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Production of butanol from starch-based waste packing peanuts and agricultural waste

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We examined the fermentation of starch-based packing peanuts and agricultural wastes as a source of fermentable carbohydrates using *Clostridium beijerinckii* BA101. Using semidefined P2 medium containing packing peanuts and agricultural wastes, instead of glucose as a carbohydrate source, we measured characteristics of the fermentation including solvent production, productivity, and yield. With starch as substrate (control), the culture produced 24.7 g l^{-1} acetone–butanol–ethanol (ABE), while with packing peanuts it produced 21.7 g l^{-1} total ABE with a productivity of 0.20 g l^{-1} h^{-1} and a solvent (ABE) yield of 0.37. Cell growth in starch, packing peanuts, and agricultural wastes medium was different, possibly due to the different nature of these substrates. Using model agricultural waste, 20.3 g l^{-1} ABE was produced; when using actual waste, 14.8 g l^{-1} ABE was produced. The use of inexpensive substrates will increase the economic viability of the conversion of biomass to butanol, and can provide new markets for these waste streams.
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Introduction

As a result of increasing oil prices (currently two to four times higher than in July–August 2000), various bioconversion programs have been initiated to produce biochemicals and bioenergy in the US. To hasten research and development and find solutions to this crisis, US President Clinton issued Executive Order 13134, entitled "Developing and Promoting Biobased Products and Bioenergy" (64 FR 44639, August 16, 1999). A primary goal of the order is to triple the nation's use of biobased products and bioenergy by 2010, generating as much as US$20 billion a year in new income for farmers and rural communities.

Similarly, a critical need for development of commodity chemicals, energy, and materials from biobased feedstocks was recognized in the Biomass Research and Development Act of 2000 that was passed by the 106th US Congress. In recognition of this need, the Midwest Consortium for Sustainable Biobased Products and Bioenergy was recently established. In addition, other programs to develop microorganisms, which can hydrolyze starch and lignocellulosic substrates efficiently, have been initiated to economize the process of fuels and chemicals production (e.g., Department of Energy/National Renewable Energy Laboratory (DOE/NREL)-sponsored research on developing cultures to produce cellulases that can hydrolyze cellulosic biomass, and DOE-sponsored research to develop high-efficiency enzymes to lower the cost of converting biomass to fermentable sugars that, in turn, will be converted into biofuels/biobased products). The goal is to convert more of the cellulose in corn stover, paper, rice straw, bagasse, and wood chips into glucose.

In the US, two substrates are abundantly available (starch and agricultural waste), in addition to various industrial wastes such as starch-based packing peanuts, which were developed to biodegrade after use. Several years ago, our laboratory developed a hyper-amylolytic culture (*Clostridium beijerinckii* BA101) to hydrolyze starch and use it for the production of butanol. It is well known that the conversion of starch to simple sugars requires enzymatic and/or acid hydrolysis prior to fermentation. The production of amylolytic enzymes (amylases and amyloglucosidases) by a single strain that also metabolizes the sugars produced to the desired end product has an advantage over those systems where hydrolysis and subsequent fermentation of starch occur separately. Additionally, acid hydrolysis can result in the inhibition of some fermentations.

As a result of the above initiatives, we have intensified our research on butanol production and recovery, which includes upstream and downstream processing and fermentation. Butanol, which is an excellent biofuel, has numerous other applications in food, plastics, and chemical industries [12]. In a recent economic study, we determined that fermentation substrate was one of the most important factors which influenced the price of butanol [11]. Other factors responsible for butanol price were acetone–butanol–ethanol (ABE) yield and byproduct credit. Since fermentation substrate cost influences butanol price most, it was our intention to investigate fermentation of starch-based packing peanuts and agricultural wastes. The starch-based peanuts also contain other undisclosed chemicals that may interfere with fermentation. Successful bioconversion of these wastes would not only convert these waste substrates to useful chemicals such as butanol, thus economizing the process of biofuel production, but also solve waste disposal problems faced by relevant industries.

Materials and methods

Microorganism

A stock culture of *C. beijerinckii* BA101 was maintained as a spore suspension in distilled water at 4°C. Spores were heat shocked in cooked meat medium (CCM) (Difco Laboratories, Detroit, MI)

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containing 30 g l^{-1} glucose at 80°C for 10 min. The culture was found to be growing actively within 16–18 h. This was followed by transferring 5 ml of actively growing culture to 100 ml of tryptone–glucose–yeast (TGY) extract medium [4]. Cells were grown anaerobically for 16–18 h at 34°C before they were transferred into solvent production medium containing starch, packing peanuts, or model/actual agricultural waste.

