BACTERIAL CELLULOSE PRODUCTION BY SOYBEAN WHEY BASED MEDIUM

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ABSTRACT

Bacterial cellulose, produced by *Acetobacter*, is a novel biomaterial. Soybean whey is the byproduct of the soybean protein. A medium containing soybean whey for producing bacterial cellulose by *Acetobacter xylinum* C5 was optimized to both reduce the cost of cellulose production and utilize the soybean whey. The results showed that sucrose, ammonium sulfate, peptone, $MgSO_4 \cdot 7H_2O$ and citrate are suitable for the production of cellulose. The best fermentation medium for the strain is sucrose 7%, peptone 0.5%, ammonium sulfate 0.5%, $MgSO_4 \cdot 7H_2O$ 0.2%, and citrate 0.1%. The yield of bacterial cellulose was increased when fermented in the medium reported in this paper.

Keywords: bacterial cellulose, soybean whey, *Acetobacter xylinum*

1. INTRODUCTION

Bacterial cellulose, which is also called microbial cellulose, was first found and reported by Brown in 1886. When *Acetobacter xylinum* is cultured statically, a gelatinous membrane of bacterial cellulose forms on the air-liquid interface. While it is cultured in agitated condition, small aggregates of bacterial cellulose are obtained.

Bacterial cellulose is a novel biomaterial and is the focus of this study. It can be applied in the manufacture of food additives and functional food, as a temporary substitute for human skin, and as acoustic transducer diaphragms [1-3]. Coco-de-nata, a popular food in the Southeast Asia, is bacterial cellulose cultured in coconut water.

Soybean whey is a byproduct of soybean protein. It contains solids 1.5 %, which comprise saccharides 0.97 %, and protein 0.32 %. The pH is 4.5 [4]. Production of one ton of soybean protein is accompanied by 10 tons of soybean whey as a byproduct. If the soybean whey is discarded without any treatment, the source will be wasted and will pollute the environment. To produce bacterial cellulose with soybean whey can both reduce the cost of production and protect the environment.

In this study, we investigated producing bacterial cellulose by *A. xylinum* with soybean whey.

2. MATERIAL AND METHODS

Cellulose–producing strain

Acetobacter xylinum strain C5 used in this study was given by Mrs. Duangjai Ochaikul (Dept. of Applied Biology, King's Mongskute Institute of Technology Ladkrabang). It was isolated in Thailand.

Culture media

The soybean whey medium (SW) consisted of 3% w/v sucrose, 1% w/v peptone, 0.1% w/v citrate, and the soybean whey was used instead of distilled water.

For the experiment to examine the effect of carbon source on cellulose production, SW medium was used as basal medium, but without sucrose, which was replaced by 8 individual sugars and 2 combinations (1:1 wt/wt) at a concentration of 3%.

For the experiment to examine the effect of nitrogen source on cellulose production, SW medium was used as basal medium, but without peptone, which was replaced by 4 individual organic or inorganic nitrogen source and 2 combinations (1:1 wt/wt) at a concentration of 1%.

For the experiment to examine the effect of inorganic salts on cellulose production, SW medium was used as basal medium, but without $MgSO_4 \cdot 7H_2O$, which was replaced by 7 individual inorganic salts at a concentration of 0.2%.

Culture conditions for cellulose production

Static culture for cellulose production was carried out using 100-ml conical flasks. The volume of the culture medium was 40 ml. The broths were inoculated at 28 °C for 6 days.

Experimental design

A three-level, three-factor design was used. Table 1 presents the coding of the levels of independent variables.

All experiments were repeated 2 times.

Table 1. Fermentation variables and their levels

	A Sucrose (%)	B Peptone+$(NH_4)_2SO_4$ (%)	C Citrate+$MgSO_4.7H_2O$ (%)
1	5.0	0.3+0.3	0.05+0.1
2	6.0	0.4+0.4	0.1+0.2
3	7.0	0.5+0.5	0.15+0.3

Analytical procedures

Measurement of cellulose production, bacterial cellulose was collected by filtration using a Buchner funnel with a glass filter, washed with distilled water, suspended in 4 % NaOH solution, and incubated at 100 °C for 20 min. The product was washed successively with distilled water, 0.5 % acetic acid, and distilled water, dried at 80 °C and weighted after cooling to room temperature [5]. The cellulose production was expressed as the amount of cellulose production per liter of medium.

pH assay

The pH of the medium before and after cellulose production was determined using a Digital Ionalyzer.

Cell concentrations

Cell growth was measured by the optical density at 600 nm.

3. RESULTS

Effects of carbon source on the production of bacterial cellulose

Experiments showed that the production of bacterial cellulose is affected by the kind of sugar present in the medium (Figure 1). Among the carbon sources that were used to test their effect on cellulose production, the highest productivity was obtained by using mannitol as carbon source followed by fructose and sucrose. All the other carbon substrates, such as maltose, galactose, starch proved be poor subatrates because of relatively low product yield.

