EFFECT OF ADDITIONAL SUPPLEMENTS IN THE CULTURED MEDIUM OF BACTERIAL CELLULOSE ON THE PRODUCTION YIELD

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Abstract

Bacterial cellulose has been used as biopolymer for bio-composite material. So far, many works have been reported that carbon, nitrogen and some supplement sources can affect bacterial growth and cellulose production. Here, we addressed the two supplements (coconut and pineapple juices) which were used for study on bacterial cellulose production. Then, *Acetobacter xylinum* strain TISTR 975 was cultured in static condition using different percents of supplement (0, 10, 30, 50, 70, 100). The results showed that both coconut and pineapple juice could increase both bacterial growth and cellulose production around 3-fold. The amount of supplements in culture media that provided the highest cellulose yield was 30% of pineapple juice and 50% of coconut juice. Next, the physical property of the bacterial cellulose will be further investigated.

Keywords: bacterial cellulose production, coconut juice, pineapple juice, *Acetobacter xylinum* strain TISTR 975

Introduction

Bacterial cellulose is synthesised by various bacteria belonging to the genera *Acetobacter*, *Rhizobium*, *Agrobacterium*, and *Sarcina* (Jonas and Farah 1998) in different form such as extracellular pellicle cellulose ribbons, cellulose fibrils and amorphous cellulose. However, the most efficient producers are Gram-negative, acetic acid bacteria *Acetobacter xylinum* (Bielecki et al. 2004). In a culture medium containing carbon and nitrogen sources, cultivated bacteria produce extracellular cellulose, an ultrafine ribbons network structure in the form of a highly hydrated pellicle (Barud et al. 2007; Iguchi et al. 2000). Dimensions of the ribbons are roughly 3-4 nm (thickness) and 70-130 nm (width). They are made from cellulose chains aggregated to form sub-fibrils, which have a width of approximately 1.5 nm and then the sub-fibrils are crystallized into microfibrils, which subsequently form bundles, while the latter form ribbons (Jonas and Farah 1998; Yamanaka et al. 2000). The unique properties of bacterial cellulose; i.e. high purity, high crystallinity, high mechanical strength, high water-holding capacity, high porosity and good biocompatibility have made it find a multitude of applications in paper, textile, and food industries, and as a biomaterial in cosmetics and medicine (Bielecki et al. 2004; Zhou et al. 2007).
To efficiently produce bacterial cellulose for approaching applications, the effect of additional supplements in the cultured medium of bacterial cellulose on the production yield were investigated in this work.

Methodology

Bacterial cellulose cultivation in the medium with additional supplements

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 investigate the effect of additional supplements in the cultured medium of bacterial cellulose on its production yield, the supplements (i.e. pineapple and coconut juices) were used in replacement of the volume of distilled water which is normally used in the control culture medium. For example, in a litter of the 10% v/v pineapple juice medium, the volumes of pineapple juice and distilled water used are 100 ml and 900 ml, respectively. The media of 10%, 20%, 30%, 50%, 70% and 100% v/v of both supplements were prepared. Then, the strain of *Acetobacter 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 the media containing different amounts of supplements were then reported as the yield ratio in relation to the BC yield from the medium without addition of the supplement (the control sample).

Results and discussion

Effect of additional supplements in the cultured medium of bacterial cellulose on the production yield.

Because the composition of juices have been reported that contains rich component such as free sugars content (mono- and disaccharides), trace element (magnesium, Mg^{2+} and manganese, Mn^{2+}), vitamins (such as nicotinic acid, biotin and pyridoxin) and some hormones (Hui and Muhamad 2007). These compounds play a crucial role in cellulose synthase gene activity i.e. promoter and transcription factors (Heo and Son, 2002). In this work, we interested to add pineapple or coconut juice in culture media due to they are locally available and cheap. However, other juices such as tomato juice were also investigated but low yield and costly were found. So that both pineapple and coconut juice would be used as efficient supplements rely on the complex of nutrient and cheaper compare to another one.

The effect of additional supplements (i.e. pineapple and coconut juices) in the cultured medium of bacterial cellulose on its production yield was investigated. *Acetobacter xylinun* strain TISTR 975 were grown in culture media containing 50g of sucrose, 5g of yeast extract, 5g of $(NH_4)_2SO_4$, 3g of KH_2PO_4, 0.05g of $MgSO_4$ and supplied with either pineapple or coconut juice (varied from 10, 20, 30, 50, 70 and 100% (v/v)) at the initial adjusted pH of 5.0 and 30 ºC in static culture condition. After cultivation for 7 days, bacterial cellulose pellicle was collected, purified and dried in order to determine the production yield of bacterial cellulose from each culture medium. The relative yield of BC cultured from each medium was also calculated in comparison to the yield of BC obtained from ‘the control media’ (0% addition or no juice added media). The effect of amount of added pineapple and coconut juices in the culture medium (%v/v) on relative yield of the BC production are shown in Figure 1 and 2, respectively. It was found that the addition of pineapple juice of 30% v/v and coconut juice of 50% v/v were the optimum amounts to supply into the culture media and the highest bacterial cellulose productivity, at approximately 3-fold increase in yield as compared to ‘the control media’, was obtained.