Media preparation

Cell growth of *C. beijerinckii* BA101 and butanol production were tested when the culture was grown on starch, starch-based packing peanuts, or agricultural wastes. A sugar solution equivalent to 48–96 g l^{-1} starch containing 1 g l^{-1} yeast extract (Difco Laboratories) was sterilized at 121°C for 15 min. On cooling to room temperature, filter-sterilized P2 medium stock solutions (buffer: KH_2PO_4, 50 g l^{-1}; K_2HPO_4, 50 g l^{-1}; ammonium acetate, 220 g l^{-1}; vitamin: *para*-amino-benzoic acid, 0.1 g l^{-1}; thiamin, 0.1 g l^{-1}; biotin, 0.001 g l^{-1}; mineral: $MgSO_4 \cdot 7H_2O$, 20 g l^{-1}; $MnSO_4.H_2O$, 1 g l^{-1}; $FeSO_4 \cdot 7H_2O$, 1 g l^{-1}; NaCl, 1 g l^{-1}) [10] were added (10 ml of each of these solutions to 970 ml of autoclaved medium containing substrate and 1 g l^{-1} yeast extract) followed by transferring the medium to an anaerobic chamber (Coy Laboratories Products, Ann Arbor, MI) where it was kept for 24 h prior to inoculation with actively growing TGY cell suspension. Butanol was produced in 125-ml to 2-l screw-capped bottles/vessels.

Packing peanuts [67.57 cm^3 g^{-1} (average volume), starch content 88.4 wt.%, average diameter 1.54 cm, length of peanuts 2–3 cm; Storopack, Cleveland, OH] were dissolved in distilled water to give a final concentration of 55–80 g l^{-1} total solids. When necessary, concentrated substrate solution was made, which was diluted to give the desired carbohydrate concentration. To facilitate rapid dissolution of packing peanuts, distilled water was stirred with a magnetic stir bar while peanuts were added. The solution was filled in bottles to 70% volume before autoclaving at 121°C for 15 min. The stock solutions were filter sterilized through a 0.2-μm pore size polyethersulfone (PES) membrane filter unit (Nalgene, Rochester, NY). The methods of preparation of medium (P2) and stock solutions have been given elsewhere [10].

In order to prepare model agricultural waste fermentation medium, 10 g of packing peanuts, 30 g of cracked corn, 150 g of apples, and 150 g of pears were blended in a blender. The volume of the mixture was adjusted to 1 l. The mixture had a sugar concentration equivalent to 96.4 g l^{-1} starch. The concentrated mixture was stored at 4°C until fermented. The pH of the mixture was not adjusted prior to fermentation. When necessary, the medium was diluted to give a carbohydrate concentration equivalent to 60 g l^{-1} starch.

The actual agricultural waste medium was prepared as follows: a total of 300 g of apple drops (approximately 5 cm cubes), 30 g of cracked corn, and 10 g of packing peanuts were mixed with 500 ml of distilled water in a Warring blender and blended for approximately 1 min. The final volume was adjusted to 1 l. The apples (drops) were collected from a local orchard (Curtis Orchard, Champaign, IL) and were of varying quality and variety, exhibiting significant signs of decay. After collection, the apples were rinsed to remove any soil and autoclaved at 121°C for 15 min to prevent further decay.

Growth conditions

The temperature for cell growth (on agar plates) and fermentation was controlled at 36±1°C. The plates were incubated for 24–48 h before counting cells as individual colonies. Fermentation pH was not controlled. However, in the cases of starch and packing peanuts, it was initially adjusted to approximately 6.8 by addition of buffer solution.

Analytical procedures

Due to the opaque nature of starch, peanut, and agricultural waste solutions, cell concentration measurement by optical density was not possible. Hence, cell population was measured as colony-forming units per milliliter. In order to enumerate cells, 1-ml samples were taken from the vessels and serially diluted (to 10^8) in TGY liquid medium followed by spreading 0.1 ml (from the last dilution) on a TGY agar plate containing 15 g l^{-1} agar as solidifying agent.

ABE and acids (acetic and butyric) were measured using a 6890 Hewlett-Packard gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector (FID) and 1.8 m×2 mm glass column (10% CW-20 M, 0.01% H_3PO_4, support 80/100 Chromosorb WAW). Productivity was calculated as total ABE concentration (g l^{-1}) divided by fermentation time (h). Fermentation time was defined as the time period when a maximum ABE concentration was reached. ABE yield was calculated as total ABE produced (g) divided by total carbohydrate (as starch) utilized (g). Being a biological system, fluctuations in ABE concentrations often occurred and they were of the order of 6–11%. The results presented here are averages of triplicate experiments.

Glucose concentration was determined using a hexokinase and glucose-6-phosphate dehydrogenase (Sigma, St. Louis, MO) coupled enzymatic assay. In order to measure glucose/reducing sugars, the fermentation broth was centrifuged (microfuge centrifuge) at 16,000 xg for 3 min at 4°C. A portion of the supernatant (10 μl) was mixed with glucose (HK 20) reagent (1.0 ml) and incubated at room temperature for 5 min. Standard solutions of anhydrous D-glucose containing 1–5 mg ml^{-1} glucose in distilled water were prepared. Ten microliters of standard solution was mixed with glucose (HK 20) reagent (1.0 ml) and incubated at room temperature for 5 min. A blank (deionized water) (10 μl) was incubated with the reagent and was used for zero adjustment of the spectrophotometer. After 5 min, the absorbance was measured at 340 nm and the glucose content in the sample was computed by least squares linear regression using a standard curve.

