

Production of high concentration bioethanol from cassava stem

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キャッサバの茎を原料とした高濃度バイオエタノール発酵

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本研究はキャッサバの茎からの高濃度エタノール発酵を目的としてキャッサバの茎の酸加水分解法を検討した。キャッサバの茎の酸加水分解液を再利用することで液中のグルコース濃度を従来の約 2 倍に濃縮することに成功した。濃縮した酸加水分解液に *Saccharomyces cerevisiae* IAM4178 を接種してエタノール発酵することで最大 40 g/L 程度のエタノールを得ることができ、ほとんどが有効利用されていないキャッサバの茎から高濃度のエタノールを生産する方法を示した。

1 Introduction

Recently, there is a growing concern about the depletion of petroleum based-fuels reserves. Carbon dioxide which is produced in combustion of petroleum based-fuels tends to increase the temperature of the planet. It leads to many negative effects to environment. Furthermore, the intensive demand of petroleum based-fuels increases the crude oil price. These factors have heightened the need to search for alternative energy resources.

Bioethanol is an attractive solution to address above problems as it is a carbon neutral energy source. Bioethanol is produced by the fermentation of sugar by microorganism. Today, almost all bioethanol is produced from corn and sugarcane. However, the production of bioethanol from food crops causes interference between food demands.

Agricultural residues are collecting attentions as alternative substrate of bioethanol fermentation, owing to its low cost and abundant amount in the world. It is estimated that 491 billion liters of bioethanol can be produced from sum of agricultural residues and food wastes in the world which is 16 times higher than the current total production. During bioethanol fermentation, the ethanol concentration should reach at least approximately 40-50 g/L, because several studies have concluded that this target concentration can make the process economically feasible, especially by saving energy for the distillation [1]. So, it is important to produce high concentration ethanol in fermentation of agricultural residue.

Agricultural residue is composed of lignocellulose. Starch in the crops can be fermented directly, but lignocellulose has to be pretreated before fermentation. The acid hydrolysis by hydrochloric acid or sulfuric acid is the most common method to break the lignocellulosic structure. However, acid hydrolysis pretreatment produces fermentation inhibitors such as furfural and phenolic compounds which can lead low

ethanol productivity. So, it is required to remove these inhibitors by detoxification.

The research purpose is to produce around 40 g/L of bioethanol from cassava stem. In this study, cassava stem was used as bioethanol substrate. The amount of cassava stem produced in a year is 35 million tons, but about 80% of cassava stem is abandoned or burned in the wild [2]. For the purpose, fermentation of synthetic broth, fermentation of cassava stem broth and fermentation of condensed cassava stem broth were conducted.

2 Materials and methods

2-1 Fermentation of synthetic broth

The fermentation ability of IAM4178 on glucose and xylose was examined using synthetic broth. IAM4178 was precultivated in 50 mL of YM broth at 30°C for 2 days. Then, 50 mL of the preculture was centrifuged at 6,000 rpm for 5 min. The supernatant was discarded and the wash solution which was composed of 5 g/L of peptone and 3 g/L of yeast extract was added to wash the pellet. The mixture of wash solution and the pellet was centrifuged again in the same condition. After centrifuging, the supernatant was discarded and the pellet was inoculated to 50 mL of synthetic broth which was composed of 40 g/L of glucose, 20 g/L of xylose, 5 g/L of peptone and 3 g/L of yeast extract. Fermentation was conducted at 30°C, 120 rpm.

The chemical formulas of ethanol production from glucose is shown below.



According to the formula, 0.51 g of ethanol is produced from 1 g of glucose. The ethanol fermentation efficiency was calculated for all experiments based on an assumption that the ethanol concentration calculated from above formula is 100%.

