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Production of Acetone Butanol Ethanol from Degermed Corn Using *Clostridium beijerinckii* BA101

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Abstract

In this article we report on acetone butanol ethanol (ABE) fermentation characteristics of degermed corn when using *Clostridium beijerinckii* BA101. Recent economic studies suggested that recovery of germ from corn and hence corn oil would help to make the ABE fermentation process more economical. *C. beijerinckii* BA101 ferments corn mash efficiently to produce ABE under appropriate nutritional and environmental conditions. Corn mash contains germ/corn oil that is, possibly, ancillary to the production of butanol during the ABE fermentation process. Since the presence of corn oil is not a critical factor in solvent fermentation, it can be removed and this will allow for byproduct credit. Batch fermentation of degermed corn resulted in 8.93 g/L of total ABE production as compared with 24.80 g/L of total ABE when supplemented with P2 medium nutrients. During the course of the germ separation process, corn steeping is required prior to grinding and removing the germ. It is likely that some nutrients from the corn are leached out during the steeping process. This may reduce the rate of fermentation and impact the final concentration of butanol/ABE that can be achieved. Fermentation of degermed corn with corn steep liquor resulted in the production of 19.28 g/L of ABE.

Index Entries: Degermed corn; butanol; *Clostridium beijerinckii* BA101; corn steep liquor; acetone butanol ethanol; fermentation.

Introduction

Butanol, a fermentation product of *Clostridium acetobutylicum/C. beijerinckii*, is a superior fuel and has more calorific value than ethanol. In addition to fuel applications, butanol can be used in the manufacture of

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plastics, and extraction of food flavors. It can be produced from renewable agricultural resources including cane molasses, agricultural biomass/wood hydrolysate, corn, dairy industry waste (whey permeate), wheat, rice, rye, cassava starch, and Jerusalem artichoke *(1)*. Because Illinois is a corn-producing state, we are interested in producing butanol from corn, which would benefit not only farmers in Illinois but those all over the United States.

The economics of butanol production is affected by the type of bioreactor used, the butanol recovery technique, byproduct credit, yield, solvent concentration, and productivity *(2–4)*. In a recent economic study examining butanol production from corn, a number of byproducts were identified, and their impact on the economics of butanol production was evaluated *(2)*. These products included germ/corn oil and corn fiber, in addition to cell biomass and gases (CO_2 and H_2). The study suggested that recovery of byproducts such as germ improves the economics of butanol production from corn. For a butanol production plant with a capacity of 121.6×10^6 kg/yr, the total annual production cost was $\$102.5 \times 10^6$ *(2)*. This plant would produce 19.9×10^6 kg of corn oil derived from germ annually, resulting in a credit of $\$8 \times 10^6$. In a similar exercise, the recovery of germ prior to fermentation to ethanol was also studied and shown to reduce the cost of ethanol production by up to \$0.03/L *(5,6)* as a result of the value of the recovered oil and the increased fermentor capacity. For the same size ethanol plant (121.6×10^6 kg/yr) the germ credit would be $\$4.6 \times 10^6$. This is lower than the credit obtained for the butanol plant owing to the fact that butanol yield is 0.33 (acetone butanol ethanol [ABE] yield 0.42) as compared to an ethanol yield of approx 0.48 (theoretical yield of 0.51). A higher yield would require less corn and would result in less germ and hence less byproduct credit. Furthermore, removal of a significant percentage of the germ (thus oil) from the mash also resulted in significantly less fouling of heated surfaces as may occur in an evaporator *(7)*.

Since the recovery of germ results in better economics of butanol fermentation, we initiated research on fermentation of degermed corn. It is anticipated that corn oil does not play any role in butanol fermentation nor in the metabolism of *C. acetobutylicum/C. beijerinckii*. In addition, during the wet-milling process (to separate germ), corn is soaked in water and then the water is drained. In this process, some of the important nutrients may be removed, which may reduce growth and affect the fermentation adversely. The overall objective of this research was to produce butanol from degermed corn using *C. beijerinckii* BA101 and evaluate whether degermed corn requires nutrient supplementation.