The reducing sugars in model and actual agricultural waste were measured using the 3,5-dinitrosalicyclic acid (DNSA) method [2]. A standard curve was prepared using standard glucose solution. A blank containing 1 ml of distilled water was heated for 5 min in boiling water with 3 ml of DNSA reagent. This was used to zero the spectrophotometer. In order to prepare a standard curve, standard solutions containing anhydrous D-glucose (0.1–0.8 mg ml^{-1} in distilled water) were heated with 3 ml each of DNSA for 5 min in a boiling water both followed by measuring optical density and plotting a graph of glucose concentration (as reducing sugar) *versus* optical density. In order to measure the reducing sugar concentration in the agricultural wastes, 250 mg of mixture (waste) was suspended in 15 ml of distilled water in a 50-ml beaker. One milliliter of the diluted suspension was heated prior to measuring optical density as mentioned above (with DNSA reagent). The reducing sugar concentration was read from the standard curve using least squares linear regression.

Starch concentrations of the samples were determined using a modified method of Holm *et al* [6]. The sample (250 mg) was

suspended in distilled water (15 ml) in a 50-ml beaker. Heat-stable α-amylase (100 μl) (Sigma) was added and mixed gently with a magnetic stirrer. The beaker was placed in a boiling water bath for 30 min with mixing every 5 min. The suspension was allowed to cool under continuous agitation on a magnetic stirrer and was transferred to a 25-ml volumetric flask followed by diluting it with water to the volume. One milliliter of the solution was transferred to a test tube followed by adding 2.9 ml of 0.1 M sodium acetate buffer (pH 4.75) and 100 μl of amyloglucosidase (Sigma). The mixture was incubated for 60 min at 55°C with mixing every 5 min. The sample was then transferred to a 50-ml volumetric flask and diluted to volume with distilled water. Ten microliters of the solution was assayed for glucose according to the hexokinase and glucose-6-phosphate dehydrogenase assay method described above:

$$\% \text{ starch} = \frac{\text{mg of glucose} \times 25^a \times 50^a \times 0.9^b \times 100}{\text{sample weight (250 mg)}}$$

where a=dilution factor; b=correction glucose→glucan. At the time of these studies, it was not possible to analyze starch-based packing peanuts for all of its constituent components.

Results and discussion

In order to evaluate the fermentation characteristics of starch solution, a control experiment was run using 60 g l^{-1} initial starch concentration in P2 medium. The fermentation was rapid and *C. beijerinckii* BA101 produced 24.7 g l^{-1} ABE in 72 h (Figure 1a). This included 7.7 g l^{-1} acetone, 15.8 g l^{-1} butanol, and 1.2 g l^{-1} ethanol. After 72 h of fermentation, ABE concentration started decreasing due to evaporative loss of solvents. There was no acetic acid production, while butyric acid was 0.88 g l^{-1}. The residual concentration of starch was 3.83 g l^{-1}, indicating that 93.6% starch was utilized during the fermentation (Figure 1b). As shown in this figure, starch utilization was rapid. The culture could not utilize all the starch because of the toxic effect of solvents (at approximately 22–25 g l^{-1} total ABE in the fermentation broth, the culture ceased to ferment) [13]. At zero time, there were 1.5×10^6 cells ml^{-1} (Table 1). A maximum concentration of *C. beijerinckii* BA101 cells of 8×10^7 cells ml^{-1} was observed at 24 of growth. After 24 h, the number of cells started decreasing. These results indicate that hyperamylolytic *C. beijerinckii* BA101 is capable of utilizing starch efficiently for the production of butanol. The initial pH of the fermentation broth was 6.8 while the final pH was 5.08 (Table 1). It should be noted that an ABE productivity of 0.34 g l^{-1} h^{-1} and yield of 0.44 were obtained in this control experiment.

In order to investigate production of ABE from starch-based packing peanuts, 55.0 g l^{-1} peanuts were fermented in P2 medium using *C. beijerinckii* BA101. This produced a medium containing 47.7 g l^{-1} total starch. *C. beijerinckii* BA101 grew to a peak cell density of 2.5×10^7 cells ml^{-1} after 18 h of growth (Table 1) followed by decreased cell numbers. After 115 h of fermentation, 13.3 g l^{-1} total solvents had been formed, with a maximum of 10.1 g l^{-1} butanol. At the end of the fermentation, the concentrations of acetic and butyric acids were 2.1 and 0.4 g l^{-1}, respectively. Starch utilization was 100%, as no starch was detected at the end of the fermentation (Figure 2b). Although the results of the fermentation of this waste are encouraging, fermentation was slow (Figure 2a) and a productivity of 0.14 g l^{-1} h^{-1} was

