Effect of inoculum size and glucose concentration for bacterial cellulose production by *Lactobacillus acidophilus*

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Effect of inoculum size and glucose concentration for bacterial cellulose production by *Lactobacillus acidophilus*

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Abstract. Bacterial cellulose (BC) has gained interest as new industrial materials because of its unique properties compared to other cellulose sources. Intense researches have been done to study the production of BC and finding a new strain source to meet the requirement of high yield of production with a low economic cost. In this work, the potential of *Lactobacillus acidophilus* as a new source of bacterial cellulose was studied by observing the effects of inoculum size and glucose concentration on the production of BC using the one-factor-at-time method. *L. acidophilus* was cultured in HS medium for 14 days at various cultivation conditions according to the experimental set-up. The results obtained indicate that the glucose concentration in the medium and the inoculum size of the bacteria had a significant role in the BC production. The highest BC of 1.843 g/L was achieved at 1.5 w/v% glucose concentration and 0.856 g/L at 6 v/v% of inoculum size. Although the amount of BC produced was comparatively low than BC produced from other bacterial strains, these results demonstrated the potential of *L. acidophilus* as a new strain source for BC production. Further study on other cultivation parameters is essential for the optimization of BC production by this *L. acidophilus* strain.

1. Introduction

The accelerating demand for cellulose in the papermaking industry has been related to the forest depletion. To replace the plant cellulose, more alternative sources of cellulose must be explored. Aside from plant cellulose, cellulose can also be produced by microorganisms like fungi, bacteria, and algae [1]. Bacterial cellulose is known to be more chemically pure, has a higher degree of polymerization, higher tensile strength and water holding capacity compared to plant cellulose [2].

Bacterial cellulose is produced by several bacteria species, such as Gluconacetobacter (formerly Acetobacter), Enterobacter, Klebsiella, Rhizobium, Agrobacterium, and Sarcina. To date, *Gluconacetobacter xylinus* was the most widely studied producer of extra-cellular pure cellulose [3]. As the interest for BC has increased in recent years, the potential use of other strains of bacteria to produce BC has also increased. Recently, researchers discovered that several lactic acid bacteria such as *Lactobacillus hiilgardi, Lactobacillus plantarum* and *Lactococcus lactis* have the capabilities to produce the cellulose [4–6]. Therefore, the potential of bacterial cellulose production from the new strain of lactic acid bacteria, *Lactobacillus acidophilus* was studied.

Though currently enormous researches on bacterial cellulose (BC) mainly focused on the biosynthetic process to achieve low-cost preparation and high cellulose production, the best cultivation condition for BC production was also in search. Depending on the cultivation methods, different BC

shapes with various characteristics were produced [3]. BC production appears to depend on a complex relationship involving cultivation conditions such as inoculum size, agitation rates, carbon and nitrogen sources, pH and also temperature. The selection of the best inoculum level that will affect microbial growth will be a crucial influence to achieve a higher yield of cellulose production [7]. Glucose was recommended as the ideal carbon source for most microbial cellulose production, however, the optimum glucose concentration particularly for Lactobacillus strain was not known. It was found out that when the initial glucose concentration is increased, the microbial cellulose production will be decreased. Thus it is desirable to begin the BC production with a low glucose concentration in batch cultures [8].

Lactic acid bacteria (LAB) is a group of gram-positive bacteria. Lactobacillus is rod-shaped, facultative anaerobic, non-spore-forming lactic acid bacteria which mostly found in fermented food [9]. *Lactobacillus plantarum* was one of the most dominant species. Various species of Lactobacillus genera such as *L. lactis*, *L. hilgardii*, and *L. brevis* has been proven to be the potential bacterial sources for BC synthesis [5,10]. Some of the Lactobacillus strains also showed their potential to produce BC in co-culture such as *Lactobacillus mali − Glucoacetobacter xylinus* and *Lactococcus lactis − Glucoacetobacter hansenii* [11,12].

As the production of BC is highly dependent on the bacterial activities and fermentation techniques, cost-effective production of BC is greatly anticipated. Thus, in this study, the potential of BC synthesized by *L. acidophilus* at different inoculum size and glucose concentration was investigated.