Materials and Methods

Culture and Maintenance

Spores of *C. beijerinckii* BA101 were stored at 4°C in sterile distilled water. The spores were heat shocked at 80°C for 10 min in cooked meat

medium (Difco, Detroit, MI) for preparation of inoculum. This was followed by incubation at 35°C in an anaerobic chamber for 12–16 h.

Preparation of Media

Butanol was produced from degermed corn using *C. beijerinckii* BA101. Degermed corn slurry (starch content of 214.3 g/L) was diluted with water to approx 55–60 g/L of starch followed by supplementation with 1 g/L of yeast extract (Difco) and sterilization at 121°C for 15 min. On cooling to room temperature the medium was transferred to an anaerobic chamber (Coy, Ann Arbor, MI) for 24 h prior to inoculation with actively growing cells. When necessary, filter-sterilized P2 medium stock solutions *(8)* were added to the fermentation medium prior to inoculation. Concentrated stock solutions were prepared, and 10 mL of each of the solutions (buffer, vitamin, and mineral) was added to 990 mL of medium. P2 medium did not require any pH adjustment. In the beginning of the fermentation, the pH was 6.8, which decreased to 5.0–5.3 by the end of fermentation. Except for the first fermentation, degermed corn was boiled for 10–15 min to make a homogeneous starch slurry/mixture. In the absence of boiling, starch settled to the bottom and did not ferment. For fermentation with corn steep liquor (CSL), cysteine hydrochloride (1 g/L) and ferrous sulfate (12 mg/L) were added to CSL prior to boiling the degermed corn. Sodium hydroxide (1 *M*) solution was used to adjust the initial pH to 6.8 when necessary. Degermed corn mash and CSL took on the consistency of a thick slurry.

Fermentation

Batch fermentation studies were conducted in 150-mL screw-capped bottles containing 125 mL of medium. The bottles were inoculated with 5% (v/v) tryptone-glucose-yeast extract medium (30 g/L of tryptone [Fisher, Fair Lawn, NJ], 20 g/L of glucose [Sigma, St. Louis, MO], 10 g/L of yeast extract [Difco], and 1 g/L of L-cysteine [Sigma]), grown culture of *C. beijerinckii* BA101 and incubated at 35°C for 84–96 h in an anaerobic chamber with a modified atmosphere of 80% N_2, 15% CO_2, and 5% H_2. One-milliliter samples were taken intermittently, followed by centrifugation in a microcentrifuge and storage at –18°C until analyzed for ABE/acids. For starch determination, samples were diluted 10 times and refrigerated without centrifugation.

Statistical Analyses

Solvents (ABE) and acids were determined by gas chromatography (Hewlett Packard Gas Chromatograph 6890) using a flame ionization detector and a capillary column (crosslinked free fatty acid phase; dimension of 30×0.53 mm and 1-µm film thickness). Starch was hydrolyzed enzymatically *(9)* prior to enzymatic measurement of glucose (Sigma Diagnostics Kit, Glucose HK; Sigma). The experimentally determined concentration of glucose was converted to starch by dividing by 1.1. For the determination of

glucose, absorbance was measured at 340 nm using a Beckman DU-40 spectrophotometer. The yield was calculated as the total ABE produced divided by the total starch utilized and is expressed as grams/gram. ABE productivity was calculated as the total ABE produced (grams/L) divided by the fermentation time. Although fermentations were run up to 96 h, for the purpose of calculations, fermentation time was taken as the time when fermentation ceased. In the glucose-based fermentation, the cell concentration was measured by reading optical density (OD) of the cell suspension. When using glucose as the substrate and P2 medium, batch cultures of *C. beijerinckii* BA101 resulted in a cell concentration of approx 3 g/L. Since degermed corn contains corn fiber, OD measurements were not possible for these studies. For this reason, cell concentration was not reported.

Results and Discussion

Initially, an experiment was conducted without boiling the degermed corn (prior to sterilization) and fermented with *C. beijerinckii* BA101. Nutrients were not added to the mash nor was the degermed corn suspension homogenized. After 72 h of fermentation, 0.56 g/L of acetone, 2.14 g/L of butanol, and 0.04 g/L of ethanol were produced. The concentration of acids was high at 6.45 g/L (acetic acid) and 1.06 g/L (butyric acid). The fermentation resulted in the production of 2.74 g/L of ABE and 7.51 g/L of acids, because the starch did not remain in solution, suggesting that boiling and homogenization of the degermed corn is necessary prior to sterilization. Fermentation was poor because settled starch was unavailable for use by *C. beijerinckii* BA101.