This result suggested that pineapple and coconut juices are the effective supplement sources that can be used in bacterial cellulose production. Since the composition of juices has been identified that they contain various carbon and nitrogen sources, trace elements (e.g. magnesium and manganese), vitamins and growth hormones, these would undoubtedly affect cell activity and cellulose yield in *A. xylinum* cultivation (Kurosumia et al., 2008). For example, magnesium (Mg^{2+}) or manganese (Mn^{2+}) ion as a cofactor is necessary for enzyme activity or control regulatory gene in cellulose biosynthesis e.g. glucosyltransferases and cellulose synthase enzymes (Ross et al., 1987; Jonas and Farah, 1998; Vandamme et al., 1997). In addition, the magnesium (Mg^{2+}) ion plays an important role in maintaining cellular metabolism for BC synthesis of *Acetobacter* strains (Heo and Son, 2002). The vitamins, pyridoxine, nicotinic acid and biotin are also the influence of cell growth and cellulose production. The other trace elements (e.g. acetate, citrate and succinate) showed highly effective in stimulating cellulose synthesis by *A. acetinenum* in defined medium (Dudman, 1959). These compounds and other intermediates of the tricarboxylic acid cycle are all readily oxidized to CO_2 by washed suspensions of *A. xylinum*. The buffering property of these substances can maintain the cultural pH value within the optimum range for cellulose synthesis (Schramm et al., 1957).

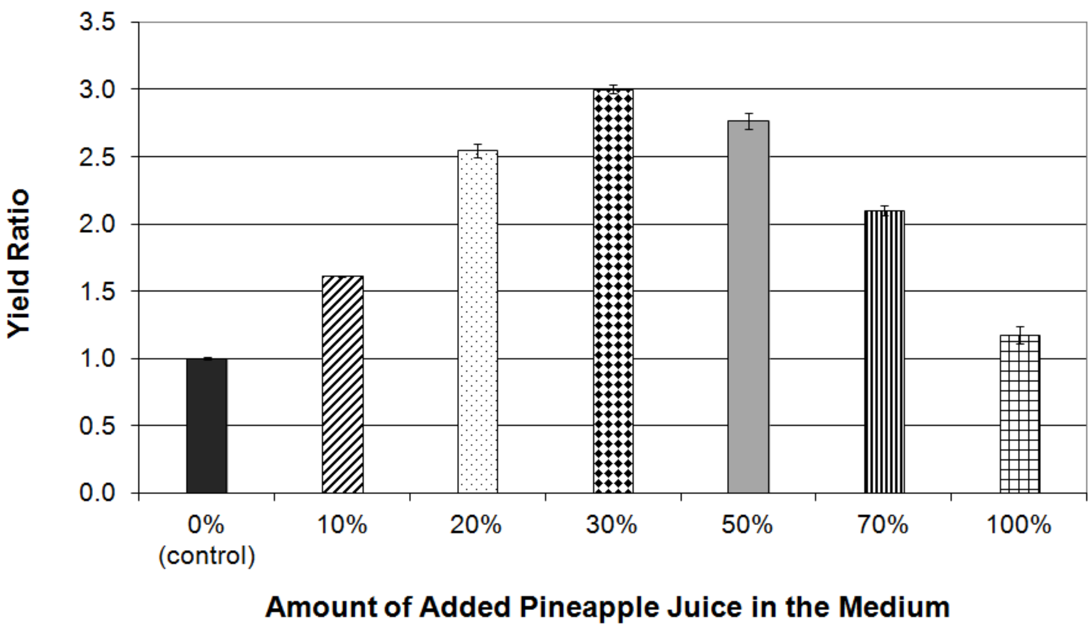

Figure 1 The effect of amount of added pineapple juice in the culture medium (%v/v) on yield ratio of the bacterial cellulose (BC) production

Furthermore, since nitrogen is the main component of proteins and necessary in cell metabolism, additional nitrogen sources from juices could probably help to promote cell metabolism in cellulose synthesis by *A. xylinum* (Chawla et al., 2009). On the other hand, carbons source is a key component necessary for both cell growth and cellulose production. Because of additional various sugars in juices supplemented in culture media, this may increase cell activity and the synthesis to have more amount of a building block to produce cellulose. Consistently, this present work showed that addition of optimum amounts of juices in the cultured medium of bacterial cellulose positively affected bacterial cellulose yield (i.e. addition of 10-30% v/v of pineapple juice and 10-50% v/v of coconut juice). In contrast, at high amount of juices, it caused the reduction in cell growth and cellulose production (see Figure 1 and 2). Here, more free sugars content (either mono or disaccharides) can create saturated carbon source environment that may directly inhibit cell activity (Kurosumi et al., 2009) and consequently, cellulose yield (i.e. the cultured medium supplied with pineapple juice of 50% v/v and higher and coconut juice of 70% v/v and higher).

