the relationship of crystallinity and performance of BC/IGB composite as water absorbent, XRD analysis was further carried out for combination of BC and IGB. As shown in Fig. 6, the crystallinity of samples decreased gradually as the IGB concentration to be

Fig. 6 The X-ray spectra of BC, IGB and BC + IGB samples

higher. However, the BC water holding capacity was higher with suitable IGB addition in HS medium when compared with that without IGB addition. Obviously, the crystallinity of BC/IGB composite related little to its performance as water absorbent. To explore the relationship of structure and performance of BC/IGB composite as water absorbent, more characterization such as BET, FT-IR, etc. should be carried out in future researches.

Conclusions

In this study, in situ modification of BC has been accomplished in both HS medium and corncob hydrolysate, and the BC/IGB composite was successfully made. The BC/IGB composite obtained in HS medium had higher water holding capacity while BC/IGB composite obtained in corncob hydrolysate had no similar performance. However, the water retention capacity (showed by water release rate) was better for the BC/IGB composite obtained in corncob hydrolysate by in situ modification. Obviously, the effect of BC/IGB composite depends on the environment of in situ modification. Also, the crystallinity of BC/IGB composite related little to its performance as water absorbent. Overall, this “green” technology is suitable for further production of composite of BC and other clay materials.

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