

수송용 바이오 부탄을 생산을 위한 미강발효의 최적화

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Fermentation of rice bran and defatted rice bran for butanol production using *Clostridium beijerinckii* NCIMB 8052

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Abstract : We examined butanol fermentation by *Clostridium beijerinckii* NCIMB 8052 using various hydrolyzates obtained from rice bran which is one of the most abundant agricultural by-products in Korea and Japan. In order to increase the amount of fermentable sugars in the hydrolyzates of rice bran, various hydrolysis procedures were applied. Total eight different hydrolyzates were prepared using rice bran (RB) and defatted rice bran (DRB) with enzyme or acid treatment and both. Each hydrolyzate was evaluated in terms of total sugar concentration and butanol production after fermentation by *C. beijerinckii* NCIMB 8052. Acid treatment yielded more sugar than enzyme treatment and combined treatment with enzyme and acid yielded even more sugars as compared to single treatment with enzyme or acid. As a result, the highest sugar concentration (33 g/L) was observed from the hydrolyzate from DRB (100 g/L) with combined treatment using enzyme and acid. Prior to perform fermentation of the hydrolyzates, we examined the effect of P2 solution containing yeast extract, buffer, minerals, and vitamins on production of butanol during the fermentation. Fermentation of the hydrolyzates with or without addition of P2 was performed using *C. beijerinckii* NCIMB 8052 in a 1 L anaerobic bioreactor. Although the hydrolyzates RB were able to support growth and butanol production, addition of P2 solution into the hydrolyzates significantly improved cell growth and butanol production. Highest butanol production (12.24 g/L) was observed from the hydrolyzate of DRB with acid and enzyme treatment after supplementation of P2 solution.

1. Introduction

As a result of increasing oil prices, various bioconversion programs have been initiated to produce biochemicals and bioenergy in many countries. Accordingly, interest in butanol fermentation using clostridia has been renewed due to high demands of alternative fuels. Butanol is one of the metabolic products of solventogenic clostridia and recognized as one of candidates for an alternative transportation fuel as well as a fuel extender. Butanol has many advantages over ethanol as a biofuel. For instance, butanol is less miscible in water and has higher energy content than ethanol. Therefore, butanol can be blended with gasoline at higher ratio than ethanol, and the combustion

properties of butanol are more similar to gasoline than ethanol [3, 6]. Currently the value-added fermentation process is paid more attention due to several economic and environmental reasons. The economics of butanol (and liquid fuels) has been studied extensively and the cost of substrate/raw material is one of the most influential factors impacting the economics of fermentation-derived liquid fuels [10]. In order to reduce production cost, it has been attempted to produce butanol from renewable agricultural resources including cane molasses, agricultural biomass, corn, and dairy industry

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waste [1, 7, 10]. However, the production of biofuels from edible substrates, such as starch or sucrose would not be appropriate because of food shortage. Therefore, it is necessary to find substrates which is consistently supplied and contains high fermentable sugars and can be hydrolyzed with simple pretreatment.

Rice bran, the residue of brown rice during the production of white rice is abundantly available in Korea and Japan and this agricultural by-product contains a number of carbohydrate and other nutrients such as proteins, lipid, fiber, Ca^{2+} , Mg^{2+} , phosphate, silica, Zn^{2+} , thiamin, and niasin. The amount of rice bran produced annually in Korea is estimated to be about 4.8×10^5 ton per year (National Agricultural Product Quality Service, Korea, 2007) but its industrial applications are only limited to animal feed additive, or production of rice bran oil. The residue after extraction of rice bran oil from rice bran (RB) is called defatted rice bran (DRB). Thus, there are two different kinds of rice bran available from rice processing industry; rice bran (RB) and defatted rice bran (DRB). DRB still contains many carbohydrates and cellulosic polysaccharides [11].

