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Low Cost Production of Bacterial Cellulose from Food Processing Residues

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Abstract

Apple pomace and soy pulp (okara) were discharged from food processing companies in Aomori Prefecture, and the additional value for utilization of these residues has been requested for the long time. It was known that the cellulose produced by some strains of bacteria, the cellulose was called Bacterial Cellulose (BC). However, the costs of production were high, so the utilization of the BC cannot become widespread. In this study, we investigated the conditions to produce the BC at low-cost, and the following five experiments were carried out; 1) screening of BC producers, 2) production of the BC by using the commercial reagents at low-cost, 3) screening of the commercial crude cellulase suitable for apple pomace, 4) production of the BC by using the saccharified solutions of apple pomace, and 5) production of the BC by using the saccharified solutions of apple pomace and okara. As results, strains of *Gluconacetobacter xylinus* NBRC-16682 and NBRC-16644 with high productivity and NBRC-13693 with good productivity at lower pH area were selected. The range of optimized pH condition was from 5 to 7. The BC would be produced from the apple pomace and the okara without the nitrogen source.

Key words: Bacterial Cellulose, Apple pomace, Okara (soy pulp), Saccharification

1. Introduction

A large amount of apple pomace has been discharged from apple juice processing companies in Aomori Prefecture. Recently, apple pomace has been utilized as livestock feed and soil conditioner, the amount of food processing residues for industrial waste has decreased every year. However the additional value for utilization of apple pomace has requested from these companies. Additionally, the processing industry using soybean has been working all the year round in Aomori Prefecture. The soy pulp (okara) discharged from the process to make tofu etc., and the utilization of the okara has been required too. Therefore, we are able to utilize these industrial wastes for biomass resources.

The cellulose produced by some strains of bacteria was called Bacterial Cellulose (BC), it is known to nano-fiber. The BC has very unique properties such as three-dimensional network, very high Young's modulus, high crystallinity, and high purity compared with plant cellulose.[1] For example, an elastic food "nata-de-coco" was contained the BC, and high quality loudspeakers were prepared by using the BC. For the last few years, the BC has been expected to be used in new medical and industrial field.[2] As the production costs were high, the utilization of the BC could not become widespread.

In this study, we investigated the conditions to produce the BC at low-cost, and the following five experiments were carried out; 1) screening of BC producer, 2) production of the BC by using the commercial reagents at low-cost, 3) screening of the commercial crude cellulase suitable for apple pomace, 4) production of the BC by using the saccharified solutions of apple pomace, and 5) production of the BC by using the saccharified solutions of the apple pomace and the okara.

2. Materials and Methods

2.1 Materials

The apple pomace and the okara were provided by food processing companies in Aomori Prefecture. The discharged samples from manufacturing processes were stored at -20°C before usage.

2.2 Bacterial strains

Strains of *Gluconacetobacter xylinus* NBRC-13772, NBRC-16644, NBRC-16670, NBRC-16672, NBRC-16682, and NBRC-13693 were obtained from the National Institute of Technology and Evaluation (NITE) Biological Resource Center (NBRC), and the BC-1106 was isolated from apple-vinegar fermentation (Table I).

Table I. Strains of BC producers

	Strain
S-1	NBRC-13772
S-2	NBRC-16644
S-3	NBRC-16670
S-4	NBRC-16672
S-5	NBRC-16682
S-6	NBRC-13693
S-7	BC-1106

2.3 Preculture and culture medium

The medium was comprised of 0.5% (w/v) polypepton, 0.5% (w/v) yeast extract, 0.5% (w/v) glucose, 0.5% (w/v) mannitol, 0.1% (w/v) $MgSO_4 \cdot 7H_2O$, and 0.5% (v/v) ethanol. This medium of No.350-medium has been recommended for an *acetobacter xylinus* medium by the NBRC. A preculture

medium was the No.350-medium controlled at pH6.8, and a culture medium was No.350-medium controlled at pH3, 5, 7, 9, and 11.

2.4 Purification of the BC-produced by bacteria

The products by the fermentation were purified by 11 steps (Table II). The purified BC was white or translucent color, and elasticity of the BC increased with increasing the amount.

