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Fermentation of Rice Bran and Defatted Rice Bran for Butanol Production Using *Clostridium beijerinckii* NCIMB 8052

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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. Eight different hydrolyzates were prepared using rice bran (RB) and defatted rice bran (DRB) with enzyme or acid treatment or 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 with 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 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 RB hydrolyzates were able to support growth and butanol production, addition of P2 solution into the hydrolyzates significantly improved cell growth and butanol production. The highest butanol production (12.24 g/l) was observed from the hydrolyzate of DRB with acid and enzyme treatment after supplementation of P2 solution.

Keywords: Butanol, fermentation, rice bran, *Clostridium beijerinckii*

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As a result of increasing oil prices, various bioconversion programs have been initiated to produce biochemical and bioenergy in many countries. Accordingly, interest in butanol fermentation using clostridia has been renewed owing to high demands for alternative fuels. Butanol is one of the metabolic products of solventogenic clostridia and recognized as one of the candidates for an alternative transportation fuel as well as a fuel extender. Butanol has many advantages over ethanol as a biofuel. For example, butanol is less miscible in water and has a higher energy content than ethanol. Therefore, butanol can be blended with gasoline at a higher ratio than ethanol, and the combustion properties of butanol are more similar to gasoline than ethanol [7, 12]. The fermentation of carbohydrate to acetone, ethanol, and butanol (ABE) by solventogenic clostridia is well known [13, 15]. The fermentation undergoes two phases; acidogenesis, the production of acids (acetic and butyric acids), followed by solventogenesis, the production of solvents (ABE in the ratio of 3:6:1). It has been one of the most important biotechnological processes for the production of solvents during the first half of the 20th century [5]. Advances in the chemical synthesis of these solvents and high substrate costs led to the decline of the ABE fermentation, but the scientific interest in this process had been revived whenever there was fluctuation of oil price. Although most of the fermentation plants for butanol production no longer remained by the 1980s, enormous progress has been made with respect to the genetics and physiology of solvent production by clostridia owing to advances in biological sciences in 1980s. This allows us a better understanding of the mechanisms to produce acid and solvent. Like other bioprocesses, production of butanol by fermentation has revealed several limitations for its commercialization, such as low product yields, high substrate cost, and high recovery cost [23, 25].

Currently, value-added fermentation processes are receiving more attention owing 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 [9, 23]. In order to reduce production cost, it has been attempted to produce butanol from renewable agricultural resources including cane molasses, corn, and dairy industry waste [7, 25, 29]. 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 that are consistently supplied and contain high fermentable sugars and can be hydrolyzed with simple pretreatment.

Rice bran (RB), 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 niacin (National Agricultural Product Quality Service, Korea, 2007). The amount of rice bran produced 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 is called defatted rice bran (DRB). Thus, there are two different kinds of rice bran available from the rice processing industry; rice bran and defatted rice bran. DRB still contains many carbohydrates and cellulosic polysaccharides [27].

The solventogenic clostridia are capable of utilizing a wide spectrum of carbon sources for ABE fermentation [14, 15]. *Clostridium beijerinckii*, a Gram-positive, anaerobic, spore-forming bacterium, is recognized as a member of the major solvent-producing clostridia, which have been tested for the production of many value-added products using renewable substrates, such as wheat straw and agricultural wastes [24, 26]. 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.

MATERIALS AND METHODS

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 clostridia medium (RCM) (BD, U.S.A.), 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 [2, 8], incubated for 16–18 h at 35°C. The glucose P2 culture (50 ml) was transferred to 1 l of RB or DRB (10%, w/v)-containing fermentation media. P2 solution contained 0.5 g/l KH_2PO_4, 0.5 g/l K_2HPO_4, 2.2 g/l ammonium acetate, 0.001 g/l *para*-aminobenzoic acid, 0.001 g/l thiamin, 0.00001 g/l biotin, 0.2 g/l $MgSO_4\cdot7H_2O$, 0.01 g/l $MnSO_4\cdot H_2O$, 0.01 g/l $FeSO_4\cdot7H_2O$, 0.01 g/l NaCl, and 1 g/l yeast extract. A 5% (v/v) inoculum and an anaerobic condition were used in all fermentation experiments.

