EFFECT OF CARBON AND NITROGEN SOURCES ON BACTERIAL CELLULOSE PRODUCTION FOR BIONANOCOMPOSITE MATERIALS

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Abstract
Bacterial cellulose is one of biopolymers that are an interesting alternative material to use for reinforcement in bio-based composites as green products. Here we reported the production of bacterial cellulose using *Acetobacter xylinum* strain TISTR 975. The results revealed that carbon source and nitrogen source could affect both the bacterial cellulose yield and its physical property. The use of fructose and mannitol as carbon source gave a higher amount of cellulose yield (3.5 and 5.0 fold, respectively) when compared to sucrose. In addition, the use of yeast extract as nitrogen source in various ratios also enhanced cellulose production. Next, structure and properties of the bacterial cellulose will be further investigated.

Keywords: bacterial cellulose production, *Acetobacter xylinum* strain TISTR 975, carbon source, nitrogen source

Introduction

The use of nano-scale fillers as reinforcement in biobased composites is another technology that has been extensively investigated. With their nanometric size effect and extremely high specific-surface area, nano-fillers have the potential for significant reinforcement in composite materials at very small filler loadings and providing some unique outstanding properties as compare to their conventional microcomposite counterparts (e.g. natural fibre reinforced composites). Studies incorporated clay, chitin or cellulose whisker as reinforcement into biodegradable polymers, polyvinyl alcohol (PVA), polylactic acid (PLA), polycaprolactone (PCL), polyvinyl acetate (PVAc), polyhydroxy butyrate (PHB), cellulose acetate butyrate (CAB), starch and aliphatic polyesters to create bionanocomposites have been reported (Garcia de Rodriguez *et al*. 2006; Jung *et al*. 2007; Lu *et al*. 2006; Oksman *et al*. 2006; Roohani *et al*. 2008;).

Lately, bacterial cellulose, which presents a unique network structure of a random assembly of ribbon shaped nanofibres, has also drawn scientific attention as reinforcement for polymers. Bacterial cellulose has recently been incorporated in hydroxyapatite (HAp), polylactic acid (PLA), polyvinyl alcohol (PVA), cellulose acetate butyrate (CAB) and also as a hybrid material in apple and radish pulp (Gea *et al*. 2007; Millon and Wan 2006; Wan *et al*. 2007). An example of the high strength and high transparency composites of bacterial cellulose sheets reinforced phenolic resin attaining an impressive Young's modulus of 28 GPa has as well been reported (Nakagaito *et al*. 2005).

To effectively produce bacterial cellulose for forthcoming uses, the effect of carbon and nitrogen sources on a production of bacterial cellulose by *Acetobacter xylinum* strain TISTR 975 were investigated in this work.

Methodology

Refreshment of the *Acetobecter xylinum* and preparation of the starter culture
The solid medium was prepared by dissolving 5% w/v of sucrose, 0.5% w/v of yeast extract, 0.5% w/v of $(NH_4)_2SO_4$, 0.3% w/v of KH_2PO_4 , and 0.005% w/v of $MgSO_4.7\ H_2O$ in a litter of distilled water. Then 1.5% w/v of agar and 10% v/v of coconut juice were added into the prepared medium. It was then adjusted the pH to 5.0 by using acetic acid and sterilized at 121°C for 15 minutes. Next, this medium was poured into a Petri dish for later use as a solid medium. It was used to grow initial inoculums at 35°C in incubator for 1 week. Thereafter, a single colony was picked up and put into the control culture medium at 35°C in incubator with stirring condition (150 rpm) for 1 week. This incubated culture medium was then used as the starter culture for the growth of bacterial cellulose.