2. Materials and methods

2.1. Materials

The chemicals used were D-glucose, peptone, yeast extract, disodium hydrogen phosphate, citric acid and nutrient agar. All chemicals that be used in this experiment were analytical grade (99.9%). The *Lactobacillus acidophilus* was obtained from prebiotic drinks, Vitagen.

2.2. Pre-cultivation of inoculum

Freshly cultivated *Lactobacillus acidophilus* was inoculated and then cultured at 30°C, static condition for 72 h in a 50 mL centrifuge tube containing 100 µL of Vitagen and 10% sterilized soybean medium. The same cultivated medium was used as inoculum for further culture in parameter inoculum size and glucose concentration.

2.3. Inoculation of Lactobacillus acidophilus for bacterial cellulose production

HS broth medium containing 2.0% (w/v) glucose, 0.5% (w/v) peptone, 0.5% (w/v) yeast extracts, 0.27% (w/v) disodium phosphate and 0.15% (w/v) citric acid dissolved in 500 mL of distilled water (Mahdieh et al., 2019). The HS medium was sterilized using autoclave at 121 °C and 1 bar for 15 minutes. The HS medium is then poured into 250 mL conical flask containing 100 mL of HS medium for each flask. The inoculum is then poured into each flask, according to the designated parameter. The mixed medium will be incubated in shaking condition with 150 rpm for 14 days under 30°C incubator temperature.

2.4. Determination of bacterial cellulose

The crude BC was treated according to the method by Castro et al. [13]. The BC production was calculated using the following formula:

$$\text{BC production}\left(\frac{\text{g}}{\text{L}}\right) = \frac{\text{dry film weight}}{\text{the initial volume of fermentation medium}} \times 1000 \quad (1)$$

3. Results and discussion

3.1. Effects of inoculum size

To study the effect of inoculum size of *L. acidophilus*, inoculum size ranging from 2%, 4%, 6%, and 10% (v/v) was examined for BC production. Figure 1 shows that maximum BC production at 0.856 g/L occurred with 6% v/v inoculum size, but a further increase of 8% v/v and 10% v/v of inoculum resulted in reduced BC yield. Meanwhile, at the lowest inoculum size of 2% v/v, no BC was produced. High inoculum size has been proven in increasing BC production to a certain extent before being detrimental to bacterial growth. The excess inoculum in the culture medium will lead to competition between bacteria in using nutrients which eventually disrupted bacterial growth and BC production [7]. Besides, the presence of cellulose-negative mutants induced by agitation in the inoculum during the BC fermentation may also lead to lower cellulose production [14].

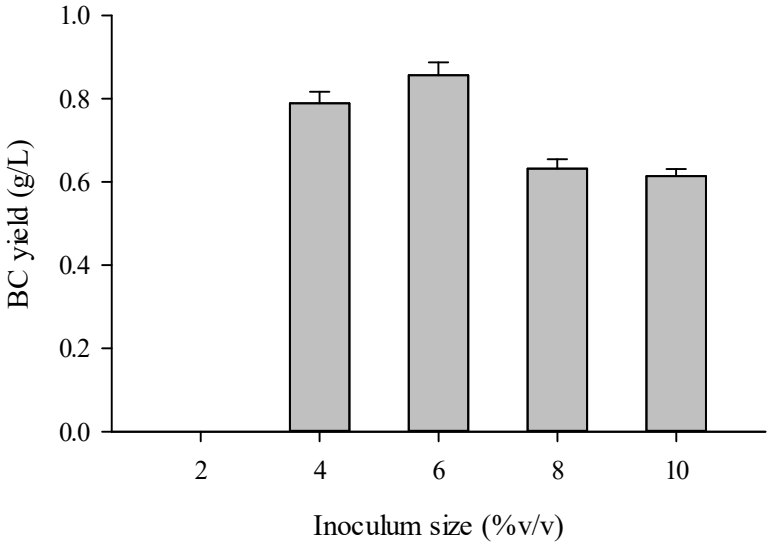

Figure 1. BC production at various inoculum size.