Rice Bran and Defatted Rice Bran Pretreatment and Media Preparation

RB was obtained from a local grocery and DRB was obtained from SERIM Inc. (Korea). In order to hydrolyze RB and DRB, 100 g (dry weight) of RB or DRB powder was suspended in 1 l water. Optimum conditions for hydrolysis of RB and DRB were obtained from test experiments of hydrolysis using various conditions. Enzyme mixtures (300 unit each) 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. For the enzyme treatment, α-amylase (500 μl) was added into the RB or DRB solution in pH 6.0 and the mixture was incubated at 30°C for 4 h. After incubation, the treatments of β-amylase (15 mg) and amyloglucosidase (1 ml) were followed at 37°C for 4 h. Then, the enzyme-treated mixture was sterilized at 121°C for 15 min. For acid treatment, 1% (v/v) HCl was used for acid hydrolysis at 80°C for 3 h. The acid-treated mixture was then cooled to room temperature followed by adjusting to pH 6.0 with 10 N NaOH and sterilized at 121°C for 15 min. For the combined treatment of enzyme and acid, the acid treatment was performed prior to the enzyme treatment under the same condition.

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. Total of eight fermentation media containing the hydrolyzates of RB and DRB were prepared through different hydrolysis methods [RB and DRB, no treatment (except for possible heat treatment during sterilization); ERB and EDRB, with enzyme treatment; ARB and ADRB, with acid treatment; AERB and AEDRB with both acid and enzyme treatment]. Glucose was used as a carbon source for a standard butanol fermentation experiment. All 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.

Analysis

Solvent, acid production, and the corresponding cell growth and pH values during batch fermentation by *C. beijerinckii* NCIMB 8052 were observed and analyzed. Cell growth in a RB or DRB containing medium was indirectly evaluated by pH measurement instead of measuring optical density of the culture, owing to the opaqueness of RB (DRB) medium. The amount of sugars in the media and culture were determined using an HPLC equipped with a refractive index detector (RID) and a 4.6×250 mm carbohydrate column. The mobile phase was 75% acetonitrile and was run at a flow rate of 0.8 ml/min. Glucose concentration was determined using HPLC equipped with a refractive index detector (RID) and a 300×7.80 mm Rezex ROA–organic acid column. The total run time was 30 min for each sample. The concentrations of ABE and acids were determined by gas chromatography (GC) equipped with an HP–FFAP column and flame ionization detector (FID). The initial and final temperatures were 50°C and 160°C by temperature increasing 20°C/min. Both injector and detector temperatures were 260°C. Samples (1 μl) were injected into the GC and the run time was 8.5 min.

Results and Discussion

Hydrolysis of RD and DRD with Enzyme and Acid Treatment

Prior to fermentation experiments, both RB and DRB were treated with acid and enzyme to hydrolyze polysaccharides contained in RB and DRB. Eight hydrolyzates were prepared (RB, RB without treatment; DRB, DRB without treatment; ERB, RB with enzyme hydrolysis; EDRB, DRB with enzyme hydrolysis; ARB, RB with acid hydrolysis; ADRB, DRB with acid hydrolysis; AERB, RB with both enzyme and acid hydrolysis; AEDRB, DRB with both enzyme and acid hydrolysis). The sugar composition in each hydrolyzate is listed in Table 1. After the hydrolysis of 100 g/l of RB and DRB under the above-mentioned conditions, total amounts of fermentable sugars in the hydrolyzates ranged from 20 to 30 g/l depending on the treatment conditions. Interestingly, small amounts of sugars were detected in control hydrolyzates (RB and DRB) without any treatments. These small amounts of sugars might have been released from rice bran through thermal degradation of polysaccharides during sterilization. This result means that there was a hidden hydrolytic effect by heat treatment, although we did not intend it. Total sugar concentration was consistently higher in the hydrolyzates from DRB than those from RB. The most abundant sugar in the hydrolyzates was glucose. As the second most abundant sugar, significant amounts of fructose were observed only in the hydrolyzates treated with acid. It is likely that fructose was produced from the hydrolysis of sucrose since the amounts of sucrose (1.39 g/l and 1.98 g/l) in the acid-treated hydrolyzates were significantly lower as compared with the amounts of sucrose (5.85 g/l and 3.03 g/l) in the enzyme-treated hydrolyzates (Table 1). This is because we did not add invertase in the enzyme mixture. According to our observation, acid treatment yielded more sugars than enzyme treatment. This result makes a sense because acid treatment can hydrolyze any glycosidic bonds present in polysaccharides, but enzyme treatment only hydrolyzes specific glycosidic bonds that can be catalyzed by the added enzymes. Combined treatment with enzyme and acid yielded more sugars as compared with single treatment with enzyme or acid. As a result, the highest sugar concentration (glucose: 33 g/l; total carbohydrates: 42.9 g/l) was observed from the hydrolysate from DRB with both acid and enzyme treatment (100 g/l) (Table 1).