Bacterial Cellulose Production
Carbon source type
The control culture medium (adapted from Yamanaka *et al*. 2000) contains 5% w/v of sucrose, 0.5% w/v of yeast extract, 0.5% w/v of $(NH_4)_2SO_4$, 0.3% w/v of KH_2PO_4 , and 0.005% w/v of $MgSO_4.7\ H_2O$ in a litter of distilled water. In order to study the effect of carbon source type on bacterial cellulose production, sucrose in the culture medium was replaced with other carbon sources (i.e. lactose, glucose, fructose, glycerol and mannitol) for the bacterial cellulose production.
The strain of *A. xylinum* TISTR 975, 5.0% v/v, was inoculated into this culture medium. The growth condition is at 35°C in incubator with static condition for 7 days. Thereafter, the bacterial cellulose was harvested and purified by immersing in running water, 2% w/v NaOH, then 0.5% w/v NaOCl and finally running water, each step for 24 hours, respectively. In order to determine the dry weight or yield of the obtained bacterial cellulose from the culture, the purified bacteria cellulose pellicle was dried by using hydraulic hot press at 115°C for 5 minutes. The bacterial cellulose (BC) yield from each culture medium was averaged from 3 replicates. The BC yields from different types of carbon source were then reported as the yield ratio in relation to the BC yield from the medium containing sucrose (the control sample).

Nitrogen source type
The control culture medium (adapted from Yamanaka *et al*. 2000) contains 2 types of nitrogen source, 0.5% w/v of yeast extract (YE) and 5% w/v of $(NH_4)_2SO_4$. To observe the effect of each nitrogen source separately on the bacterial cellulose production, the culture medium contain either yeast extract or $(NH_4)_2SO_4$ were prepared for the bacteria cellulose culture. Thereafter, the source of nitrogen that provides the higher yield percentage of the culture bacteria cellulose was determined and used in combination with other nitrogen sources (i.e. peptone, polypeptone, $NH_4H_2PO_4$ and casein hydrolysate) to prepare the culture medium for the bacteria cellulose production. The dry weight of the obtained bacterial cellulose of each culture medium was averaged from 3 replicates. The BC yields from different types of nitrogen source were then reported as the yield ratio in relation to the BC yield from the medium containing yeast extract (YE) and $(NH_4)_2SO_4$ (the control sample).

Statistical and data analysis

All the treatments were performed in triplicate. Microsoft excel 2007 was used for calculating ANOVA and Duncan's new multiple range test of the experimental data obtained.

Results and discussion

Effect of a carbon source type on bacterial cellulose production.

This part is to address the effect of a carbon source on cellulose production in *A. xylinum* strain TISTR 975. Carbon is necessary for both cell growth and cellulose production. To investigate the effect of carbon source in cellulose production, here we used various carbon sources i.e. disaccharides (sucrose and lactose), monosaccharide (glucose and fructose) and alcohol (glycerol, and mannitol). *A. xylinum* was cultivated with 50 g/l of different carbon sources and then cellulose production was determined by relative yield of cellulose production as compared to sucrose (the control sample). From this study, mannitol showed the highest relative yield (~5 fold compared to the control carbon source). However, the effect of fructose (3.5 fold), glycerol (3 fold), glucose and lactose (less than 0.5 fold) on cellulose production were also reported, respectively (Figure 1). The statistical and data analysis suggested that different types of carbon source showed the significant differences ($p < 0.05$) in cellulose production. In addition, this result indicated that disaccharides (e.g. sucrose and lactose) were not to be expected for high cellulose production yield.