The present result is partly in agreement with Ragaswamy et al., who have stated that Gluconacetobacter sp., RV28 produced 4.7 g/L of cellulose at optimum growth conditions of inoculum density 5% v/v under static condition [15]. As for other Lactobacillus species, *L. hilgardii* IITRKH159 was discovered producing 7.39 g/L of BC at 8% v/v of inoculum [6]. Other lactic acid bacteria strain, *Lactococcus lactis* showed a similar pattern with our study where the highest BC at 41.4 g/L was produced at a medial inoculum size of 100 µL. At lower and higher inoculum sizes of 30 µL and 200 µL respectively, the BC yield decreased by 90 percent [4]. Similar to other BC-producing bacteria, these findings indicate that BC produced by lactic acid bacteria vastly affected by the size of the inoculum.

3.2. Effects of glucose concentration

Aside from being a carbon source for BC production, glucose also acts as an ideal precursor for the assembly of the highly structured cellulose polymer which calls attention to the study of optimum glucose addition concentrations for BC yields [11]. To investigate the effect of glucose concentration on the production of BC, various concentrations of glucose ranging from 0.5% w/v, 1.5% w/v, 2.0% w/v, 3.5% w/v, and 5% w/v were added.

Figure 2 shows that the BC production was enhanced with increasing amounts of initial glucose concentration up to 1.5% w/v, but started to decline when the glucose concentration was above 2% w/v. The highest BC production of 1.843 g/L was obtained at a glucose concentration of 1.5 w/v%. It has

been reported that BC production decreased with the increase in the glucose concentration for other BC-producing bacteria [16]. Meanwhile, for lactic acid bacteria strains, Umamehaswari et al. reported 41.4 g/L BC yield at a low glucose concentration of 2% for *L. lactis* [4]. However, in other studies of Lactobacillus BC-producing strain, *L. hilgardii* demonstrated that a high sucrose concentration of 50 g/L was required [6].

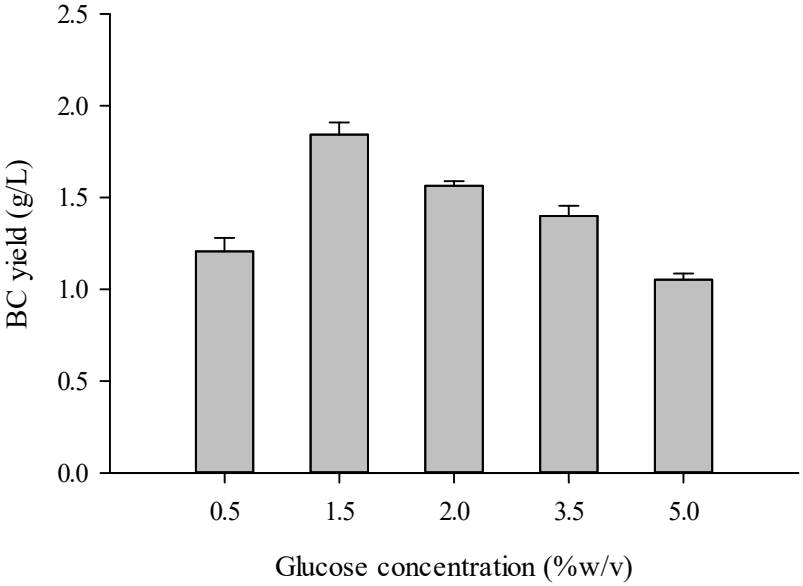

Figure 2. BC production in various glucose concentration.

Previous research reported that when glucose concentration continuously increasing and exceeding the maximum limit, the production of microbial BC will decrease. This result was observed in other previous study that found out glucose concentration at 1% w/v had the highest yield of BC production. This phenomenon might be due to the metabolism changes of the bacteria in high glucose concentration medium [17]. Moreover, the release of gluconic acid as a by-product to the medium caused by the degradation of glucose during BC synthesis notably affected the pH of the culture and eventually the BC production [18].

4. Conclusion

In conclusion, this study has shown that *Lactobacillus acidophilus* has a promising potential in producing BC. Eventhough the amount of BC produced in this study were comparatively low to other BC-producing bacteria, a further study that may enhance the BC yield has the potential to be carried out. The highest BC production of 1.843 g/L was achieved at a glucose concentration of 1.5 w/v and 0.856 g/L at 6 v/v% inoculum size. It is recommended for the optimization study of BC produced from *Lactobacillus acidophilus* and as well as the characterization study of BC production between static and agitated conditions for future works.

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