Table II. Purification of the BC from bacterial fermentation products

Step	Treatment
1	Treated by 2%SDS in a boiling water bath for 2hr
2	Washed by distilled water
3	Treated by distilled water in a boiling water bath for 2hr
4	Washed by distilled water
5	Treated by 4%NaOH in a boiling water bath for 2hr
6	Washed by distilled water
7	Treated by distilled water in a boiling water bath for 2hr
8	Washed by distilled water
9	Treated by distilled water in a boiling water bath for 2hr
10	Filtered by grass filter and washed by distilled water
11	Dried at 105°C until constant weight

2.5 Screening of the BC producer

Each of the strains was inoculated into the sterilized 20ml of the preculture medium with a platinum loop from slants stored at 4°C, and cultured with shaking (120 rpm) at 30°C for 3 days. After the preculture for 3 days, 100μl of the preculture medium was added to the sterilized 5ml culture medium in a sterilized tri-plate, and cultured statically at 30°C for a week. After that, the products by fermentation were purified, and weighted as the BC.

2.6 Production of the BC by using the commercial reagents at low cost

The improved low cost media based on the No.350-medium were controlled at pH5 and autoclaved for 30 min at 121°C (Table III).

Table III. Composition of the low cost culture media for the BC production

	Polypepton (w/v)%	Yeast extract (w/v)%	Glucose (w/v)%	Mannitol (w/v)%	Ethanol (v/v)%
M-1	0.5	0.5	0.5	2.0	0.5
M-2	0.5	0.3	0.5	2.0	0.5
M-3	0.5	0.1	0.5	2.0	0.5
M-4	0.5	0	0.5	2.0	0.5
M-5	0.5	0.5	0.5	1.0	0.5
M-6	0.5	0.5	0.5	0.5	0.5
M-7	0.5	0.5	0.5	0.1	0.5
M-8	0.5	0.5	0.5	0	0.5

Each of the selected bacteria (NBRC-13693, NBRC-16644, and NBRC-16682) was cultured in 40ml of the preculture medium at 30°C for 3 days with shaking (120 rpm). After the preculture for 3 days, 100μl of the preculture medium was added to the 5ml improved low cost media and cultured under static conditions at 30°C for a week. After a week, the products by fermentation were purified, and weighted as the BC.

2.7 Screening of the commercial crude cellulase suitable for the apple pomace

The apple pomace was mixed and mashed with an equal volume of distilled water by the blender (7010S, Waring), and autoclaved for 30 min at 121°C. After addition of individual enzymes of 0.1g (Table IV) to a sample of 200g without controlled pH, the mixture was incubated at 40°C for 48 hrs by shaking (100rpm), and the amount of pulp and reduced sugar in these samples were measured at 0 hr, 6 hrs, 24 hrs, and 48 hrs. The pulp was measured as the ratio of precipitate in a calibrated 10ml test tube, after centrifugation for 10 min at 3,000 rpm (H-100B, Kokusanenshinki). Reduced sugar was analyzed by Somogyi improved method for the determination of glucose. [3]

Table IV. The commercial crude cellulases for the apple pomace

	Enzyme name
E-1	Cellulase Y-NC (Yakult Pharmaceutical Co, Japan)
E-2	Cellulase "ONOZUKA" 3S (Yakult Pharmaceutical Co, Japan)
E-3	Sumizyme AC (Shin-Nihon Kagaku Kogyo Co, Japan)
E-4	Sumizyme C (Shin-Nihon Kagaku Kogyo Co, Japan)
E-5	Sumizyme X (Shin-Nihon Kagaku Kogyo Co, Japan)
E-6	Sumizyme ACH (Shin-Nihon Kagaku Kogyo Co, Japan)
E-7	Cellulase A "Amano" 3 (Amano Enzyme Co, Japan)
E-8	Cellulase A "Amano" 4 (Amano Enzyme Co, Japan)