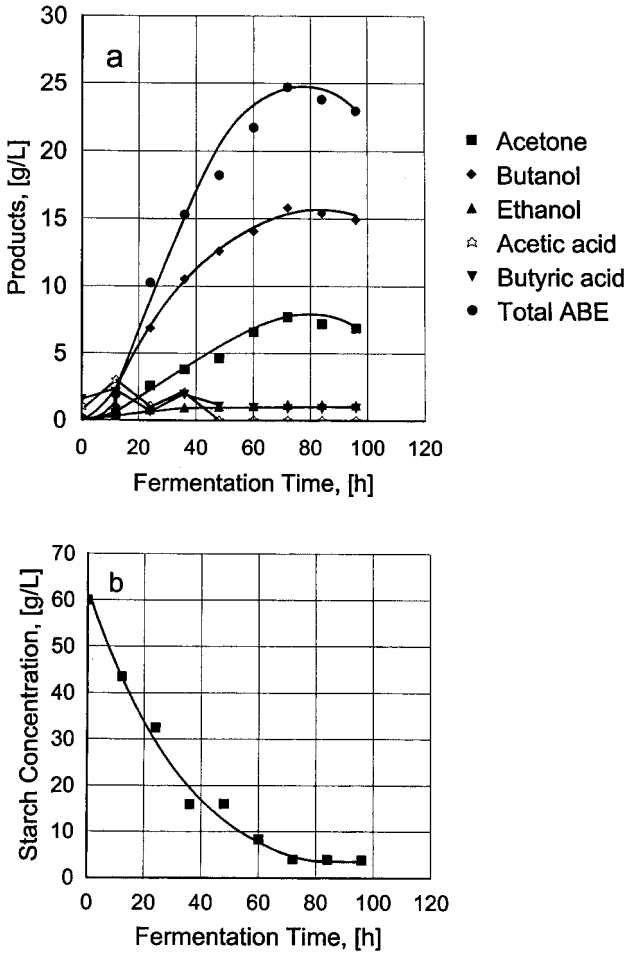

Figure 1 Production of ABE from starch by hyperbutanol-producing *C. beijerinckii* BA101 in a batch reactor. (a) Fermentation products; (b) starch concentration.

obtained. An ABE yield of 0.28 was achieved as compared to 0.44 in the control experiment. A possible reason for low ABE concentration, low productivity, and low yield may be due to the presence of toxic chemicals in the peanuts, which may have slowed down and hindered ABE production, thus resulting in the accumulation of 2.5 g l^{-1} acids. The presence of a limited amount of carbon source is known to result in acid production and hence poor ABE yield [3,14]. The initial cell concentration and pH were 7.7×10^5 cells ml^{-1} and 6.1, respectively (Table 1). The maximum cell concentration was 2.5×10^7 cells ml^{-1} at 18 h, which is comparable to the control experiment. It should be noted that initially, the culture produced acids and then reassimilated them. In ABE fermentations, acids are metabolic intermediates and are produced before they are reutilized for acetone and butanol production.

In order to examine the inhibitory effects of unidentified components of peanuts on solvent production by *C. beijerinckii* BA101, an experiment was conducted with 80 g l^{-1} peanuts. An increase in packing peanut concentration would increase substrate and any associated inhibitors' concentrations. Since substrate concentration, at the level employed, is not inhibitory, any negative affect on the culture would likely be due to the presence of inhibitors. In this experiment, the initial starch concentration was

Table 1 Cell growth (colony-forming units ml^{-1}) of *C. beijerinckii* BA101 in starch, packing peanuts (PP), and model agricultural waste fermentation media

Starch (60 g l^{-1})			PP (55 g l^{-1})			PP (80 g l^{-1})		PP (80 g l^{-1}) (2-l reactor)			Model agricultural waste (60 g l^{-1})			Model agricultural waste (96.4 g l^{-1})		
FT (h)	Cells ml^{-1}	pH	FT (h)	Cells ml^{-1}	pH	FT (h)	Cells ml^{-1}	FT (h)	Cells ml^{-1}	pH	FT (h)	Cells ml^{-1}	pH	FT (h)	Cells ml^{-1}	pH
0	1.5×10^{6}	6.80	0	7.7×10^{5}	6.10	0	3.0×10^{6}	0	1.0×10^{6}	6.60	0	6.0×10^{5}	4.34	0	6.2×10^{5}	4.21
12	3.0×10^{7}	4.69	18	2.5×10^{7}	4.75	14	4.0×10^{7}	10	2.0×10^{7}	5.18	12	4.2×10^{5}	4.51	12	2.0×10^{5}	5.33
24	8.0×10^{7}	4.83	28	2.0×10^{7}	4.81	26	1.3×10^{7}	24	1.3×10^{7}	5.49	24	2.1×10^{6}	4.73	24	7.9×10^{8}	5.22
36	7.0×10^{7}	5.17	41	1.0×10^{7}	5.07	38	1.5×10^{8}	48	3.8×10^{7}	5.47	36	1.9×10^{6}	4.94	36	6.9×10^{8}	4.92
48	7.5×10^{6}	5.03	52	1.0×10^{7}	5.12	49	5.5×10^{7}	78	1.3×10^{5}	6.05	48	5.7×10^{8}	5.18	48	6.4×10^{10}	4.87
60	2.0×10^{6}	4.92	68	8.0×10^{6}	4.96	73	5.3×10^{7}	110	2.0×10^{4}	6.10	60	1.4×10^{8}	5.11	60	3.2×10^{9}	4.88
72	4.0×10^{5}	4.99	93	4.5×10^{5}	5.10	97	5.0×10^{7}				72	8.5×10^{8}	5.13	72	1.3×10^{8}	4.90
84	4.0×10^{5}	5.03	115	2.0×10^{4}	5.07						84	6.1×10^{8}				
96	3.0×10^{4}	5.08														

