Bacterial Cellulose Production from Waste products and Fermentation conditions optimization

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Abstract. Bacterial cellulose is a new type of microbial synthetic material. It has excellent properties compared with plant cellulose and thus it has application potential on many fields including food, biomedical material, paper manufacturing. In this study, yeast lees (tan-colored dead yeast particles that collect at the bottom of a fermentation vessel) were employed as low-cost substrates to produce bacterial cellulose using Glucoacetobacter xylinum BC-11. Results indicated that yeast lees performed well for the growth of G. xylinum BC-11 and the bacterial cellulose production. The fermentation conditions were also optimized: the proportion of lees and water is 4: 5, fluid volume is 75 milliliter, initial pH is 6.0. The study showed the possibility of using the fruit processing by-products to produce high value-added bacterial cellulose and made bacterial cellulose production more cost-effective.

1. Introduction
Bacterial cellulose (BC) is a kind of pure cellulose which synthesized by microorganisms, which are widely used in many fields including food, biomedical material, paper-making and transducer diaphragms [1, 2]. Therefore, bacterial cellulose as a new material has been attracted more attention with the development of molecular biology. Despite its enormous potential in various fields, the high cost of BC production is the main drawback that hinders its industrial application.

The yield of BC was affected by various factors, such as medium component, the culture conditions, the microorganism and so on [3]. Some cheap agricultural and industrial products or wastes have been used as alternative low-cost substrates for BC production. Hong et al. [4, 5] used processed crop straw as potential cost-effective feed-stock to produce BC for the first time. And the waste from beer culture broth was also used as medium to produce bacterial cellulose by a static cultivation [6].

In this study, for the first time, yeast lees were used as substrate for bacterial cellulose (BC) production using G. xylinus in static liquid culture. After 7 days of fermentation, BC yield and property were evaluated. In addition, the optimal fermentation conditions of G. xylinus in lees were studied.
2. Material and Methods

2.1. Microorganism, culture media and growth conditions
G. xylinus BC-11 was screened in our previous study and preserved in our lab. The pre-culture was performed using HS medium [7] (g/L, glucose 20, yeast extract 5, peptone 5, disodium hydrogen phosphate 2.7, citric acid 1.15, initial pH 6.0) at 28°C and 140 rpm for 48 h. After pretreatment of the selected substrates, other nutrients (nitrogen sources or trace elements) were added into substrates to make the cultivation medium (initial pH 6.0) for BC production by G. xylinus BC-11, respectively. Then, 10% (v/v) seed culture was inoculated into the medium and the fermentation was carried out in static culture at 28°C for 7 days. Control group was also set using the classical HS medium [7] (g/L, glucose 20, yeast extract 5, peptone 5, disodium hydrogen phosphate 2.7, citric acid 1.15, and initial pH 6.0. The fermentation was performed in duplicate and an average value of BC yield was given.

2.2. BC harvest
After cultivation, the fermentation broth and BC was separated by running water. Then, the BC was treated by 0.1M NaOH at 100°C for 20 min to eliminate the entrapped cells, followed by thoroughly washing with deionized water to neutral pH 7.2. After that, the BC was weighed and dried to constant weight at 80°C for 12 h [8]. Subsequently, the dry mass of BC was weighed and the BC yield was calculated. The water holding capacity (WHC) was calculated using the following formula:

\[
\text{water holding capacity} = \frac{\text{Mass of water removed during drying (g)}}{\text{Dry weight of bacterial cellulose (g)}} \times 100
\]  

2.3. Fermentation conditions optimization

2.3.1. The ratio of lees and water. The ratio was determined by comparing BC yield and WHC in different ratio gradient (lees: water=5: 5; 4: 5; 3: 5) lees mediums at suitable conditions as above. Medium with better performance is selected for next experiments.

2.3.2. Liquid volume. The liquid volume was determined by comparing BC yield and WHC in different volume gradient (50 mL, 75 mL, 100 mL, 125 mL) lees mediums at suitable conditions as above. Medium with better performance is selected for next experiments.

