



Different Media Formulation on Biocellulose Production by *Acetobacter xylinum* (0416)

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ABSTRACT

Biocellulose (BC), produced by *Acetobacter xylinum* (0416), was carried out using three types of medium composition under static surface culture. The media used in this experiment included CWHSM (Coconut water in Hestrin-Schramm medium), CM (Complex medium) and HSM (Hestrin-Schramm medium). CWHSM and CM used coconut water from agro-waste as the main source of sugar. The fermentation was conducted for 12 days and the results of BC dry weight, cell entrapped, pH medium and productivity were evaluated and compared. The results show that CWHSM is the most suitable medium for BC production with a productivity of up to 0.044 g l⁻¹ day⁻¹.

Keywords: *Acetobacter xylinum*, agro-waste, biocellulose, coconut water, production medium

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INTRODUCTION

It is known that some *Acetobacter* strains produce cellulose. This cellulose is called bacterial cellulose (BC) (Ross *et al.*, 1991). BC is a chemically pure form of cellulose and it is free from hemicellulose, pectin, and lignin, which are associated with plant cellulose and are difficult to eliminate (Bielecki *et al.*, 2002; Jung *et al.*, 2005). In addition, BC is extremely pure and it exhibits a high degree of polymerisation and crystallinity. BC has many applications

which include temporary artificial skin for the therapy of burns, ulcers and dental implants; it is also used as non-woven paper or fabric to improve latex or other binders and repair old documents, as sensitive diaphragms for stereo headphones, cellulose for immobilisation of proteins and chromatographic techniques, stabiliser for emulsions in cosmetics, food and coating compositions and edible cellulose for addition to food (Jonas & Farah, 1998).

BC production has been demonstrated from glucose, sucrose, fructose, glycerol, mannitol and arabitol, among which mannitol and fructose are better carbon sources (Masaoka *et al.*, 1993; Oikawa *et al.*, 1995a; Oikawa *et al.*, 1995b; Ross *et al.*, 1991; Shoda & Sugano, 2005). The high economic cost of mannitol and fructose, as well as the relatively low-yield production with these carbon sources, limits industrial production and extended commercial applications of BC. Therefore, it is challenging and meaningful for us to look for a new approach to prepare a carbon source for high-yield BC production (Hong *et al.*, 2007). There are many sources of carbon that have been experimentally verified as substrates but the elimination is in the method of preparation, which involves highly toxic chemicals and time consuming steps such as hydrolysis and detoxification. It is necessary for the substrate to be detoxified before being utilised.

In this research, a relatively low-cost carbon source of culture media was successfully developed from agro-waste. The results indicate that coconut water, waste from copra and coconut milk industries with additional nutrient formulation could serve as a feedstock or potential media for bacterial cellulose production. Meanwhile, direct application of coconut water without involving any hydrolysis and detoxification steps makes it very economical as a main carbon source of media for BC production. The usage of coconut water as a medium substrate has also been suggested by Budhiono *et al.* (1999), but the formulation is totally different from the proposed medium in this study. Other than the nutrient, the significant difference was in the initial pH value of 4.5 for them and 6.0 for this study. With the aim to manipulate the waste into a valuable material, the waste from coconut water is turned into a valuable polymeric material for commercial application. The productivity and BC production rate are the important parameters in this study.

MATERIALS AND METHODS

The strain *Acetobacter xylinum* (0416) was supplied by the Malaysian Agricultural Research and Development Institute (MARDI) in Serdang, Selangor, Malaysia. Three types of media formulation for the new proposed medium are: (1) CWHSM (Coconut water in Hestrin-Schramm medium), (2) CM (Complex medium) as suggested by MARDI, and, (3) HSM (Hestrin-Schramm medium) as suggested by Hestrin and Schramm (1954). The composition of each medium is stated in Table 1. The fermentation was carried out in 250 ml conical flasks containing 100 ml of the medium for 12 days. Each flask was inoculated with a 3-day old preculture grown statically at 30°C. The dry weight of BC, cell entrapped within the pellicle, pH of the medium and productivity for each medium were evaluated and compared.