FT = fermentation time.

70.7 g l^{-1}. During the fermentation, 15.2 g l^{-1} ABE were produced in 97 h (Figure 3). Since this is higher than 13.3 g l^{-1} (previous experiment), it was disregarded that the presence of any inhibitory components was a result of slow fermentation, low ABE concentration, and low ABE yield. If inhibitory components were present, this fermentation run would have been much slower than previous one. At zero time, 3.0×10^{6} cells ml^{-1} were present (Table 1). This experiment indicated that the previous fermentation was deficient in substrate.

Next, an experiment was conducted where ABE production was scaled-up from 125-ml to a 2-l vessel to study if scaling up had an affect on ABE fermentation of packing peanuts. The fermentations were conducted with 1.4 l of medium volume. The concentration of peanuts was 80 g l^{-1}, equivalent to 69.6 g l^{-1} starch. Solvent production reached its peak at 110 h, and consisted of 5.7 g l^{-1} acetone, 15.7 g l^{-1} butanol, and 0.3 g l^{-1} ethanol for a total of 21.7 g l^{-1} solvents (Figure 4a). An ABE productivity of 0.20 g l^{-1} h^{-1} was achieved in this fermentation. After 110 h, the amount of starch remaining in the fermentation broth was 11.1 g l^{-1} (Figure 4b), resulting in an ABE yield of 0.37. While improved solvent titres and ABE yield were obtained, fermentation still remained slow. At this stage, we do not know reasons for the slow fermentation. The concentrations of acids were low at

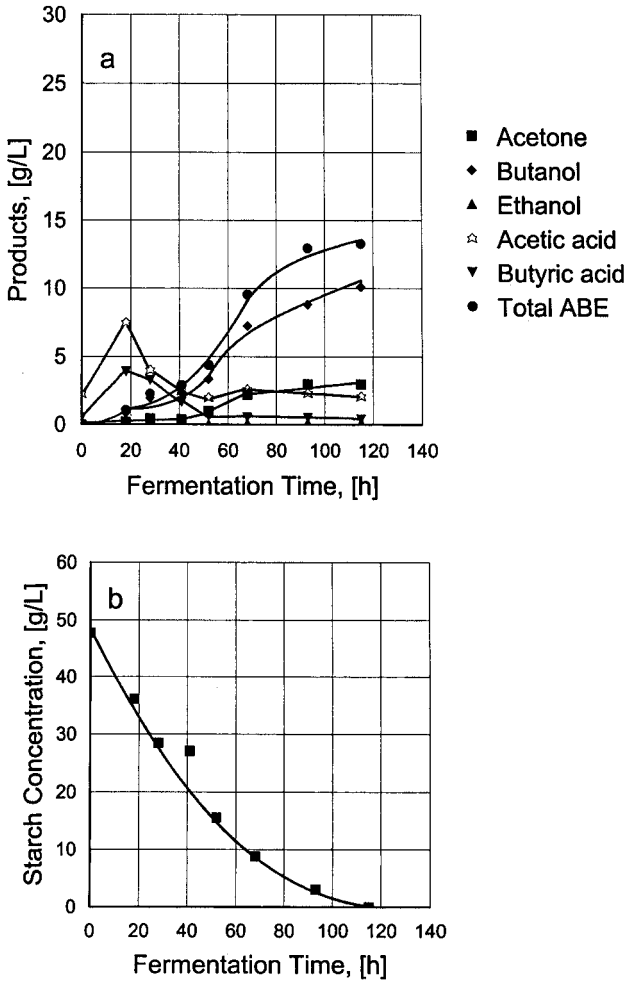

Figure 2 Production of ABE from starch-based packing peanuts (55 g l^{-1}) by hyperbutanol-producing *C. beijerinckii* BA101 in a batch reactor. (a) Fermentation products; (b) starch concentration.