2-2 Fermentation of cassava stem broth

2-2-1 Fermentation of CS 200

Cassava stem hydrolysate was fermented by IAM4178 to investigate the fermentation ability of IAM4178 in the cassava stem hydrolysate and compare the fermentation characteristic of the CS broth and synthetic broth. Cassava stem (CS) was obtained from Trang Bom region, Dong Nai prefecture, Vietnam. Firstly, CS was ground to make the particle size smaller than 0.5 mm. The ground CS was stored in the desiccator. Ground CS and 72wt% sulfuric acid were mixed at the weight ratio 1:1.25. The mixture was put in the incubator at 30°C for 1 hour. During incubation, the inside content was stirred every 15 min to make it uniformity. After 1 hour, distilled water was added to decrease the acid concentration to 16.4% and the mixture of CS and acid was heated in autoclave at 111°C for 1 hour. The hydrolysate was kept stand at room temperature for 1 hour, and calcium carbonate was added to adjust pH around 5.5. The hydrolysate after neutralization was centrifuged at 6000 rpm for 5 min and supernatant was filtered with a paper which has pore size of 0.45 µm. Then, 5 g/L of peptone and 3 g/L of yeast extract were added to the filtered hydrolysate. The hydrolysate after these procedures was used as CS 200. The theoretical sugar concentration is shown in Table 1. The value was calculated using CS composition measured by NREL method [3]. IAM4178 was inoculated to CS 200 in the same manner with chapter 2-1.

2-2-2 Fermentation of CS 400

In order to produce high concentration of ethanol, condensation of sugar in CS broth by two times hydrolysis and fermentation characteristic of the condensed CS broth were investigated. Ground CS and 72wt% sulfuric acid were mixed at the weight ratio 1:1.25. The mixture was put in the incubator at 30°C for 1 hour. During incubation, the inside content was stirred every 15 min to make it uniformity. After 1 hour, distilled water was added to decrease the acid concentration to 16.4% and the mixture of CS and acid was heated in autoclave at 111°C for 1 hour. The hydrolysate was kept stand at room temperature for 1 hour, and filtered with a paper which has pore size of 0.45 µm.

Ground CS and 72wt% sulfuric acid were mixed and incubated for 1 hour in the same manner, and the filtered hydrolysate mentioned above was added to the

mixture. The volume of the added hydrolysate was same with the volume of distilled water which was added before the first heating. Acid concentration after adding the hydrolysate became 28%. The mixture of CS and hydrolysate was heated in autoclave at 111°C for 1 hour. The hydrolysate was kept stand at room temperature for 1 hour, and calcium carbonate was added to adjust pH around 5.5. The hydrolysate after neutralization was centrifuged at 6000 rpm for 5 min and supernatant was filtered with a paper which has pore size of 0.45 µm. Then, peptone and yeast extract, 5 g/L and 3 g/L each were added to the filtered hydrolysate. The hydrolysate after these procedures was used as CS 400. The theoretical sugar concentration is shown in Table 1. The value was calculated using CS composition measured by NREL method. IAM4178 was inoculated to CS 400 in the same manner with chapter 2-1. After the fermentation started, IAM4178 was inoculated again in 72h and 144h as the cell density decreased.

2-3 Analytical methods

Sugar, ethanol, and furfural were measured with a HPLC system with a Shodex Sugar SH1011 column and a refractive index detector. Mobile phase was 5 mM H₂SO₄, flow rate was 0.6 mL/min and the temperature in the column oven was 60°C. Cell density was measured by dilution plating method with YM agar plate. Incubation of the plates was conducted at 30°C, for 2 days. Phenolic compound was measured by Folin-Ciocalteu method referred from [4].

3 Results and discussion

3-1 Fermentation of synthetic broth

The courses of sugars, ethanol and cell density are shown in Fig. 1 and 2. IAM4178 consumed 40 g/L of glucose within 1 day and 20 g/L of xylose was decreased to 10 g/L in 96h. As a result, ethanol and xylitol were produced. The ethanol fermentation efficiency was 84.4%. The cell density was stable around 10⁸ CFU/mL. These results indicated that IAM4178 can produce ethanol from glucose at high efficiency and can produce xylitol from xylose as a byproduct. The present study also demonstrated that the xylitol production from 5 g/L of xylose by IAM4178 in the existence of 10 g/L of glucose was higher than 5 g/L of xylose alone (data not shown). So, the xylitol production was enhanced possibly by the existence of glucose.