Glucose Fermentation by *C. beijerinckii* NCIMB 8052

In order to evaluate the fermentation characteristics of hydrolyzates from rice bran by *C. beijerinckii* NCIMB 8052, a control fermentation experiment was run using glucose as a substrate. Fermentation, products, cell growth and pH values during batch fermentation by *C. beijerinckii* NCIMB 8052 grown in semi-defined P2-containing medium containing 6% glucose are shown in Fig. 1. As glucose is known as one of the favored substrates by solventogenic clostridia [17], the fermentation was rapid, showing a clear phase transition from acidogenesis to solventogenesis (pH 4.8 at 12 h). Product profiles also demonstrated a typical biphasic growth of *C. beijerinckii* NCIMB 8052. Acids (acetate and butyrate) were mainly produced during the early exponential phase, acidogenesis (up to 12 h), and solvents (acetone and butanol) were produced the during the late exponential phase, solventogenesis (from 12 h). The residual concentration of glucose was 11 g/l, indicating that 49 g/l glucose (83%) was utilized during fermentation.

Effect of P2 Supplementation on Fermentation Hydrolyzates

First, we tested whether or not the hydrolyzates can support growth and ABE fermentation by *C. beijerinckii* NCIMB 8052 without any nutritional supplementation, since rice bran was known as a rich source of varied carbohydrates, minerals, and vitamins. The P2 solution containing buffer, minerals, vitamins, and yeast extract was originally designed as part of a semisynthetic medium containing a sugar for saccharolytic clostridia [2]. Previous studies reported that supplementation of P2 enhanced butanol production using

Table 1. Carbohydrate composition (g/l) of rice bran and defatted rice bran media with different treatments (before fermentation: 0 h).

(0 h)	RB	DRB	ERB	EDRB	ARB	ADRB	AERB	AEDRB
Glycerol	1.11±0.03	0.64±0.02	1.31±0.15	0.82±0.12	1.35±0.18	1.20±0.10	1.24±0.20	1.14±0.15
Ribose	0.77±0.02	0.98±0.09	0.56±0.08	0.99±0.12	0.58±0.05	1.01±0.07	0.93±0.11	1.12±0.14
Xylose	-	0.20±0.05	0.26±0.04	0.27±0.09	0.29±0.08	0.31±0.09	0.37±0.01	0.48±0.09
Arabinose	0.80±0.02	0.83±0.03	0.88±0.02	0.89±0.15	1.14±0.30	1.33±0.26	1.44±0.34	1.19±0.25
Fructose	0.22±0.05	0.16±0.09	2.50±0.12	0.69±0.02	5.22±0.60	6.05±0.50	6.41±0.80	6.78±0.50
Glucose	2.21±0.50	5.22±0.80	6.20±0.80	7.70±1.00	12.66±1.20	14.84±1.10	21.74±1.00	27.26±1.50
Sucrose	6.66±1.20	4.68±0.80	5.85±1.00	3.03±0.90	1.39±0.50	1.98±0.80	1.00±0.30	0.21±0.10
Innositol	1.01±0.05	1.34±0.09	1.32±0.08	2.48±0.30	1.79±0.50	1.52±0.30	1.03±0.40	1.75±0.20
Stachyose	1.32±0.30	0.94±0.20	1.32±0.40	4.30±1.20	1.64±0.80	3.07±0.50	2.04±0.30	1.25±0.40

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.