Acetobacter xylinum C5 can grow and produce bacterial cellulose in the soybean whey without the addition of any carbon substrates, which indicates that soybean whey contains some oligosaccharides that can be utilized by *A. xylinum*.

Effects of nitrogen source on the production of bacterial cellulose

Acetobacter xylinum C5 can produce bacterial cellulose in soybean whey without adding any nitrogen substrates. Adding some nitrogen substrates can increase the yield of cellulose

(Figure 2). A combination of peptone and ammonium sulfate was found to be the most suitable for cellulose synthesis, and its effect was better than using peptone or ammonium sulfate alone. Adding yeast extract resulted in no increase in the amount of cellulose produced. Ammonium nitrate inhibited the growth of cells and cellulose production.

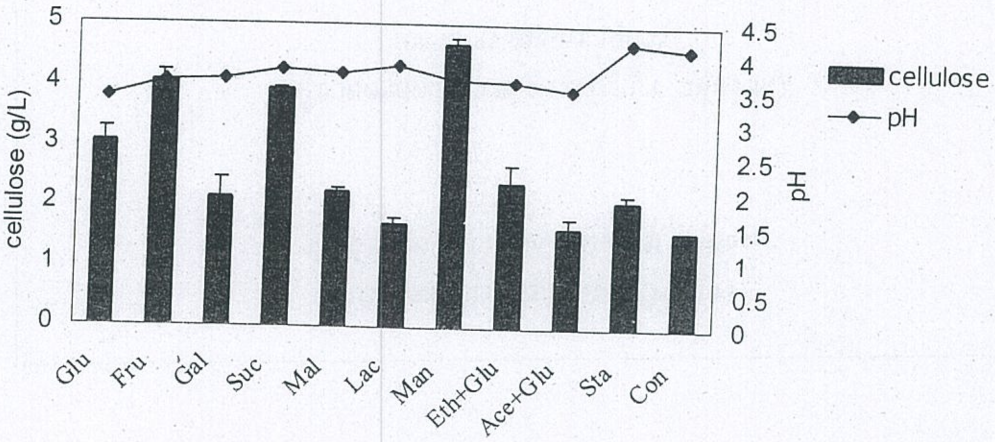

Fig.1 Effects of Carbon Source on the Production of Bacterial Cellulose
Glu=glucose; Fru=fructose; Gal=galactose; Suc=sucrose; Mal=maltose; Lac=lactose; Man=Mannitol; Eth+Glu=ethanol+glucose; Ace+Glu=acetic acid+glucose; Sta=starch; Con=control

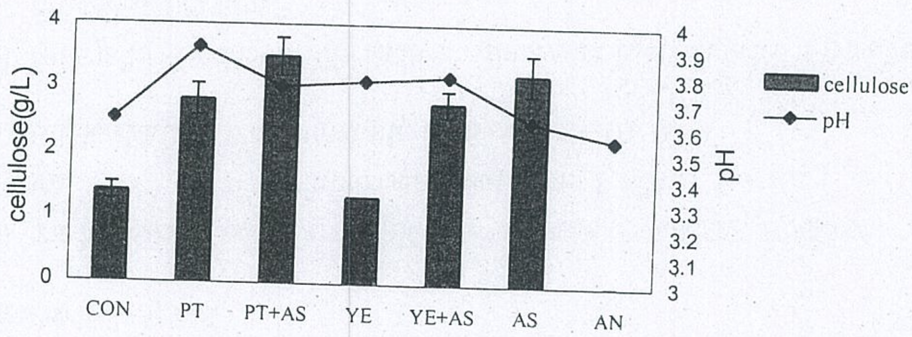

Fig.2 Effects of Nitrogen Source on the Production of Bacterial Cellulose
CON=control, PT=peptone, YE=yeast extract, AS=ammonium sulphate, PT+AS=peptone+ammonium sulphate, YE+AS=yeast extract+ammonium sulphate, AN=ammonium nitrate

Effects of salts on the production of bacterial cellulose

Among 7 salts tested, $MgSO_4 \cdot 7H_2O$ gave the best yield, followed by citrate (Figure 3). NaCl, $CaCl_2$, KH_2PO_4 had fewer effects on the production of cellulose. $ZnSO_4$ inhibited the synthesis of cellulose completely, at the same time the pH of medium became very low, but the cells could grow.