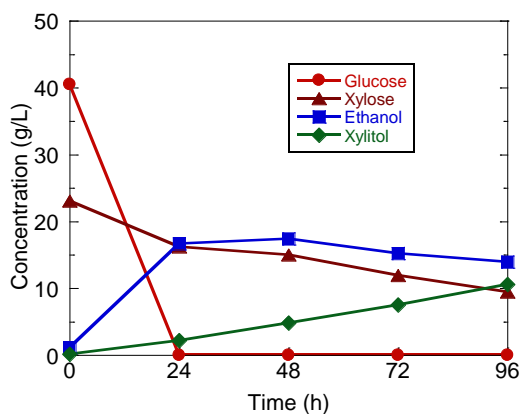


Fig. 1 The courses of sugars and ethanol

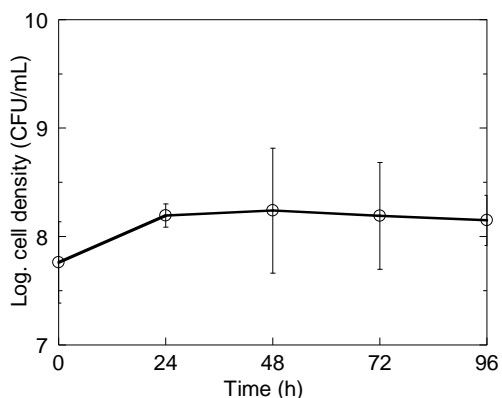


Fig. 2 The course of cell density

3-2 Fermentation of CS 200

The courses of sugars, ethanol, furfural and cell density are shown in Fig. 3 and 4. The initial sugar concentration of CS200 was 63 g/L, which was about 90% of the theoretical value (see Table 1). All glucose was consumed within 12h while about a half (i.e. 10 g/L) of xylose was consumed in 36h. Ethanol was produced with the decrease of glucose but xylitol was not produced although xylose decreased. Schirmer-Michel, Ângela Cristina, et al. [5] reported that the *Candida guilliermondii* produces xylitol from xylose in the synthetic broth, but does not produce xylitol in the rice hull hydrolysate. The author speculated the existence of inhibitor of xylose reductase activity in the rice hull hydrolysate. In this study, CS 200 might also contained xylose reductase inhibitor. The ethanol fermentation efficiency from CS200 was 73.4%, and the cell density was almost stable at 10^8 CFU/mL (see Fig. 4). These results indicate that IAM4178 can produce ethanol in the CS hydrolysate without any detoxification. However, the ethanol fermentation efficiency was 10% lower than that of the synthetic broth. It is possibly because of the delay in glucose consumption by furfural [6], and/or the consumption of ethanol by the yeasts.

Run	Glucose (g/L)	Xylose (g/L)
CS 200	73.94	31.08
CS 400	136.79	57.50

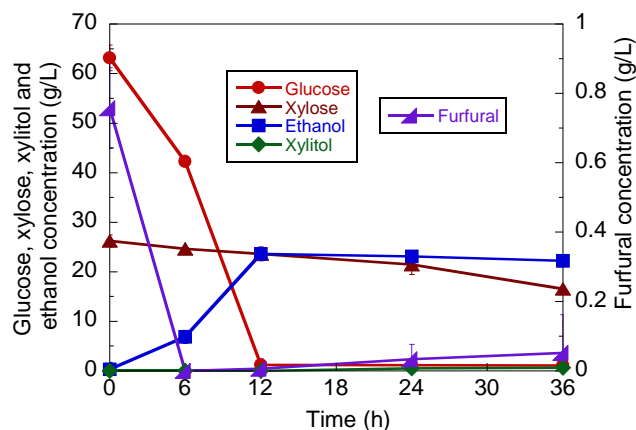
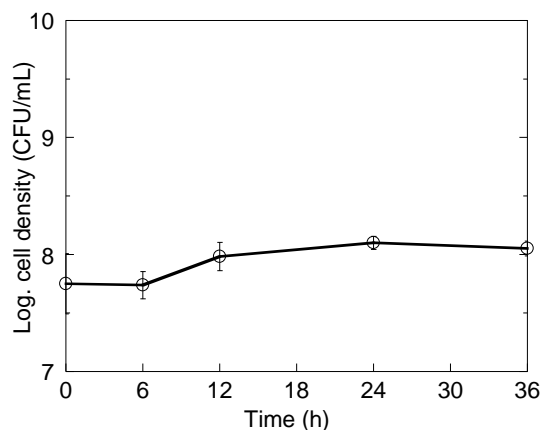


Fig. 3 The courses of sugars, ethanol, and furfural



3-3 Fermentation of CS 400

The courses of sugars, ethanol, furfural, cell density, and phenolic compound are shown in Fig. 5, 6 and 7. The sugar concentration in CS 400 was about 70% of theoretical value in Table 1. This is possibly because some sugar was degraded excessively by two times hydrolysis. From 0h to 48h, about 30 g/L of glucose was consumed. However, the glucose consumption was ceased from 48h to 72h and cell density decreased. The glucose consumption begun after IAM4178 was inoculated in 72h. But the glucose consumption ceased and cell density decreased again in 144h. After IAM4178 was inoculated in 144h, remaining glucose was consumed all.