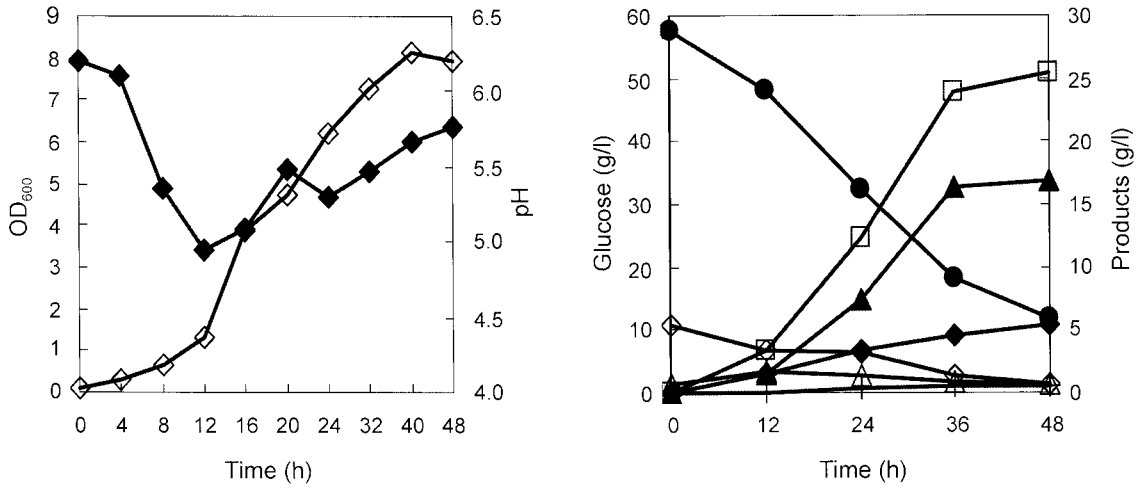

Fig. 1. Fermentation of 6% (w/v) glucose [left, growth OD_{600} (◇) and pH (◆); right, fermentation production (◆, acetone; +, ethanol; ▲, butanol; ◇, acetate; △, butyrate; □, total ABE) and glucose utilization (○)].

a variety of substrates such as maltodextrin, soy molasses, and starch packing peanuts as well as carbohydrates [6, 8, 25]. As shown in Table 3, addition of P2 solution into the hydrolyzates consistently improved ABE fermentation by *C. beijerinckii* NCIMB 8052, which is consistent with the previous findings above. About 2-fold increase in butanol production was observed after addition of P2 solution. This result suggests that supplementation of P2 solution into the hydrolyzates is required for the complete transition from acidogenic to solventogenic phases (Table 3). Therefore, we performed fermentation experiments using the hydrolyzates with addition of P2 solution.

The phase transition from acidogenesis to solventogenesis is a unique nature of solventogenic clostridia [3, 16]. When cells do not undergo the phase transition, (i) cells are not able to produce solvents, (ii) only acid can be produced and accumulated in the culture, and (iii) fail to survive and start to sporulate earlier than normal, which is called "degeneration." Thus, the transition to solventogenesis is a strategy for solventogenic clostridia to survive and solvent production is required for complete growth of solventogenic clostridia. Although the study of initiation of phase transition to solventogenesis is not well understood, one of the factors associated with the phase transition is nutritional limitation [11, 16, 18]. Supplementation of P2 seems to contribute nutritional requirement to conduce to the transition to solventogenesis in RB and DRB cultures.

Fermentation Characteristics Using the Hydrolyzates of RB and DRB as Substrates

In the fermentation of the hydrolyzates by *C. beijerinckii* NCIMB 8052, cell growth was estimated based on pH changes over time rather than optical density change, because the optical density of the culture was not measurable owing to the characteristics of the hydrolyzates. Since the culture undergoes biphasic growth, it is possible to evaluate cell growth by pH change over time of growth in the hydrolyzates (Fig. 2). It was observed that growth in the hydrolyzates was different from one in the glucose P2. The phase transition represented by the pH shift was not as

Table 2. Carbohydrate composition (g/l) of rice bran and defatted rice bran media with different treatments (after fermentation: 48 h).

(48 h)	RB	DRB	ERB	EDRB	ARB	ADRB	AERB	AEDRB
Glycerol	0.40±0.02	-	0.02±0.02	-	-	-	0.03±0.03	0.12±0.12
Ribose	1.00±0.08	0.67±0.06	1.01±0.01	0.94±0.13	0.66±0.05	0.61±0.05	0.85±0.05	0.90±0.04
Xylose	-	0.10±0.05	0.10±0.10	0.10±0.10	0.20±0.04	0.29±0.05	0.24±0.02	0.26±0.10
Arabinose	0.21±0.21	-	-	-	-	-	-	-
Fructose	-	-	-	-	-	-	-	-
Glucose	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-
Innositol	0.49±0.49	-	-	-	-	-	-	-
Stachyose	-	-	-	-	-	-	-	-

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.

Table 3. Effect of P2 solution on ABE production (g/l) from rice bran and defatted rice bran media.