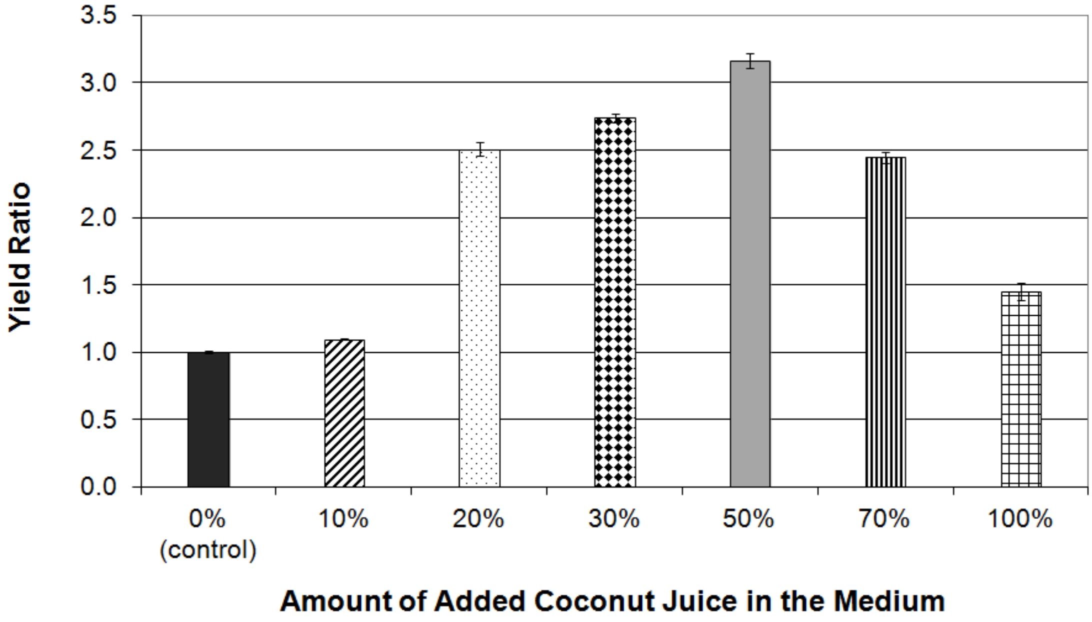

Figure 2 The effect of amount of added coconut juice in the culture medium (%v/v) on yield ratio of the bacterial cellulose (BC) production

In comparison between both supplements (pineapple and coconut juices), the results suggested that both supplements can efficiently improve the bacterial cellulose yield but pineapple juice is a slightly more effective supplement because at low amount of juice addition (i.e. 10-30% v/v), the higher yield was obtained in this media. This described that pineapple juice may have richer and higher concentration of components including carbon and nitrogen sources, trace elements, vitamins and growth hormones, to promote cell growth and bacterial cellulose production by *Acetobacter xylinum*.

Conclusions

The effect of additional supplements (i.e. pineapple and coconut juices) in the cultured medium of bacterial cellulose (BC) on its production yield was investigated. It was found that addition of optimum amounts of juices in the cultured medium of BC positively affected bacterial cellulose yield (i.e. addition of 10-30% v/v of pineapple juice and 10-50% v/v of coconut juice). This presumably due to various carbon and nitrogen sources, trace elements, vitamins and growth hormones which contain in the juices increase cell activity and consequently, cellulose yield. The addition of pineapple juice of 30% v/v and coconut juice of 50% v/v were the optimum amounts and the highest cellulose productivity at approximately 3-fold increase in production yield as compared to 'the control media' (no juice added media) was obtained. However, at higher amounts of juice addition (i.e. pineapple juice of 50% v/v and higher and coconut juice of 70% v/v and higher), the reduction in cell growth and cellulose production was found. Here, more free sugars content from the added juices can create saturated carbon source environment that may directly inhibit cell activity and subsequently, cellulose yield. In conclusion, the results suggested that both supplements can efficiently improve the cellulose yield but pineapple juice is a slightly more effective one. Pineapple juice may possibly have richer and higher concentration of components including carbon and nitrogen sources, trace elements, vitamins and growth hormones, to promote cell growth and bacterial cellulose production by *Acetobacter xylinum*.

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