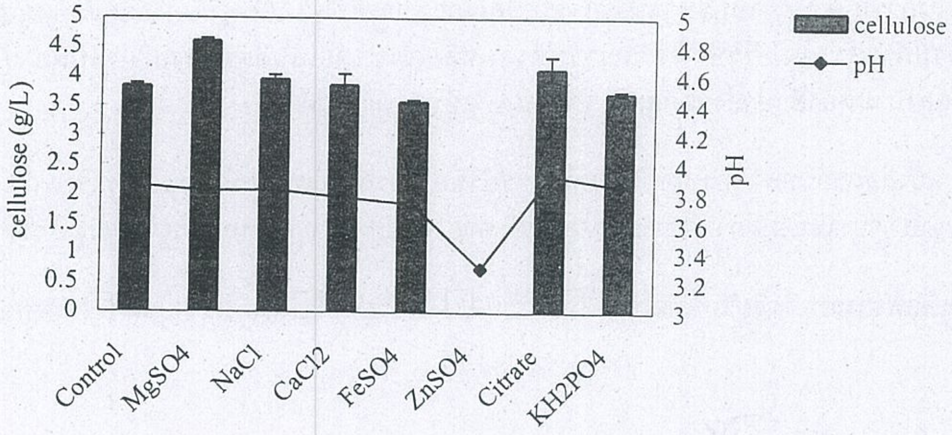

Fig 3 Effects of sults on the production of bacterial cellulose

Optimization of the soybean medium for bacterial cellulose

According to the experiments above, we chose sucrose, peptone+ $(NH_4)_2SO_4$, citrate+$MgSO_4 \cdot 7H_2O$ as the components of the medium. A $L_9(3^4)$ orthogonal design was used to optimize the medium (Table 1). The treatments, the experimental data and range incidence analysis are summarized in Table 2.

All the components we chose affected the production of cellulose. The contribution they gave to the yield of cellulose is, sucrose > peptone+$(NH_4)_2SO_4$ > citrate+$MgSO_4 \cdot 7H_2O$. The effect of sucrose on the production of cellulose was highly significant. With the increase of the sucrose concentration, the yield of cellulose increased too.

The range incidence analysis showed the optimum soybean medium was, sucrose 7.0 %, peptone 0.5 %, $(NH_4)_2SO_4$ 0.5 %, citrate 0.1 %, $MgSO_4 \cdot 7H_2O$ 0.2 %.

Bacterial cellulose fermented with soybean whey

The production of cellulose by *Acetobacter xylinum*C5 was compared between the media prepared with soybean whey and distilled water separately (Table 3). The results showed that when using the soybean whey medium, the yield of bacterial cellulose and the cell growth were better than those using the normal medium. We presumed it was because there were some accelerative factors in the soybean whey that could increase both the synthesis of bacterial cellulose and the growth of the cells.

Table 2. Experimental data for $L_9(3^4)$ orthogonal analysis

	A	B	C	Yield (g/L)
1	1	1	1	3.95
2	1	2	2	4.3
3	1	3	3	4.538
4	2	1	3	4.513
5	2	2	1	4.425
6	2	3	2	4.675
7	3	1	2	5.088
8	3	2	3	4.688
9	3	3	1	5.088
K1	12.788	13.551	13.463	
K2	13.613	13.413	14.063	
K3	14.864	14.301	13.739	
k1	4.263	4.517	4.488	
k2	4.538	4.471	4.688	
k3	4.955	4.767	4.58	
R	0.692	0.199	0.2	
Order from more importance to less importance		A>C>B		

Table 3. Comparison on cellulose yield between soybean whey and normal medium

Medium	Yield (g/L)	OD_{600}
Soybean	5.888±0.25	1.893
Normal	3.375±0.3	0.223

4. DISCUSSION

Acetobacter xylinum can utilize many kinds of carbon sources. In the experiment we found that mannitol was suitable for cellulose production, which is the same as Oikawa *et al* [5]. Others reported that sucrose [6, 7], glucose [8], fructose [9] or lactose [10] was the best carbon source for the production of cellulose. We think it depends on the differences in strains of *A. xylinum*.

Although the bacterial cellulose is polymerized with the glucoside, the productivity of cellulose was lower when glucose used. *A. xylinum* is known to convert glucose to gluconic acid. Embuscado *et al.* [9] presumed that gluconic acid production was the primary biochemical reaction in the glucose-containing media. Oikawa *et al* [5] found the final pH of glucose medium decreased to acidic pH (3.4) while the D-mannitol medium did not, and they presumed this was one of the reasons for high productivity of cellulose from D-mannitol by *A. xylinum*. But our results showed that the final pH of 11 media, including D-mannitol medium, were decreased to acidic pH (3.4~4.3). The principal reason for the difference in cellulose production remains unclear. We chose sucrose as the carbon source because of the low cost of sucrose.

Embuscado [9] found that organic nitrogen sources gave higher yields than inorganic sources. Yu [11] reported that *A. xylinum* could not utilize the inorganic nitrogen. Our result showed that *A. xylinum* C5 can utilize the organic nitrogen in the soybean whey well and inorganic nitrogen, such as $(NH_4)_2SO_4$, is better than organic nitrogen for bacterial cellulose production.

In this study, we found that there was no difference on the color, odor, and tissue between the bacterial cellulose prepared with the soybean whey and that prepared with normal medium. So the soybean whey bacterial cellulose can be applied to the food process.

The substrate specificity of *A. xylinum* is advantageous to remove organic pollutants in the sewage treatment. This is the first report on producing bacterial cellulose with the soybean whey. This study helps in soybean process and food industrial sewage treatment.

5. ACKNOWLEDGEMENTS

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