2.3.3. Initial pH value. Setting the initial pH value of mediums as 4.0, 4.5, 5.0, 5.5, 6.0 and fermentation was carried out in different initial pH at suitable conditions as above. BC yield and WHC were calculated.

3. Results and discussion
In this study, we selected yeast lees as low-cost cultivation medium for the growth of G. xylinus BC-11. The results showed that BC could be produced using this medium, shown in Figure1. BC produced with lees have similar properties (colour, thickness and flexibility) compared to the HS medium (Figure 1 A, B). The micro-structures of BC produced with the two different mediums were also analyzed by using SEM (shown in Figure 1 C, D). Clear networks of cellulose fibers produced in both of the two different culture media were observed, indicating that the micro-structure of the BC produced in lees were similar to the one produced with HS medium.

The mechanical properties of BC including the Young's modulus, tensile strength and strain at break) were also evaluated. As shown in Table 1, the BC samples obtained from lees showed no significant differences in Young's modulus, tensile strength and strain at break compared to BC produced with HS medium at Day 5 or Day 7 (p < 0.05). However, there was a positive correlation between the mechanical properties of BC and cultivation time, indicating that extending the fermentation time might increase
the polymerization degree of the cellulose, by which the mechanical properties of BC could be improved [9].

![Figure 1. Bacterial cellulose produced with yeast lees (A, C) and HS medium (B, D)](image)

| Samples   | Young's modulus (MPa) | Tensile strength (MPa) | Strain at break (%) |
|-----------|------------------------|------------------------|---------------------|
| BCHS-5th  | 7128±484\(a\)         | 59.3±4.84\(a\)         | 1.23±0.44\(a\)     |
| BCHS-7th  | 11218±681\(b\)        | 129.49±24.04\(b\)      | 4.66±0.94\(b\)     |
| BClees-5th| 8122±424\(a\)         | 64.1±11.04\(a\)        | 1.19±0.31\(a\)     |
| BClees-7th| 12182±364\(b\)        | 135.3±24.4\(b\)        | 5.23±0.14\(b\)     |

Note: BC samples obtained in HS medium and lees after 5 and 7 days of fermentation; Data are mean values ± SD (n = 6); ANOVA and Fisher's LSD were carried out at 5% significance; superscripts within the columns represent significance of difference.
Figure 2. Fermentation conditions optimization: loading volume (A), ration of lees and water (B), initial pH (C)

Fermentation conditions of bacterial cellulose synthesis were optimized, and the results were shown in Figure 2. The WHC and yields of BC decreased with the increase of loading volume obviously (figure 2A). It may result in the decline of the oxygen concentration in the media, which may lead to cell death and hinder BC production [10]. Glucose synthesis includes two main pathways: the pentose phosphate cycle and the Krebs cycle [11]. But high oxygen partial pressure may switch the pathway from the Krebs cycle to the pentose cycle, which causes lower BC production with the formation of glycolic acid [11]. In conclusion, the optimal liquid volume is 75 milliliters.

With the increase of lees concentration, BC dry weight decreased and WHC increased rapidly, which performed different changing trends (figure 2B). The main reason can be the existence of ethanol and nutritional components which may lead to the acceleration of BC production and promotion of BC quality in a certain concentration or be the opposite in higher concentration. With the comprehensive consideration, the optimal ratio of lees and water was 4:5.

As shown in the figure 2C, the BC yields were enhanced with increasing of pH from 4.0 up to 6.0, however, WHC of BC performed opposite trend. The lowest yield was obtained at pH 4.0, possibly due to the inhibitory effect of low pH on BC accumulation in the fermentation medium [12]. With the development of fermentation, pH of culture fluid continues to decline due to the acidic compounds metabolism. In this study, the optimal initial pH is 5.5.

4. Conclusion
In this study, the yeast lees, a kind of low-cost wine processing by-products, were employed as nutrients for the production of bacterial cellulose. Based on our results, the micro-structure and mechanical properties of BC produced from lees were similar to that produced with conventionally used chemical medium HS medium, indicating that yeast lees could be a cost-effective substrate to provide enough nutrients for the growth of G. xylinum BC-11 to produce high quality and quantity of bacterial cellulose.
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