	Without P2				P2			
36 h	RB	DRB	AERB	AEDRB	RBP2	DRBP2	AERBP2	AEDRBP2
Acetone	1.91	1.79	1.42	1.84	1.80	1.63	3.14	3.27
Ethanol	-	-	-	-	-	-	-	0.22
Butanol	1.28	2.21	4.03	5.50	2.18	3.34	9.54	10.38
Acetate	2.35	2.33	4.31	3.33	2.23	2.80	4.05	4.37
Butyrate	4.25	4.57	5.92	3.53	1.81	5.64	4.58	4.50
48 h	RB	DRB	AERB	AEDRB	RBP2	DRBP2	AERBP2	AEDRBP2
Acetone	1.98	1.79	1.69	1.76	1.59	1.08	3.01	3.49
Ethanol	-	-	-	-	-	-	-	-
Butanol	1.05	2.30	5.75	6.78	2.22	3.97	9.32	9.95
Acetate	2.46	2.36	4.12	3.10	1.45	2.23	4.93	4.55
Butyrate	4.06	4.36	6.35	3.84	1.81	5.37	4.64	4.90

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.

dramatic in the hydrolyzates as in the glucose culture. Moreover, the transition from acidogenic to solventogenic phases occurred in earlier time of growth in the hydrolyzates as compared with glucose culture. It is likely that the cells could not achieve complete growth in the hydrolyzates for some reasons. The observation that the phase transition starts earlier and is not complete is usually found in a degenerated culture under unfavorable conditions such as nutritional limitation. As a result, the solvent production in a degenerated culture decreases, while acid production, especially butyrate, goes on during the late exponential phase. This is consistent with our observation of high butyrate production even after phase transition in the hydrolyzates.

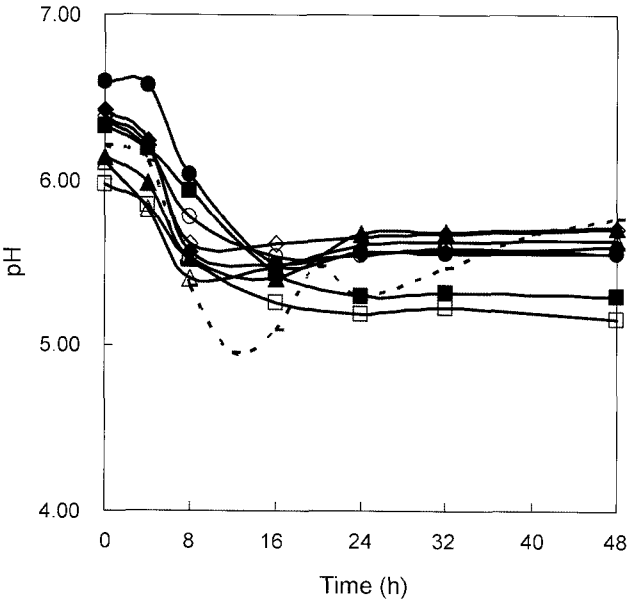

Fig. 2. pH in 10% (w/v) rice bran and defatted rice bran hydrolyzates (--, glucose 6%; ■, RB; □, DRB; ▲, ERB; △, EDRB; ●, ARB; ○, ADRB; ◆, AERB; ◇, AEDRB).

Sugar analysis (Tables 1 and 2) indicates that all fermentable sugars present in the hydrolyzates were utilized, but lower amounts of solvents were produced from the hydrolyzates compared with glucose fermentation. We speculate that the limited solvent production from the hydrolyzates was caused by the inhibitors generated during acid hydrolysis. The inhibitors seemed to have inhibitory effects on cell growth and the phase transition of the culture because relatively higher amounts of butyric acid accumulated in the cultures using acid- treated hydrolyzates as compared with both acid- and enzyme-treated hydrolyzates (Table 3).