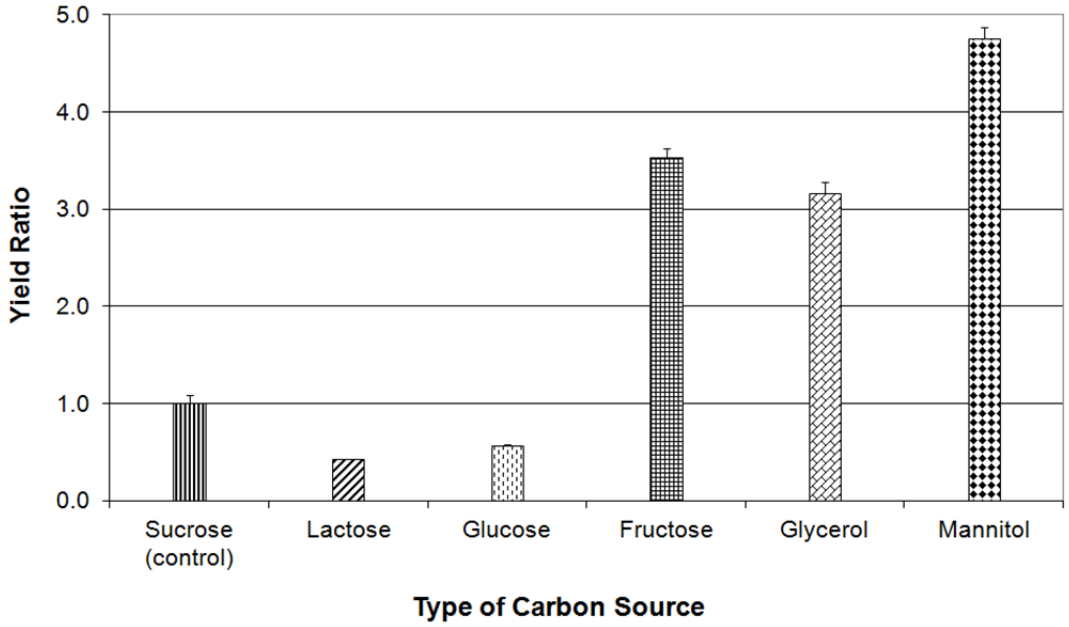

Figure 1 Yield ratio of the bacterial cellulose (BC) production with different types of carbon source.

Here, mannitol, fructose, and glycerol, were likely to be suitable for cellulose synthesis. So far many studies have been reported that bacterial cellulose productivity in *A. xylinum* could be increased yield in different carbon sources, for example, sugar alcohol (mannitol and glycerol), fructose, and glucose (Jonas and Farah 1998; Ramana *et al*. 2000; Bielecki *et al*. 2004; Panesar *et al*. 2009). Panesar and his co-workers and Jonas and Farah found that mannitol gave the high productivity for cellulose production (Panesar *et al*. 2009, Jonas and Farah, 1998). Ramana and his co-workers reported that among the carbon sources, mannitol was one that found to be suitable for optimum levels of cellulose production (Ramana *et al*.

2000). For cellulose production, carbon source is key precursor required for glucose synthesis by entering either in pentose phosphate pathway or gluconeogenesis pathway. However, mannitol may have more efficient carbon as intermediate to generate for UDP-glucose for cellulose synthesis than other carbon sources (Bielecki *et al*. 2004). Heo and his co-workers reported highest cellulose yield from *Acetobacter* sp. A9 by using glucose (Heo and Son, 2002). However, high amount of glucose concentration could inhibit cell growth and cellulose production due to the accumulation of (keto) gluconic acids (lower pH) (Vandamme *et al*. 1998).

Effect of a nitrogen source type on bacterial cellulose production.

Nitrogen is the main component of proteins necessary in cell metabolism and comprises 8-14% of the dry cell mass of bacteria. The effect of various nitrogen sources on the production of bacterial cellulose has been reported in *A. xylinum* (Chawla *et al*. 2009). In this work, different nitrogen sources were used to test the effect on BC production of *A. xylinum* strain TISTR 975. The cells were cultivated in sucrose culture medium supplement with different nitrogen sources at 5 g/l each (i.e. yeast extract, peptone, $(NH_4)_2SO_4$, polypeptone and casein hydrolyte). It was found that the combination of yeast extract (YE) + casein hydrolysate showed highest cellulose production as compared to the YE + ammonium sulphate $(NH_4)_2SO_4$) medium (used as the control sample) followed by the YE + YE medium.