2.8 Production of the BC by using the saccharified solution of apple pomace

The apple pomace was mixed with an equal volume of distilled water by the blender (7010S Waring), and warmed in a boiling water bath for 2 hrs. The sample was mashed by the blender, and autoclaved for 30 min at 121°C. After addition of 6.65g of enzymes (Sumizyme AC: Shin-Nihon Kagaku Kogyo) to 2,660g of the sample, the mixture was incubated under static conditions at 40°C for 72 hrs. The saccharified sample was boiled on a gas cooker boiling for 2 min, and the supernatant solution of centrifugation for 15 min at 6,000rpm (himac CR20F, Hitachi) was filtered through a filter paper (Advantec Toyo, No. 5C). The BC production medium was prepared by the saccharified solution composed of 0.5% (w/v) polypepton, 0.5% (w/v) yeast extract, 0.5% (w/v) mannitol, and 0.1% (w/v) $MgSO_4 \cdot 7H_2O$, and autoclaved for 30 min at 121°C. After cooling, 0.5% (v/v) of ethanol was added. This medium was divided into 6 volumes (10ml, 20ml, 40ml, 80ml, 120ml, and 240ml), and added to a culture vessel (a surface area was 58.8cm^2). The strain (NBRC-13693) was cultured in 100ml of the preculture medium at 30°C for 3 days with shaking (90 rpm). After 3 days, 2.0%(v/v) of the preculture medium was added individually to the BC production medium, and cultured under static conditions at 30°C for 4 weeks. After 4 weeks, the products by fermentation were purified, and weighted as the BC.

2.9 Saccharification of the apple pomace and the okara by the crude enzyme

The apple pomace was mixed and mashed with an equal volume of distilled water by the mixer (Kenmix KM-600, Aicohsha). The apple pomace mixed with water was represented as AW. The okara was mixed and mashed with 4 volumes of distilled water by the mixer. The okara mixed with water was represented as OW. AW and OW were mixed under the conditions of Table V, and autoclaved for 20min at 121°C. The samples were stationary treated with 0.5% (w/w) Sumizyme AC (Table V) for total weight of residues at 40°C for 24 hrs, and 4 times shaking lightly (3 hrs, 6 hrs, 9 hrs, and 12 hrs). After the enzyme treatment, the samples were autoclaved for 20min at 121°C. The supernatant solution of centrifugation for 10min at 9,000rpm (himac CR20F, Hitachi) was filtered through a filter paper (Advantec Toyo, No. 5A). The solutions were controlled at pH 5.0 to 5.5, and autoclaved for 20min at 121°C (AO-1 to AO-6).

Table V. Mixture rate of AW and OW and the amount of Sumizyme AC added to sample

	AW(g) (weight of apple pomace)	OW(g) (weight of okara)	Sumizyme AC(g)
AO-1	400 (200)	0	1.0
AO-2	100 (50)	50 (10)	0.3
AO-3	90 (45)	75 (15)	0.3
AO-4	60 (30)	150 (30)	0.3
AO-5	40 (20)	200 (40)	0.3
AO-6	0	300 (60)	0.3

2.10 Production of the BC by using the saccharified solutions of the apple pomace and the okara

Selected bacteria (NBRC-13693, NBRC-16682) were cultured with 20ml of the preculture medium for 3 days at 30°C by shaking (120rpm). After 3 days, 100μl of the preculture medium was inoculated into the AO-1 to AO-6 (5ml each) in a tri-plate sterilized, and cultured under static conditions at 30°C for a week. After a week, the products by fermentation were purified, and weighted as the BC.

3. Results and Discussion

3.1 Screening of the BC producer

The cellulose synthesis was considered as the reaction of enzyme on the surface of cells. Enzyme reaction was influenced by pH, therefore, it was required for the enzyme reaction efficiency to optimize pH for the cellulose synthesis. We investigated the production of the BC by 7strains of the BC producer. Fig.1 shows the production of the BC with the each medium (pH3, 5, 7, 9, and 11). The production was different from the strains and the pH of the medium, particularly the production increased at pH from 5 to 9. All strains could not produce the cellulose at pH11. The optimized condition was that 19.3mg was produced by NBRC-16682 with the 5ml of No.350 medium controlled to pH7. In this study, NBRC-16682 and NBRC-16644 with high productivity and NBRC-13693 with good productivity at lower pH area were selected. The optimized pH range was from 5 to 7.