TABLE 1: Composition of media used

| MEDIUM COMPOSITION | | | | | |
|---|------------------------|---|----------------------|---|------------------------|
| (1) CWHSM: Coconut water in HS medium | | (2) CM: Complex medium | | (3) HSM: HS medium | |
| Sodium hydrogen phosphate (Na ₂ HPO ₄) | 2.7 g l ⁻¹ | Sucrose | 80 g l ⁻¹ | Glucose | 20 g l ⁻¹ |
| Bacto peptone | 5 g l ⁻¹ | Ammonium sulphate (NH ₄) ₂ SO ₄ | 5 g l ⁻¹ | Sodium hydrogen phosphate (Na ₂ HPO ₄) | 2.7 g l ⁻¹ |
| Yeast extract | 5 g l ⁻¹ | Coconut water | Up to 1 l | Bacto peptone | 5 g l ⁻¹ |
| Citric acid | 1.15 g l ⁻¹ | pH | 4.5 | Yeast extract | 5 g l ⁻¹ |
| Coconut water | Up to 1 l | Strain <i>A. xylinum</i> | 100 ml | Citric acid | 1.15 g l ⁻¹ |
| pH | 6.0 | | | Distilled water | Up to 1 l |
| Strain <i>A. xylinum</i> | 100 ml | | | pH | 6.0 |
| | | | | Strain <i>A. xylinum</i> | 100 ml |

Harvesting method

After removing the BC sample from the flask, the BC was washed twice with distilled water. Then, the gel was boiled in 0.5 M sodium hydroxide solution (NaOH) for 20 minutes to dissolve the cell entrapped within the pellicle. After that, it was drained and rewashed with distilled water several times. The gel was soaked in distilled water overnight to remove alkaline solution in the BC gel. The purpose of this step is to ensure that the alkaline used in this treatment is totally removed from the gel. This step was also performed to ensure that the degradation of BC would not occur as it could disturb the consistency of the BC dry weight measurement and the quality of the BC produced. Then, the gel was slowly dried in an oven at 40°C for about 5 hours. The dried gel sheets were weighed and the data were claimed as a dry weight of the BC production. The samples were stored in airtight cases.

RESULTS AND DISCUSSION

Fig.1 shows that M1-CWHSM (Coconut water in HS medium) gave the maximum BC production in the dry weight of product, followed by M2-CM (Complex medium) and the lowest was M3-HSM (HS medium). In order to get uniform results and for the purpose of comparison, the pre-culture used throughout the experiments was obtained from the same batch. The trend of the BC production in M1 and M3 is almost the same, while M2 remains constant throughout the experiment. A major difference observed between M1 and M3 formulation is only by substituting coconut water for the distilled water and glucose. The high production rate in M1 was probably because of the monosaccharide and disaccharides present in the medium, as suggested by Unagul *et al.* (2007), and which would have affected the metabolic pathway of the BC production. The highest production rate was obtained at day-8 of M1 by 0.343 g l⁻¹, which is approximately 4.0 fold higher than M3.

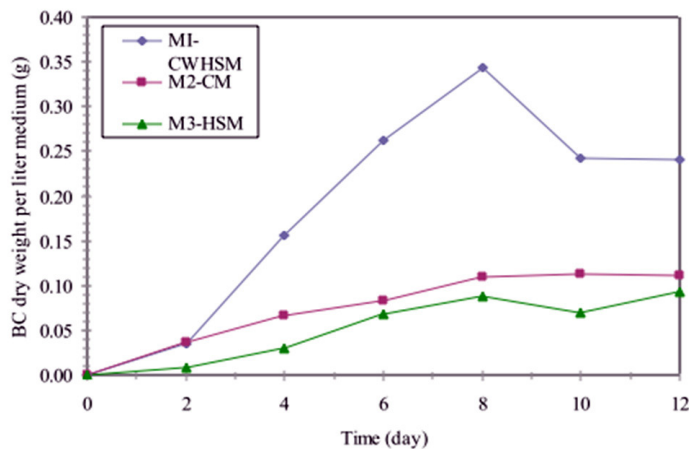


Fig.1: The dry weight of BC in different fermentation media

The metabolic pathway suggested by Serafica (1997) clearly states that glucose will directly convert to glucose-6-phosphate and phosphogluconic acid as a by-product. The precursors in cellulose synthesis are UDP-glucose and glucose, while sucrose and fructose are used as substrates for growth and BC formation. This is in a good agreement with the finding by Vandamme *et al.* (1998). When *A. Xylinum* is grown on a dextrose substrate (or a carbohydrate substrate of which it is a component), it was observed to have converted 26% of glucose into gluconic and 2-keto-gluconic acid (European Patent No. 86308092.5).