In this study, the effect of combination of YE and other nitrogen sources was not significantly different ($p > 0.05$) in cellulose production (especially, the average BC obtained between the YE + casein hydrolysate and YE + YE medium, YE + polypeptone and YE + ammonium sulphate medium and YE + peptone and only YE medium (Figure 2). However, single nitrogen showed a considerable reduction in yield of BC production. The single nitrogen source of YE exhibited the higher relative yield than the single nitrogen $(NH_4)_2SO_4$ source. The use of $(NH_4)_2SO_4$ as a single nitrogen source has also been reported to decrease growth and cellulose yields in another research (Dudman 1959). So, the combination of YE and other nitrogen sources could better improve BC production. Among various nitrogen sources evaluated, Ramana and his co-workers have reported that peptone/ammonium sulphate or casein hydrolysate was found to be suitable for cellulose synthesis (Ramana *et al*. 2000). It was also observed that addition of methionine for culturing *A. xylinum* stimulated the growth rate during the early culture period, reduced the lag time and increased the cellulose production rate (Matsuoka *et al*. 1996).

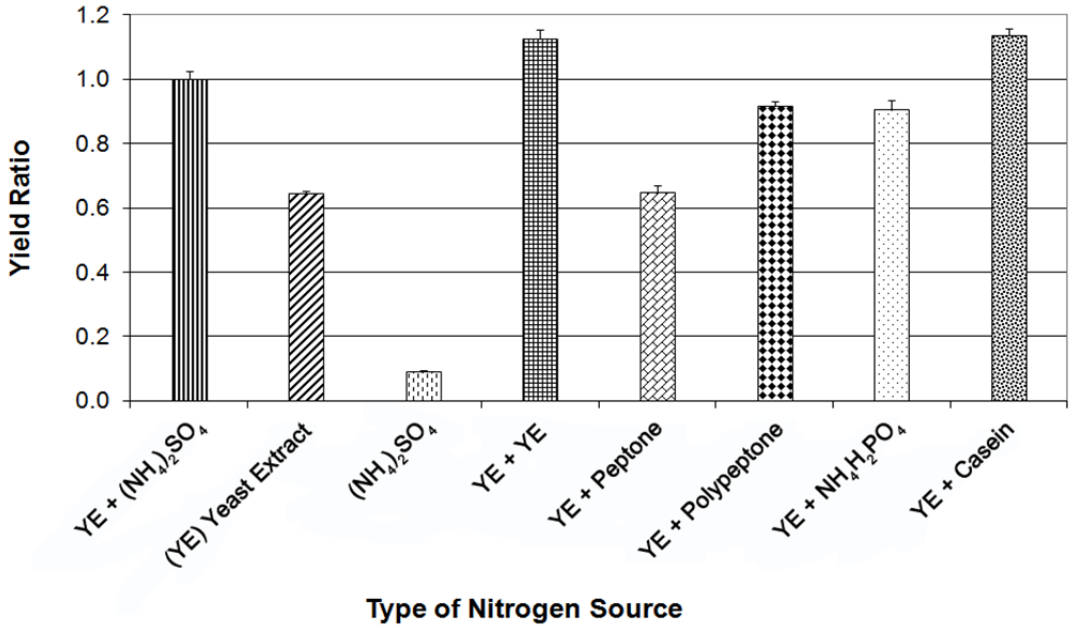

Figure 2 Yield ratio of the bacterial cellulose (BC) production with different types of nitrogen source.

Conclusions

Mannitol is found to be the most suitable carbon source for bacterial cellulose production by *A. xylinum* strain TISTR 975. It showed the highest relative yield around 5 fold compared to the control sample. However, fructose and glycerol are also having the positive effect to bacterial cellulose synthesis. Because of these carbon sources are able to entering either in pentose phosphate pathway or gluconeogenesis pathway and have more efficient to generate the intermediate; UDP-glucose, for cellulose synthesis by *A. xylinum*. The combination for yeast extract and casein hydrolysate and yeast extract and yeast extract also provided the highest relative yield as compared with the control sample. In addition, the effect of combination of yeast extract and other nitrogen sources are not significantly different in the yields. As organic nitrogen is the main component and necessary in cell metabolism and cell growth, they have an important effect on bacterial cellulose synthesis.

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