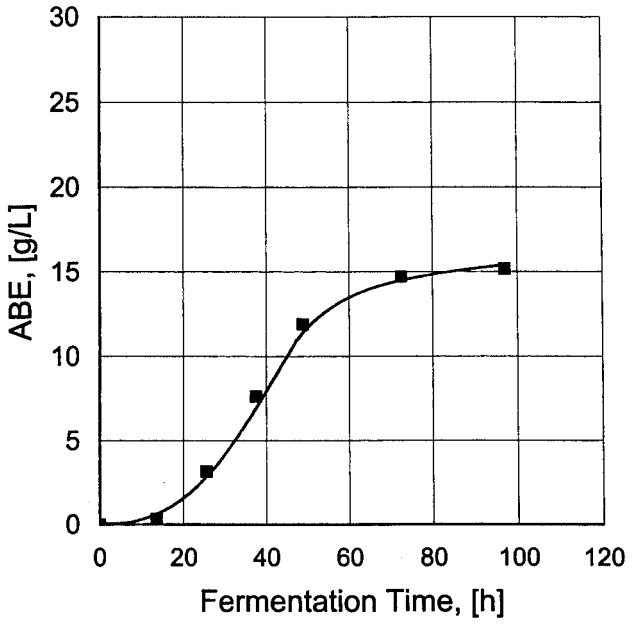

Figure 3 Production of ABE from starch-based packing peanuts (80 g l^{-1}) by hyperbutanol-producing *C. beijerinckii* BA101 in a batch reactor.

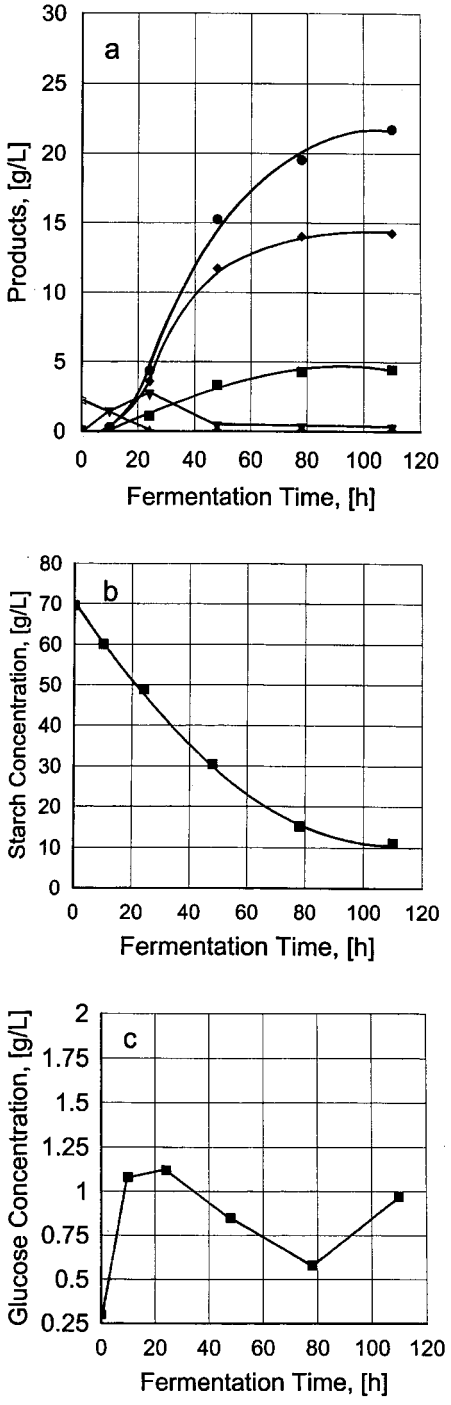

Figure 4 Production of ABE from starch-based packing peanuts (80 g l^{-1}) by hyperbutanol-producing *C. beijerinckii* BA101 in a 2-l batch reactor. (a) Fermentation products; (b) starch concentration; (c) glucose concentration.

0.0 g l^{-1} (acetic) and 0.2 g l^{-1} (butyric) at the end of fermentation, and this was the likely reason for improved ABE yield compared to the 55 g l^{-1} peanut fermentation.

In addition to investigating fermentation parameters, we also investigated whether cultures hydrolyzed starch (to glucose) faster than they utilized the latter for cell growth, maintenance, and ABE production. Cultures hydrolyzed starch efficiently and there was free glucose present in the fermentation medium throughout the fermentation (Figure 4c). More interesting was the culture's ability to hydrolyze starch even during the initial phase of cell growth (at 10 h) when there was 1.08 g l^{-1} glucose present. After 24 h, the concentration of glucose was 1.12 g l^{-1}, and at 78 h, it was 0.58 g l^{-1}. At the end of fermentation, approximately 1.0 g l^{-1} glucose was present. These results indicated that the extracellular amylases continued hydrolyzing starch throughout the fermentation. These results indicate that *C. beijerinckii* BA101 is a hyperamylase-producing culture. In the beginning of the fermentation, there were 1.0×10^6 cell ml^{-1} which grew to 3.8×10^7 after 48 h of fermentation (Table 1). These results suggested that the peanut fermentation performed better in the 2-l vessel compared to 125-ml vessel.