Sugar Utilization During Fermentation of Hydrolyzates

All hexoses (glucose and fructose) present in the hydrolyzates were completely utilized while pentoses such as ribose and xylose were barely utilized, whereas fermentation (Table 2). This result is consistent with a previous report that *C. acetobutylicum* exhibited diauxic growth in the presence of mixtures of glucose and xylose [20]. This suggests that *C. beijerinckii* NCIMB 8052 also exhibit the diauxic growth pattern; that is, pentose would not be utilized until hexoses were completely utilized when mixed sugars of hexoses and pentoses are present in a culture medium. Based on Table 2, it was also noticed that *C. beijerinckii* NCIMB 8052 preferred to utilize arabinose to xylose and ribose among pentoses. Glycerol, which cannot be used as a sole carbon source itself for *Clostridium* [10], was partially utilized with other sugars. Some oligosaccharides seemed to be utilized as monomeric sugars by the action of extracellular enzymes because the calculated yields of butanol based on sugar concentration in the hydrolyzates were a little bit higher (0.35–0.43 g solvent/g total monomeric sugars) than the yield of butanol from glucose (038 g solvent/g glucose).

Table 4. ABE production (g/l) 1 l batch fermentation of rice bran treated with different methods.

	RB	DRB	ERB	EDRB	ARB	ADRB	AERB	AEDRB	Glu
Acetone	1.10±0.05	0.74±0.23	3.05±0.52	2.50±0.48	1.66±0.34	1.79±0.49	2.94±0.21	3.84±0.42	5.43±0.72
Ethanol	0.17±0.03	0.71±0.04	0.19±0.01	0.32±0.11	0.19±0.09	0.29±0.13	0.23±0.10	0.34±0.09	0.50±0.12
Butanol	2.91±0.42	4.31±0.43	3.82±0.33	6.25±0.62	9.68±0.58	11.11±0.39	11.39±0.54	12.24±0.74	16.77±1.22
Acetate	3.06±0.24	4.82±0.41	3.64±0.23	3.89±0.21	3.83±0.39	3.50±0.51	4.62±0.13	3.53±0.26	5.96±0.32
Butyrate	1.75±0.12	4.11±1.51	1.88±0.91	4.17±0.71	4.22±0.42	5.49±1.44	3.59±0.28	3.67±1.19	2.44±1.24

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.

According to the genome sequence of *Clostridium* spp., genes coding for extracellular hydrolytic enzymes for polysaccharide degradation have been found [1, 2, 19, 28]. Solventogenic clostridia are able to utilize a variety of sugars by operation of different levels of transport systems [sugar-specific PEP-dependent phosphotransferase systems (PTS) and permeases] and by secretion of extracellular enzymes to hydrolyze polysaccharides [14, 15, 16]. For example, glucose, fructose, sorbitol, sucrose, and lactose are transported into the cell with phosphorylation *via* PTS, and pentoses are transported *via* permeases without modification [16]. Therefore, sugars available in the hydrolyzates in RB and DRB can be utilized for conversion into butanol production by *C. beijerinckii* NCIMB 8052. In order to enhance butanol production from the hydrolyzates of RB and DRB, further study should focus on efficient sugar utilization.

Fermentation Products from Hydrolyzates

As described earlier, eight different kinds of RB/DRB-based hydrolyzates were prepared using four different hydrolysis treatment methods to facilitate fermentation. Based on the sugar concentration shown in Table 1, the concentration of reducing sugar in ARB/ADRB and AERB/AEDRB was estimated to be about 2% and 3%, respectively. The differences in sugar concentration resulted in different profiles of products in each medium shown in