The solventogenic clostridia are capable of utilizing a wide spectrum of carbon source for ABE fermentation [8, 9]. *Clostridium beijerinckii*, a gram positive, anaerobic, spore-forming bacterium is recognized as a member of major solvent-producing clostridia which have been tested for many different value-added fermentations using renewable substrates, such as wheat straw and agricultural wastes [2, 10]. However, no fermentation study using rice bran has been reported for butanol production. As such, we investigated butanol fermentation by *C. beijerinckii* NCIMB 8052 using potential agricultural substrates, RB and DRB.

2. Materials and Methods

2.1 Microorganisms and culture

A stock culture of *C. beijerinckii* NCIMB 8052 was maintained as a spore suspension in distilled water at 4°C. Spores were heat shocked at 80°C for 10 min, inoculated in reinforced clostridial medium (RCM) (BD, USA), and incubated for 16–18 h at 35°C. The RCM cultures were transferred to 50 mL of glucose (6%, w/v) containing P2 media [4], incubated for 16–18 h at 35°C, and transferred to 1L of RB or DRB (10%, w/v) containing fermentation media.

2.2 Rice bran and defatted rice bran pretreatment and media preparation

Rice bran (RB) was obtained from a local grocery and defatted rice bran (DRB) was obtained from SERIM Inc. (Korea). In order to hydrolyze RB and DRB, Enzyme

mixture of α -amylase (Sigma A8220, 884 unit/g), β -amylase (Sigma A7130, 19.9 unit/mg), and amyloglucosidase (Sigma A7095, 300 unit/mL) and 1% (v/v) HCl were used to hydrolyze polysaccharides in the RB and DRB. Conditions for enzyme and acid hydrolysis were incubations at 30°C for 4 h and at 80°C for 3 h, respectively. For the combined treatment of enzyme and acid, the acid treatment was performed prior to the enzyme treatment under the same condition.

2.3 Fermentation

Batch fermentation (1 L scale) was performed on a custom made-2.5 L bioreactor using 10 % (w/v) RB or DRB as a fermentation substrate as well as 6% glucose as a control. All 1 L-fermentation experiments were carried out at 33°C under oxygen-free N_2 atmosphere in the absence of agitation or pH control. Culture samples were withdrawn to be analyzed during fermentation.

2.4 Analysis

Cell growth in a RB or DRB containing medium was indirectly evaluated by pH measurement instead of measuring optical density of the culture due to the opaqueness of RB (DRB) medium. The amount of sugars in media and culture were determined using a HPLC. The concentrations of ABE and acids were determined by gas chromatography (GC) equipped with HP-FFAP column and flame ionization detector (FID).

3. Results

3.1 Glucose fermentation

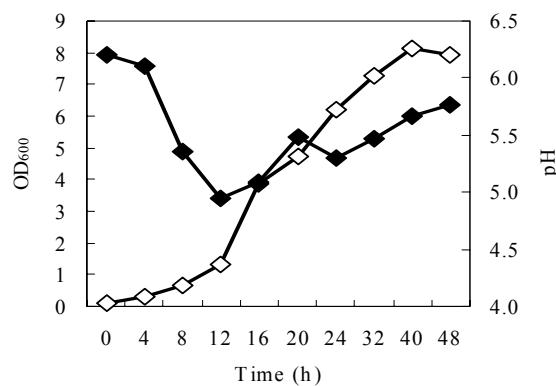


Fig 1. Standard growth of *C. beijerinckii* NCIMB 8052 in 6% glucose

3.2 Cell Growth in hydrolysates of RB/DRB

Abbreviations: RB, rice bran; DRB, defatted rice bran;

ERB, enzyme treated rice bran; EDRB, enzyme treated defatted rice bran; ARB, acid treated rice bran; ADRB, acid treated defatted rice bran; AERB, acid and enzyme treated rice bran; AEDRB, acid and enzyme treated defatted rice bran