Using the diluted model waste, *C. beijerinckii* BA101 reached a cell density of 5.7×10^8 cells ml^{-1} in 48 h of fermentation (Table 1). Solvent concentration reached a maximum of 14.7 g l^{-1} in 60 h (Figure 5). The concentrations of individual solvents were 5.2 g l^{-1} acetone, 9.2 g l^{-1} butanol, and 0.3 g l^{-1} ethanol. The final concentration of acetic acid was 0.3 g l^{-1}, while that of butyric acid was zero. No P2 medium ingredients were added to the fermentation medium and pH was not adjusted to 6.8 prior to fermentation. The initial pH of the fermentation medium was 4.34 and this may have been the reason for initial slow growth (Table 1). At 72 h, the cell population was 8.5×10^8 cells ml^{-1}, which was much higher than in the starch fermentation and packing peanut fermentations. This suggested that such a model agricultural waste is a good substrate for cell growth of *C. beijerinckii* BA101. Based on a fermentation time of 60 h (when the fermentation reached a maximum ABE concentration), the reactor productivity was 0.24 g l^{-1} h^{-1}, which is higher than packing peanut fermentations. Adjusting the pH to 6.8 prior to fermentation might have resulted in faster fermentation partly due to sufficient nutrients available in the model waste and partly due to pH.

In order to find out if the concentrated mixture was fermentable, the model waste medium containing carbohydrates (as starch) at 96.4 g l^{-1} was fermented using *C. beijerinckii* BA101. Fermentations with high sugar/carbohydrate concentrations are beneficial when integrated with simultaneous solvent removal. In such cases,

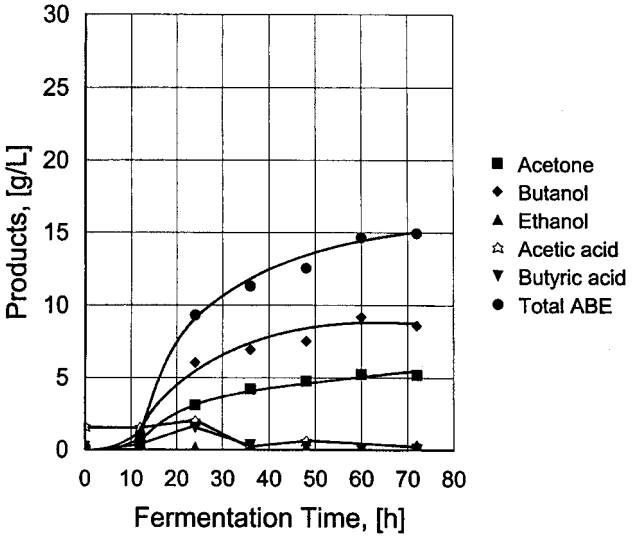

Figure 5 Production of ABE from model agricultural waste (60 g l^{-1} as starch) by hyperbutanol-producing *C. beijerinckii* BA101.

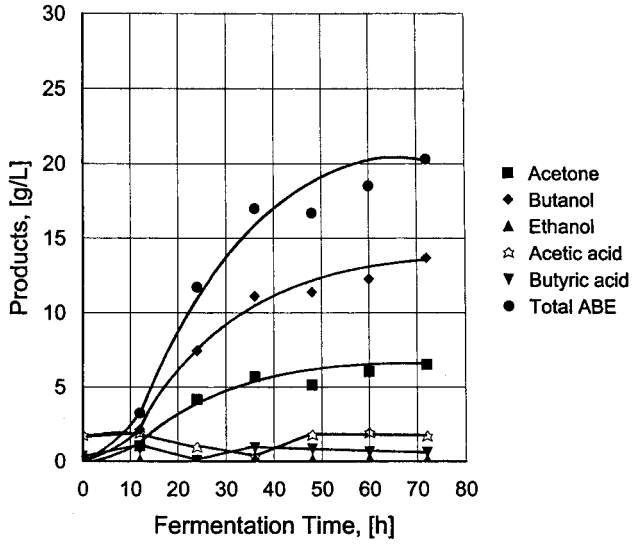

Figure 6 Production of ABE from model agricultural waste (96.4 g l^{-1} as starch) by hyperbutanol-producing *C. beijerinckii* BA101.

solvents are continuously removed until all the carbohydrates are utilized. This type of system results in an economic process due to reduction in process volume and waste disposal stream. However, the culture should not be inhibited by the initial high carbohydrate concentration. As above, neither pH was adjusted, nor P2 medium ingredients added to the medium.