Medium M3, which only contains glucose as a substrate, was consumed by about one-third into by-product and this limited the conversion to BC as a major product. This result was verified and shown in Fig.2 for pH profile for each medium. A comparison between M1 and M3 clearly showed that a significant drop in pH was observed on day-4 of M3, which is strongly believed to be due to the accumulation of gluconic acid or acidic by-products in the medium. In more specific, the accumulation of acidic by-products is inversely proportional to the BC formation. Glucose for BC production was applied for the formation of other by-products. The first reading at day-2 showed that the pH value had dropped significantly for all the media. After that, the increase in the pH of the medium was observed up to day-6 for M1; however, M2 remained constant throughout the fermentation time. Medium M1 was too flexible as compared to the others, which is strongly believed to be due to various types of carbohydrate (monosaccharide and disaccharide) that is present in the medium. This condition provides a chance for microorganisms to adapt with the mild environment and promotes growth and conversion of polysaccharide. The accumulation of one type of substrate, with high concentration results in the medium to fast conversion reduces the chances for microbes to convert glucose into BC. The profile pH for M3 almost fluctuated because of the conversion of BC and acidic by-products by the glucose substrate.

Fig.3 shows the amount of cells entrapped within the BC pellicles plus water (wet state) in different medium formulations. The assumption was made that the amount of water that could be retained in the BC pellicle was almost the same. It is strongly believed that the amount of cells entrapped plus water is reflected in the amount of cells producing BC in the sample. It

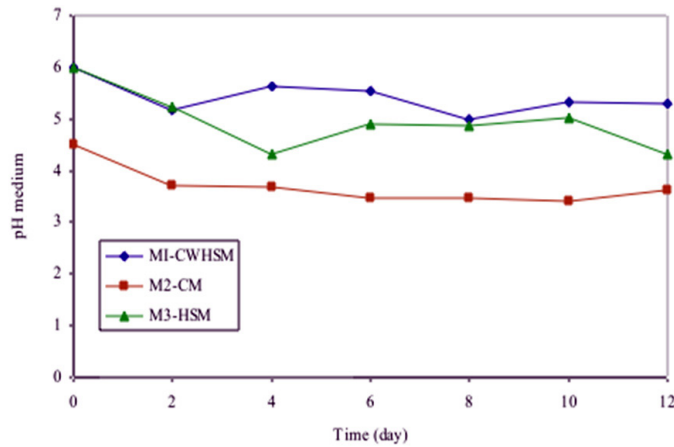


Fig.2: The pH of BC fermentation media

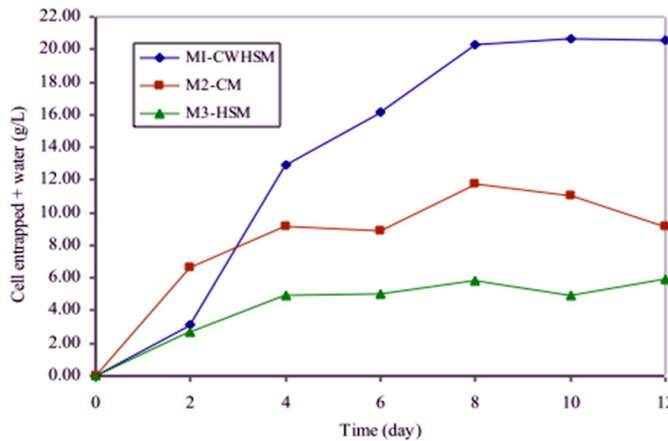


Fig.3: The amount of cells entrapped plus water in different fermentation media (wet state)

also represents active and viable cells that could synthesize BC. The BC pellicle formation mechanisms by *A. xylinum* cells is in a backward and forward motions of the *Acetobacter* cells known as reversal movements caused by synthesis of the cellulose (Shah & Malcolm, 2004). Recent research suggests that the build up of strain during the crystallisation of cellulose may be responsible for these reversals. The amount of cells entrapped within the pellicle is proportional to the BC production rate, as shown by media M1, M2 and M3. These results are in close agreement with those reported by Marx-Figini and Pion (1974); cellulose synthesis only occurs in growing bacterial populations and the yield of cellulose and the growth of bacterial populations obey the same reaction law, which is a first-order one. The fact that the cellulose synthesis only occurs when the bacteria is able to divide indicates that the production of BC must be combined with cell division itself or with some other events in the cell related to it (Marx-Figini & Pion, 1974).

Fig.4 shows the productivity of BC in different fermentation media, which is proportional to the dry weight of BC. The highest productivity was obtained by M1 at day-6 of incubation at $0.044 \text{ g l}^{-1} \text{ day}^{-1}$. This means the M1 medium formulation shows excellent productivity compared to the other media. Hestrin and Schramm (1954) and Forng *et al.* (1989) reported that although various undefined and synthetic media have been developed for *A. xylinum*, more cellulose has been produced by the undefined medium compared to the synthetic medium. M1 medium formulation serves the most complex composition compared to the others, which covers the requirements for BC production.