This experiment was followed by another fermentation in which degermed corn was boiled prior to sterilization and a homogeneous starch suspension was produced. Following sterilization, the starch did not settle but remained in solution. This was fermented without nutrient supplementation, and 84 h of fermentation resulted in the production of 8.93 g/L of total ABE and 3.75 g/L of total acids (Fig. 1A, Table 1), suggesting that boiling the degermed corn was essential for fermentation. An ABE yield of 0.38 was obtained. Of the 56.3 g/L of starch available in the beginning of the fermentation, 23.5 g/L was utilized, leaving behind 32.8 g/L. The above concentration of ABE is not toxic to the cells; up to 33 g/L of ABE has been produced using this strain *(10)*. Hence, a possible reason for the production of such a low concentration of ABE was lack of nutrients. Readers are advised that *C. beijerinckii* BA101 is a hyperamylolytic strain that does not require starch hydrolysis prior to fermentation. The culture was genetically developed to utilize starch with a potential for producing butanol from corn *(11)*.

To investigate this further, a batch fermentation was run using degermed corn (initial starch concentration of 61.1 g/L) in P2 medium. P2 medium is a rich source of nutrients, and fermentations run with 60–80 g/L of glucose containing P2 medium resulted in the production of

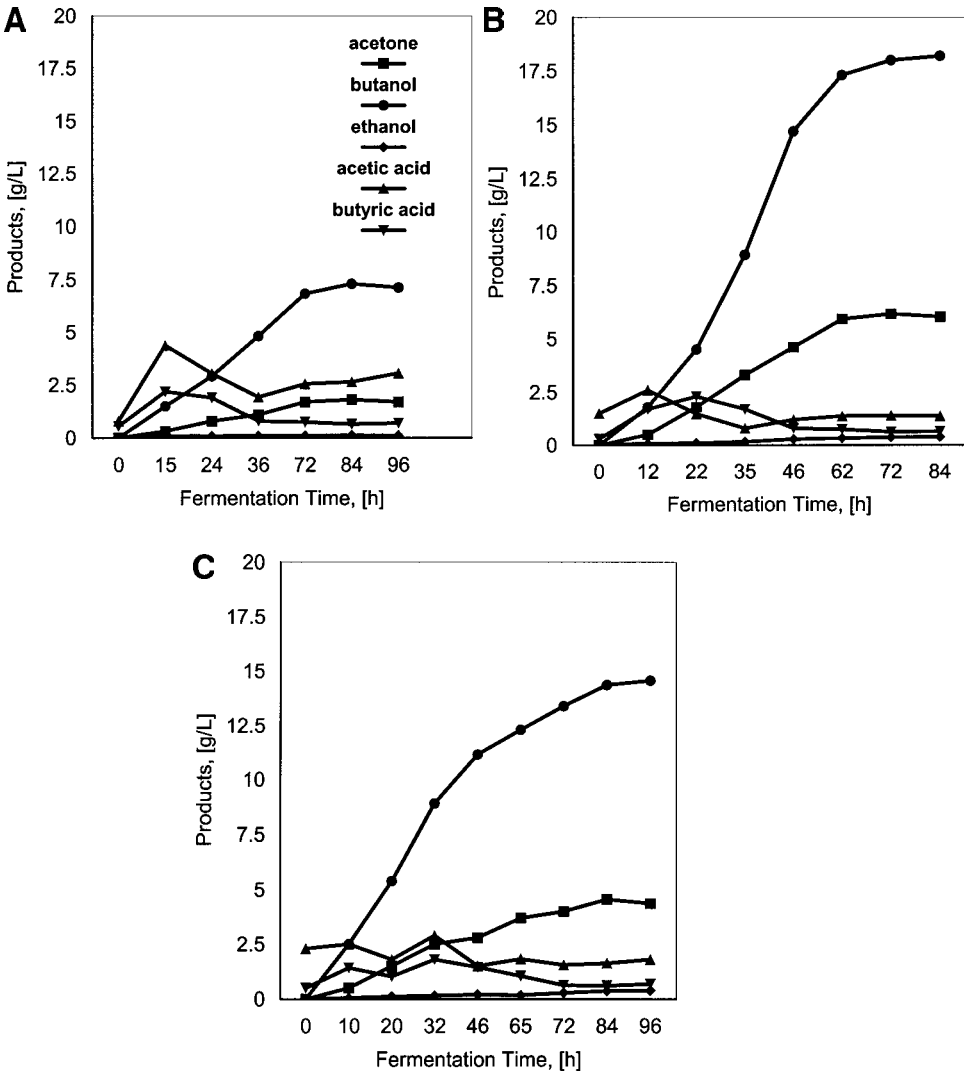

Fig. 1. Production of butanol (ABE) by *C. beijerinckii* BA101 from degermed corn: (**A**) no nutrient supplementation; (**B**) supplemented with P2 medium nutrients (symbols as in A); (**C**) supplemented with CSL (symbols as in A).

27.5–33 g/L of total ABE *(10,12)*. At the end of fermentation, 24.80 g/L of ABE and 2.03 g/L of acids were produced. (Fig. 1B, Table 1). It is anticipated that the cell concentration was approx 3 g/L (not measured). This suggested that previous fermentation was deficient in nutrients and that the fermentation of degermed corn indeed requires nutrient supplementation. Nutrient deficiency may lower butanol production owing to reduced cell growth or metabolic changes or both. The 72-h fermentation utilized 59 g/L of starch and resulted in an ABE yield and a productivity of 0.42 and 0.34 g/(L·h) respectively.

Table 1