The concentrated carbohydrate medium demonstrated good growth with a maximum cell density of 6.4×10^{10} cells ml^{-1} in 48 h of growth (Table 1). This cell growth is much higher than in any of the previous fermentations and was possibly a result of the abundance of nutrients. At 72 h of fermentation, the total solvent concentration was 20.3 g l^{-1}. The final concentrations of acetone, butanol, and ethanol were 6.5, 13.7, and 0.1 g l^{-1}, respectively (Figure 6). The final concentrations of acetic and butyric acids were 1.7 and 0.6 g l^{-1}, respectively. These levels of acids are higher than seen in the diluted medium and were possibly due to excessive of nutrients available. The initial pH of this medium was 4.21, but fermentation proceeded quite well. Natural acids that are present in fruits were responsible for the low pH. Data on feed from both of the model agricultural waste fermentations suggest that the culture used those natural acids resulting in an increase in pH. Removal of ABE from concentrated whey permeate/lactose medium has been carried out previously [7].

Fermentation of actual agricultural waste resulted in the production of 14.8 g l^{-1} total ABE in 66 h (Table 2). Cell growth was maximal at 2.64×10^{8} cells ml^{-1} at 44 h followed by a decline. At the end of fermentation, 1.3 g l^{-1} acids remained. Interestingly, only 14.8 g l^{-1} total ABE was produced compared to 20.3 g l^{-1} when using model agricultural waste.

The hyperamylolytic activity of *C. beijerinckii* BA101 was first described by Annous and Blaschek [1]. In addition, this organism is superior to other butanol-producing cultures with respect to butanol production [4,9]. However, in these studies, glucose and maltodextrin were used as fermentation substrates. Since these substrates have value as food ingredients, their use as feed for butanol fermentation is not economical. Starch-based packing peanuts, designed as a biodegradable alternative to styrofoam packing material, were originally intended to lessen the impact of waste materials in the landfill stream. In this study, we have shown that this waste material can be utilized as the sole carbohydrate source for butanol production by *C. beijerinckii* BA101. Although levels of ABE were not achieved as high as in the starch fermentation medium (24.7 g l^{-1}), they were on the order of 13.3–21.7 g l^{-1}, which is highly encouraging for waste substrates. Use of agricultural wastes is even more attractive as it requires neither nutrient supplementation nor pH adjustments. This will result in the economic production of biofuels (acetone, butanol). Our earlier studies indicated that a cheap nutrient source such as corn steep liquor (CSL) was a good replacement for P2 medium ingredients. Use of other agricultural substrates (potatoes) for economic production of biofuels such as butanol has been documented [5,8].

The growth of *C. beijerinckii* BA101 on model agricultural waste was higher than that observed on starch and packing peanuts. This was most likely due to the varied and rich nutrients available in the medium. However, butanol production did not increase with increased cell growth, perhaps due to the initial acidic pH of the medium and the apparent lack of buffering capacity of the model waste. One of the major drawbacks of these fermentations has been measurement of growing cells only. Growing cells are not indicative of fermentation characteristics because they do not produce solvents [15]. Conversely, solvent-producing cells have been reported not to support growth. In any butanol-producing system, there are four different types of cells namely: growing, butanol-producing, maintenance-requiring, and dead cells [15]. It is likely that more cells were present in the fermentation broths than reported here.

In conclusion, we have demonstrated that *C. beijerinckii* BA101 can utilize waste substrates effectively for the production of ABE. Using starch as substrate, the culture produced 24.7 g l^{-1} ABE, while with packing peanuts it produced 21.7 g l^{-1} total ABE with a productivity of 0.20 g l^{-1} h^{-1} and a solvent yield of 0.37. Using model agricultural waste, 20.2 g l^{-1} ABE was produced. We have

Table 2 Cell growth and ABE production from agricultural waste using *C. beijerinckii* BA101

Cell growth		Fermentation products (g l^{-1})						
FT (h)	Cells ml^{-1}	FT (h)	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid	Total ABE
0	1.4×10^{6}	0	0.13	0.46	0.04	0.11	0	0.63
8	4.2×10^{7}	12	0.15	0.33	0.11	1.30	0.94	0.59
12	1.6×10^{7}	18	0.61	1.14	0.10	1.56	1.22	1.85
20	1.5×10^{7}	24	1.43	2.56	0.13	0.98	0.65	4.12
26	2.32×10^{8}	42	3.99	7.32	0.19	0.65	0.65	11.50
44	2.64×10^{8}	66	4.80	9.40	0.25	1.27	0.89	14.45
66	3.03×10^{7}	84	4.76	9.80	0.20	0.81	0.51	14.76

FT = fermentation time.

fermented actual agricultural waste of similar composition to butanol using *C. beijerinckii* BA101. This produced 14.8 g l^{-1} total ABE. The use of inexpensive substrates will increase the economic viability of the conversion of biomass to butanol, and can provide new markets for these waste streams. Further, it is our intention to integrate product recovery with fermentation using these waste substrates as feed.

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