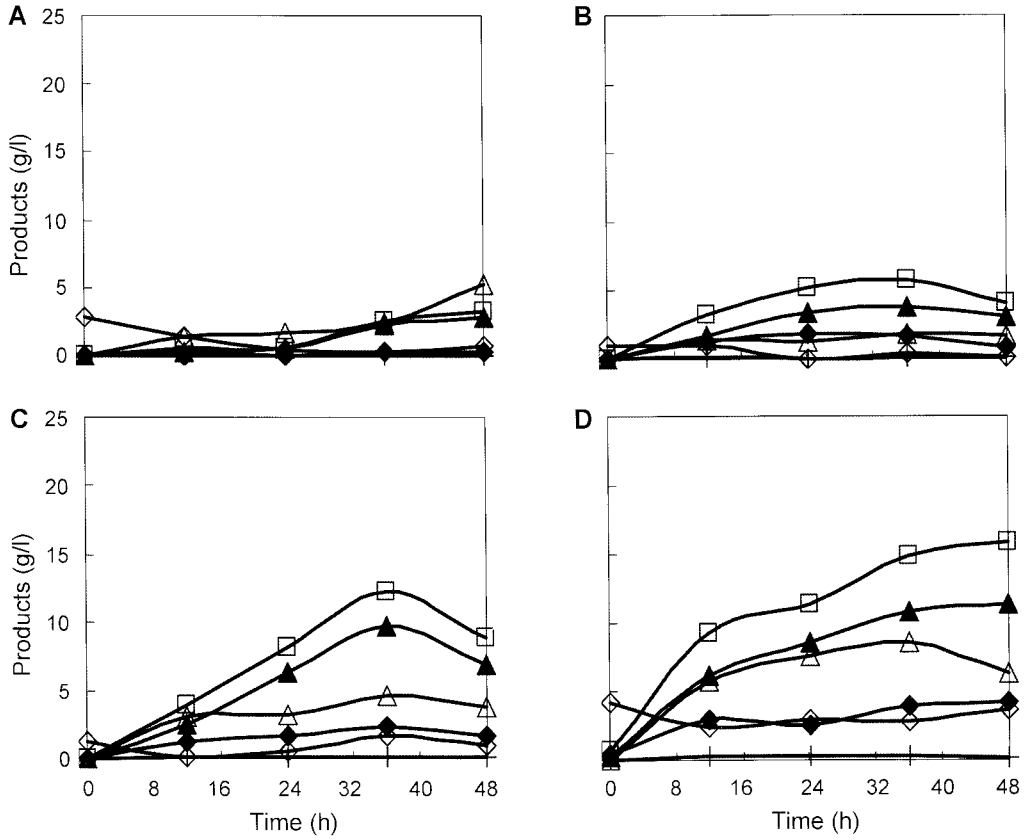

Fig. 3. Fermentation products from 10% (w/v) rice bran media (**A**, rice bran; **B**, enzyme-treated rice bran; **C**, acid-treated rice bran; **D**, acid- and enzyme-treated rice bran; ◆, acetone; +, ethanol; ▲, butanol; ◇, acetate; △, butyrate; □, total ABE).