Fermentation Products and Kinetic Parameters of Butanol Production from Degermed Corn Using *C. beijerinckii* BA101

Fermentation product and parameter	No nutrient supplementation	P2 medium	CSL
Acetone (g/L)	1.70	6.17	4.56
Butanol (g/L)	7.12	18.30	14.35
Ethanol (g/L)	0.11	0.37	0.37
Acetic acid (g/L)	3.05	1.40	1.63
Butyric acid (g/L)	0.70	0.63	0.61
Total ABE (g/L)	8.93	24.80	19.28
Total acids (g/L)	3.75	2.03	2.24
Initial starch (g/L)	56.30	61.10	— [a]
Final starch (g/L)	23.50	21.10	— [a]
Productivity (g/L·h)	0.11	0.34	0.23
ABE yield (–)	0.38	0.42	— [a]
Fermentation time (h)	84.00	72.00	84.00

[a] Not measured.

Note that the *C. beijerinckii* BA101 culture produces both acetic and butyric acids, which are reaction intermediates to acetone and butanol production, respectively. During the early stages of fermentation, only acids are produced followed by their reassimilation to produce acetone and butanol. Acids are also produced during the solvent-producing phase—however, at lower rates and concentrations. The butanol-producing cultures are known for their oscillating/fluctuating behaviors for acid production. For this reason, no particular trend is observed for acid production.

An experiment was carried out using various concentrations of degermed corn starch ranging from 30 to 60 g/L in P2 medium (Fig. 2A). At a starch concentration of 30 g/L, *C. beijerinckii* BA101 produced 8.13 g/L of total ABE and 5.15 g/L of total acids. ABE yield was low at 0.33 owing to production of a significant amount of acids. The amount of solvents produced at 40, 50, 55, and 60 g/L of starch content was 10.39, 16.70, 24.20, and 25.30 g/L, respectively. At lower starch concentrations, more acids were produced. Interestingly, the ratio of acids to solvents and butyric acid to acetic acid linearly decreased up to a starch concentration of 55 g/L (Fig. 2B). Above a starch concentration of 55 g/L, it remained constant. Previously, it has been reported that at low sugar concentration, acids are produced rather than solvents *(13)*. This experiment confirms that butanol fermentation at a degermed corn starch concentration of <55 g/L produced more acids and fewer solvents. Additionally, at a higher starch concentration, more butanol was produced, suggesting that butyric acid utilization was efficient at that level of starch.