The final ethanol concentration and ethanol fermentation efficiency of CS 400 were 37.5 g/L and 77%, respectively. It showed that high concentration of

ethanol is produced by condensation in the new hydrolysis method. The ethanol fermentation efficiency was similar value with CS 200. These results indicated that the inhibition did not affect the fermentation efficiency.

The cell density decreased from 10^8 CFU/mL in 0h to 10^7 CFU/mL in 72h. Then, the cell density recovered to 10^8 CFU/mL because of second inoculation in 72h, but it decreased again to 10^7 CFU/mL in 144h. The cell density recovered to 10^8 CFU/mL after third inoculation in 144h, and dropped rapidly after glucose depletion in 168h. The total phenol was around 2 g/L and it was almost stable. In the previous research, 2 g/L of phenolic compound could inhibit the ethanol fermentation [7]. Thus it can be said that 2 g/L of phenolic compound in the CS 400 was enough high to affect the cell increase of IAM4178. High concentration of ethanol could be produced from CS 400, but IAM4178 decreased during the fermentation. In summary, the present study indicated that the condensation of CS broth increases both fermentable sugars and inhibitors, but high concentration ethanol is produced by the addition of yeast during the fermentation. The removal of the inhibitory substances will be the next challenge.

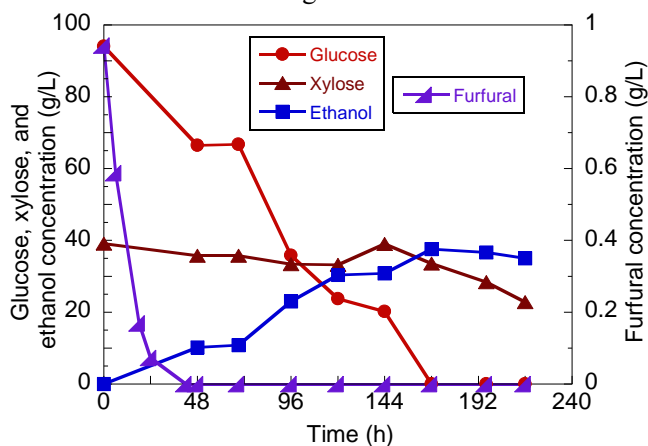


Fig. 5 The courses of sugars, ethanol, and furfural

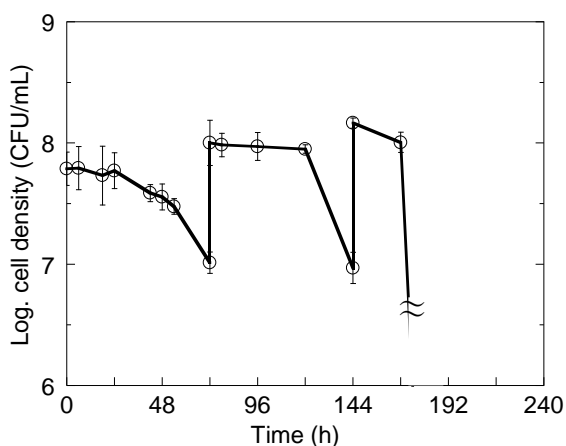


Fig. 6 The course of cell density

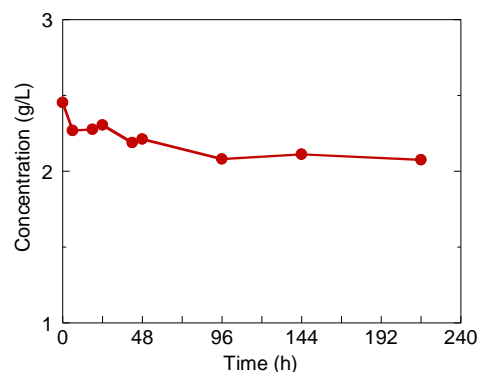


Fig. 7 The course of phenolic compound

4 Conclusions

The cassava stem broth (CS 400) was successfully condensed by two times hydrolysis. Fermentation of the condensed broth with IAM4178 achieved ethanol concentration of 37.5 g/L, which is the target concentration of bioethanol from agricultural residue.

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