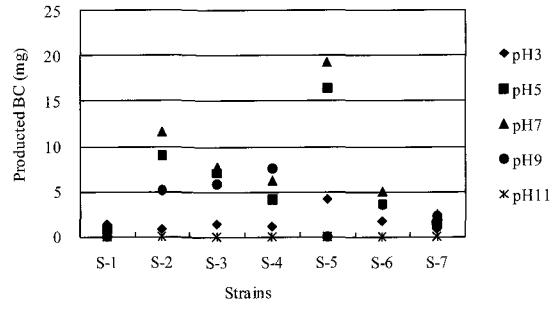

Fig.1. The production of the BC with the No.350 medium (pH3, 5, 7, 9, and 11) by 7 strains.

3.2 Production of the BC by using the commercial reagents at low cost

We investigated the cost efficiency of the medium with commercial reagents. In this study, the compounding rate of the Yeastextract (a high price reagent for this medium) and the Mannitol (concerned with a productivity increase of the BC) were changed, and NBRC-16682, NBRC-16644, and NBRC-13693 were used for producing. The results were shown in Fig.2. The most BC production was 19.6mg of the BC produced by NBRC-16682 with the 5ml of the medium M-1, but the most efficiency of producing with the medium cost was 15.7mg with the medium M-4 by NBRC-16682, in this case, the 18.0mg of the BC was expected to produce with about 1 yen of the medium cost.

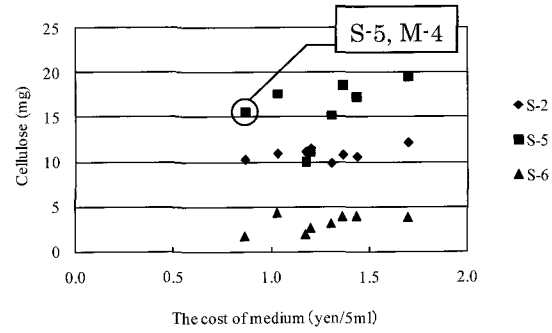

Fig.2. The relationships between the cost of improved medium and the BC production.

3.3 Screening of the commercial crude cellulase suitable for the apple pomace

For utilizing the apple pomace as the medium of the BC production, it was needed to treat with an enzyme. The apple pomace was individually treated with 8 kinds of commercial crude cellulases, and pulp (Fig.3) and reduced sugar (Fig.4) of saccharification samples were measured. The pulp decreased and the reduced sugar increased with time, because of the conversion of polysaccharides to mono-saccharaides in the apple pomace by the enzyme. Consequently, E-3 (Sumizyme AC) with high- efficiency was selected from these enzymes. After saccharification, the volume of the apple pomace treated with the E-3 decreased to 19.1 percent, and the apple pomace was changed from pasty to liquid state. Thus, the E-3 was assumed to be a practical enzyme.

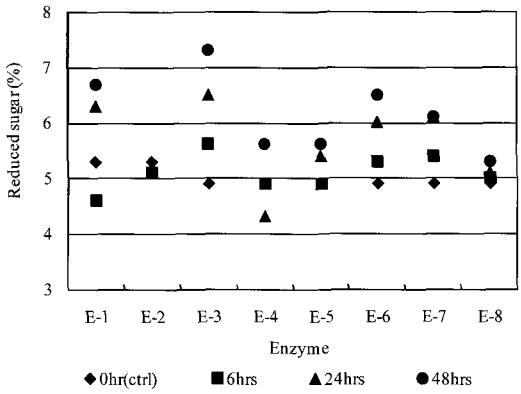

Fig.3. Pulp of saccharification samples by enzymes.