CONCLUSION

Based on the results obtained in this experiment, it can be concluded that medium formulation M1 CWHSM can be a potential medium for production of BC. The advantage of CWHSM is that the medium substrate is easy to procure, as it can be locally sourced and is inexpensive as well. The use of coconut water, without any pre-treatment or hydrolysis, makes it very effective from the economic point of view. In addition, it does not involve toxic and hazardous materials in producing BC, which is excellent and suitable for safe environments such as medical and cosmetic applications. Moreover, the static surface culture fermentation also requires a low cost operation and is easy to perform.

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REFERENCES

- Bielecki, S., Krystynowicz, A., Turkiewicz, M., & Kalinowska, H. (2002) In E. J. Vandamme, S. De Baets, & A. Steinbuechel. *Biopolymers*. Weinheim: Wiley-VCH.
- Ben-Bassat, A., Bruner, R., Shoemaker, S. P., Aloni, Y., Wong, H., Johnson, D. C., & Naogi, A. N. (1987). *European Patent No. 86308092.5*.
- Budhiono, A., Rosidia B., Tahera, H., & Iguchib, M. (1999). Kinetic aspects of bacterial cellulose formation in *nata-de-coco* culture system. *Carbohydrate Polymers*, 40, 137 - 143.
- Forng, E. R, Anderson, S. M., & Cannon, R. E. (1989). Synthetic medium for *Acetobacter xylinum* that can be used for isolation of auxotrophic mutants and study of cellulose biosynthesis. *Applied Environment Microbiology*, 55(5), 1317 - 1319.
- Hestrin, S., & Schramm, M. (1954). Synthesis of cellulose by *Acetobacter xylinum*: preparation of freeze dried cells capable of polymerizing glucose to cellulose, *Biochemical Journal*, 58, 345.
- Hong, F., & Qiu, K. (2007). An alternative carbon source from konjac powder for enhancing production of bacterial cellulose in static cultures by a model strain *Acetobacteracetii* subsp. *xylinus* ATCC 23770, *Carbohydrate Polymers*, 72(3), 545 - 549.
- Jonas, R., & Farah, L. F. (1998). Production and application of microbial cellulose. *Polymer Degradation and Stability*, 59, 101 - 106.

- Jung, J. Y., Park, J. K., & Chang, H. N. (2005). Bacterial cellulose production by *Gluconacetobacter hansenii* in an agitated culture without living non-cellulose producing cells. *Enzyme and Microbial Technology*, 37, 347 - 354.
- Marx-Figini, M., & Pion, B. G. (1974). Kinetic investigations on biosynthesis of cellulose by *Acetobacter xylinum*. *Biochimica et Biophysica Acta*, 338, 382 - 393.
- Masaoka, S., Ohe, T., & Sakota, N. (1993). Production of cellulose from glucose by *Acetobacter xylinum*. *Journal of Fermentation and Bioengineering*, 75, 18 - 22.
- Oikawa, T., Morino, T., & Ameyama, M. (1995a). Production of cellulose from D-arabitol by *Acetobacter xylinum* KU-1. *Bioscience Biotechnology and Biochemistry*, 59, 1564 - 1565.
- Oikawa, T., Ohtori, T., & Ameyama, M. (1995b). Production of cellulose from D-mannitol by *Acetobacter xylinum* KU-1. *Bioscience Biotechnology and Biochemistry*, 59, 331 - 332.
- Ross, P., Mayer, R., & Benziman, M. (1991). Cellulose biosynthesis and function in bacteria. *Microbiology and Molecular Biology Reviews*, 55, 35 - 58.
- Serafica, G. C. (1997). Production of bacterial cellulose using a rotating disk film bioreactor by *Acetobacter xylinum* (PhD Thesis dissertation). Rensselaer Polytechnic Institute.
- Shah, J., & Malcolm, B. R. Jr. (2004). *US patent 60/507,961*.
- Shoda, M., & Sugano, Y. (2005). Recent advances in bacterial cellulose production. *Biotechnology and Bioprocess Engineering*, 10, 1-8.
- Unagul, P., Assantachai C., Phadungruengluij S., Suphantharika, M., Tanticharoen, M., & Verduyn, C. (2007). Coconut water as a medium additive for the production of docosahexaenoic acid (C22:6n3) by *Schizochytrium mangrovei* Sk-02. *Bioresource Technology*, 98, 281 - 287.
- Vandamme, E. J., de Baets, S., Vanbaelen, A., Joris, K., & de Wulf, P. (1998). Improved production of bacterial cellulose and its application potential. *Polymer Degradation Stability*, 59, 93 - 99.