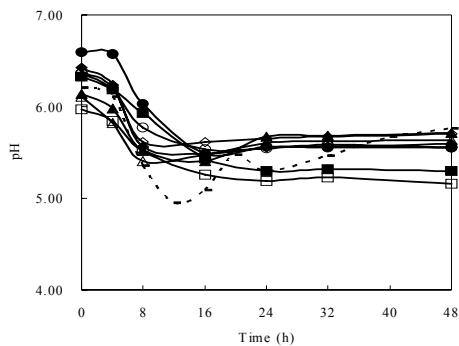


Fig 2. pH in 10% (w/v) rice bran and defatted rice bran media (● Glucose 6%, ■ RB, □ DRB, ▲ ERB, △ EDRB, ● ARB, ○ ADRB, ◆ AERB, ◇ AEDRB)

3.3. Fermentation of hydrolysates

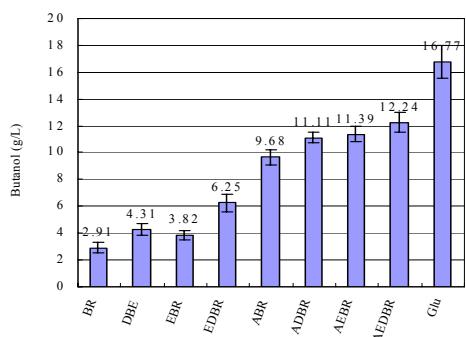


Fig 3. Butanol production by *C. beijerinckii* NCIMB 8052 using various rice bran media

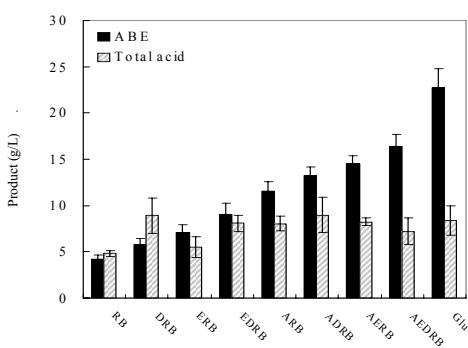


Fig 4. Fermentation products from various RB hydrolysates using *C. beijerinckii* NCIMB 8052

4. Discussion and Conclusion

In order to evaluate the fermentation characteristics of hydrolysates from rice bran by *C. beijerinckii* NCIMB 8052, a control fermentation experiment was run using glucose as a substrate. Fermentation products and cell growth and pH values during batch fermentation by *C. beijerinckii* NCIMB 8052 grown in semi-defined P2 containing medium containing 6% glucose is shown in Fig. 1. As glucose is known as one of the favored substrates by solventogenic clostridia [8], the fermentation was rapid showing a clear phase transition from acidogenesis to solventogenesis (pH 4.8 at 12 h).

The concentration of reducing sugar in ARB/ADRB and AERB/AEDRB was estimated about 2% and 3%, respectively. The differences in sugar concentration resulted in different profiles of products in each medium shown in Table 3 and Fig. 3 and Fig. 4. The hydrolysates obtained from both acid and enzyme treatment produced the highest butanol production (11.4 g/L (RB) and 12.2 g/L (DRB)). The hydrolysates from acid treatment resulted in the highest butyrate production (4.2 (RB) / 5.5 g/L (DRB)) as well as the second highest butanol production (9.7 (RB) / 11.1g/L (DRB)).

Regardless of types of hydrolysis treatments prior to fermentation, DRB produced 5-10% higher fermentable carbohydrates and butanol production when those compared to RB. This may be caused by relatively higher contents of polysaccharide in DRB after extraction of lipids. Also we can speculate that low fat content in DRB may introduce a favorable condition for either hydrolysis treatment or cell growth which led to the enhanced butanol production.

So far a variety of renewable resources has been employed for the production of butanol but fermentation of the renewable resources requires complicated pretreatments including hydrolysis and separation or supplementation of additional glucose, maltose or other sugar for better production of butanol [3, 4, 5, 10]. Low titer was also reported as a problem in whey fermentation (5 g/L of butanol produced) where pretreatment was not required. This studies suggested that both RB and DRB can be potentialrenewable substrates for production of butanol (up to 12 g/L butanol) when partial hydrolysis treatment was carried prior to fermentation since RB and DRB is a relatively abundant biomass in East Asia.

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