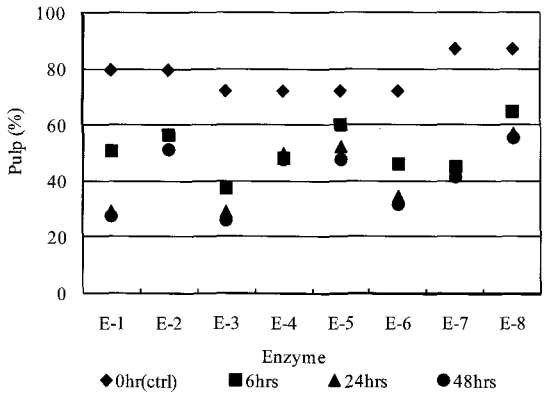

Fig.4. Reduced sugar of saccharification samples by enzymes.

3.4 Production of the BC by using the saccharified solution of the apple pomace

Fig.5 shows the BC produced from saccharified solution of the apple pomace. The relationship between the volume of medium, X, and the production of the BC, Y, was Y=2.47X. It was assumed that 3.3g of the dry BC produced from 1kg of the wet apple pomace, but it was needed to add the nitrogen source.

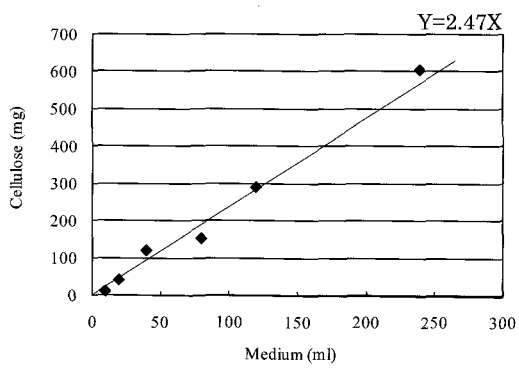

Fig.5. The BC produced from saccharified solution of the apple pomace.

3.5 Production of the BC by using the saccharified solutions of the apple pomace and the okara

We tried to produce the BC from the saccharified solutions of the apple pomace and the okara by using the NBRC-16682 and the NBRC-13693 (Fig.6). The most BC production was 5.1mg produced by the NBRC-16682 with the 5ml of a saccharified solution medium AO-4 (prepared from an equal amount of the apple pomace and the okara), the 5ml of the medium was prepared from 1.25g of the apple pomace and 1.25g of the okara. In the case of the NBRC -13693, the BC was produced by a certain amount from 100 percent of the okara when the ratio of the apple pomace to the okara was 4:1, and produced slightly with the apple pomace alone. Thus it was found that the BC would be produced from the apple pomace and the okara without the nitrogen source.

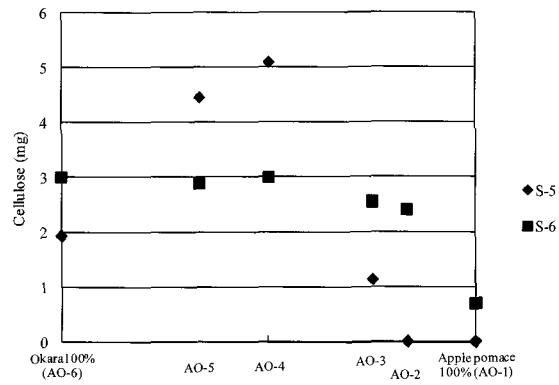

Fig.6. The BC produced from saccharified solutions of the apple pomace and the okara.

4. Conclusion

1) The strain of *Gluconacetobacter xylinus* NBRC-16682 was selected. 2) The 18.0mg of the BC was expected to produce with about 1 yen of the medium cost by using the commercial reagents. 3) Sumizyme AC was selected as the cellulase suitable for the apple pomace. After saccharification, the volume of the apple pomace treated with the enzyme decreased to 19.1 percent. 4) It was assumed that 3.3g of the dry BC produced from 1kg of the wet apple pomace, but it was needed to add the nitrogen source. 5) It was found that the BC would be produced from the apple pomace and the okara without the nitrogen source.

Furthermore, it is needed to investigate the functional compositions of the water-soluble saccharified solution or the production by the fermentation for the industrial utilization of biomass resources.

5. References

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