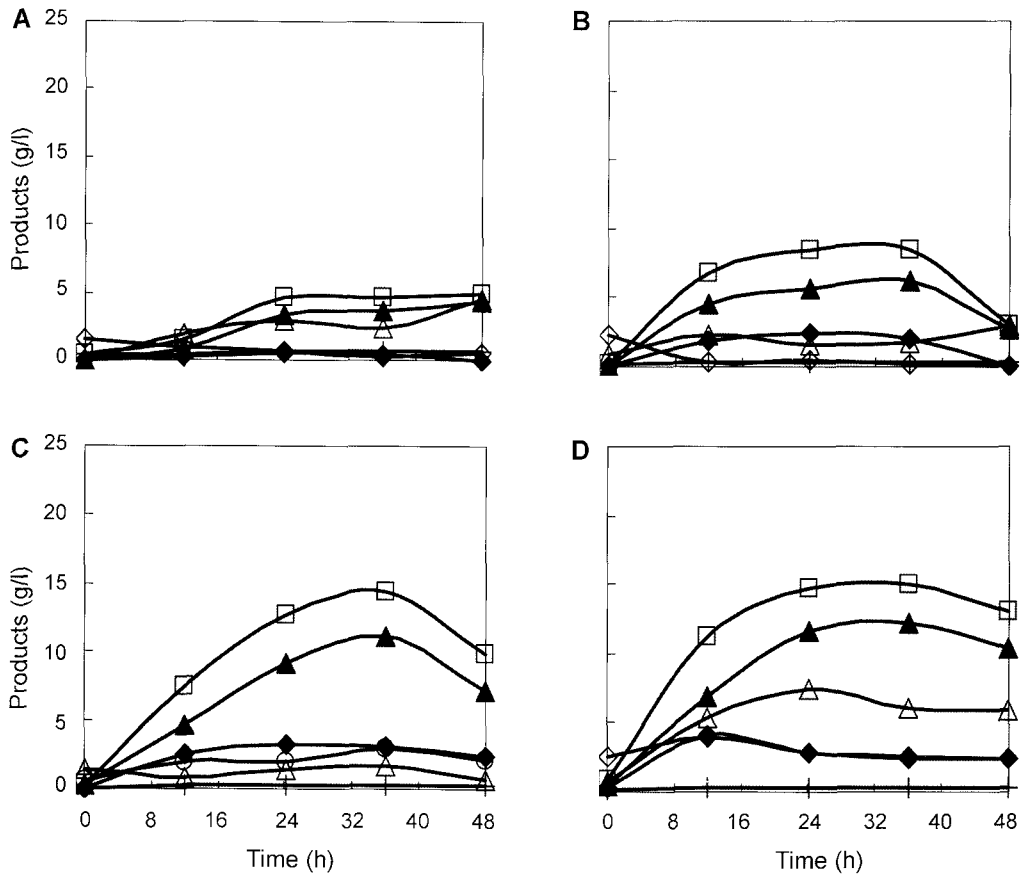

Fig. 4. Fermentation products from 10% (w/v) defatted rice bran media (**A** , defatted rice bran; **B**, enzyme-treated defatted rice bran; **C**, acid-treated defatted rice bran; **D**, acid- and enzyme-treated defatted rice bran; ◆, acetone; +, ethanol; ▲, butanol; ◇, acetate; △, butyrate; □, total ABE).

Table 4, Figs. 3, 4, and 5. The hydrolyzates obtained from both acid and enzyme treatment produced the highest butanol production [11.4 g/l (RB) and 12.2 g/l (DRB)]. The hydrolyzates 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) /11.1 g/l (DRB)]. It implies that the carbon flux from the metabolized sugars went down to both butyrate and butanol production owing to incomplete phase transition. This may be caused by growth inhibitors formed during acid hydrolysis treatment. Many problems associated with acid hydrolysis of carbohydrate are already known. For example, the formation of toxic compounds, such as furans, aliphatic acids, and phenolic compounds, has been reported [22]. However, the acid treatment has been proven to be a fast and cost-effective method for producing monomeric sugars from polysaccharides. Thus, acid-treated RB/DRB for butanol production would be recommended as inexpensive and efficient processes when the fermentation condition is optimized in this medium.

Regardless of types of hydrolysis treatments prior to fermentation, DRB produced 5–10% higher fermentable carbohydrates and butanol production compared with RB. This may be caused by the relatively higher contents of polysaccharide in DRB after extraction of lipids. We can speculate that the 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 [8, 21, 25]. Low titer was also reported as a problem in whey fermentation (5 g/l of butanol produced) where pretreatment was not required [4]. However, fermentation of RB and DRB seems to be advantageous, since the conditions for butanol production are less demanding, such as partial hydrolysis as pretreatment but no requirement of additional carbon source.

This studies suggested that both RB and DRB can be potential renewable substrates for production of butanol (up to 12 g/l butanol) when partial hydrolysis treatment was carried out prior to fermentation, since RB and DRB are relatively abundant biomasses in East Asia.

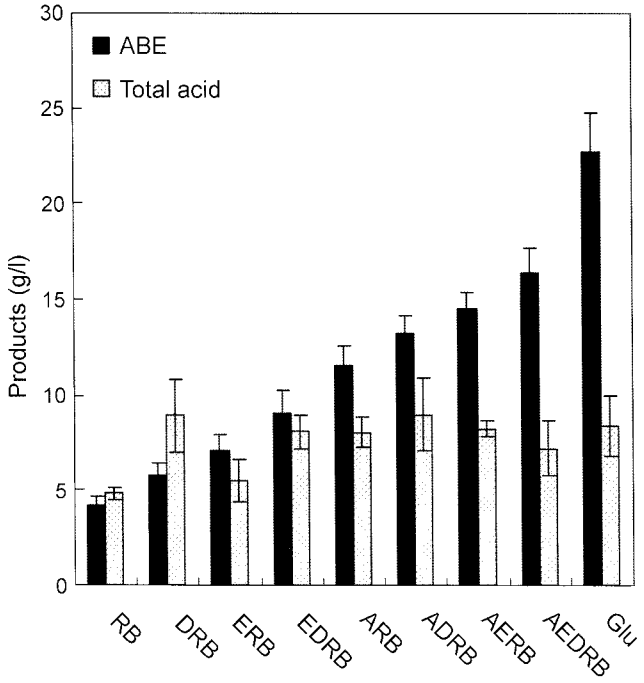

Fig. 5. Total acid and solvent (ABE) production by *C. beijernickii* NCIMB 8052 using rice bran media (10% w/v).
(RB, rice bran; DRB, defatted rice bran; ERB, enzyme-treated rice bran; EDRB, enzyme-treated defatted rice bran; ARB, acid-treated rice bran; ADRB, acid-treated defatted rice bran; AERB, acid- and enzyme-treated rice bran; AEDRB, acid- and enzyme-treated defatted rice bran).

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