Since CSL is a potentially economical nutrient source derived from corn, CSL was supplemented to the degermed corn fermentation medium

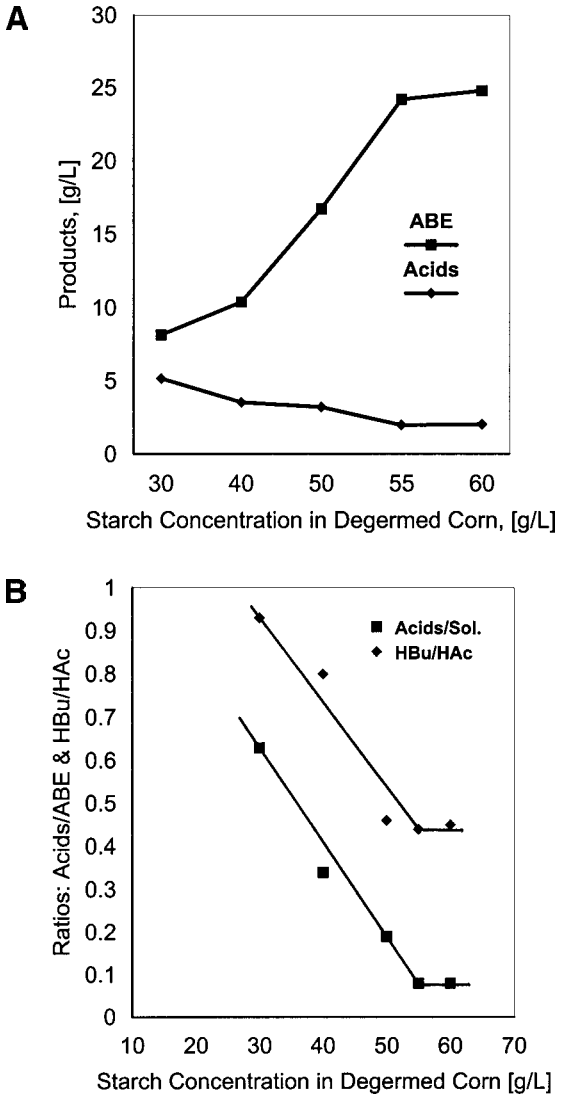

Fig. 2. Production of butanol by *C. beijerinckii* BA101 at various concentrations of degermed corn starch: (**A**) ABE and acids; (**B**) ratios of acids to solvents and butyric acid to acetic acid.

to evaluate butanol fermentation. The concentrations of degermed corn starch and CSL used were 56.7 and 50 g/L, respectively. The CSL was diluted from 500 g/L (dry wt) of solution. Following sterilization the medium became thick. Although the medium was viscous, the fermentation was rapid and produced 19.28 g/L of total ABE in 84 h (Fig. 1C, Table 1). The fermentation medium became dark brown, and it was difficult to measure starch concentration because of the color interference; hence, we were unable to calculate starch utilization and yield.

CSL is a byproduct of the corn wet-milling process. CSL is considered to be a no-cost or low-cost nutrient source compared with nutrients in P2 medium. From the perspective of fermentation, the use of CSL would result in the economic production of butanol. Recycling of CSL not only replaces expensive nutrient supplements such as yeast extract and vitamins, but also helps save significantly on energy (since CSL has to be concentrated to 50% solids for animal feed) and transportation costs. Currently, CSL is used as animal feed and is transported to the southern states of the United States from the Midwest. Although its use as a nutrient source has advantages, it is likely that during butanol recovery, fermentation broth based on degermed corn and CSL may reduce the efficiency of butanol recovery by traditional distillation. At this stage, we are not aware of the effects of CSL on distillative recovery. However, it has been documented that removal of a significant percentage of the germ (oil) from the mash results in significantly less fouling of heated surfaces *(6)*. In addition, butanol recovery experiments using pervaporation membrane suggested that CSL does not foul the membrane *(14)*.

The data on the economic study mentioned in the Introduction *(2)* are based on the ABE titer value of 26.5 g/L at the end of fermentation. During our fermentation experiment with degermed corn and CSL, the total concentration of ABE was 19.28 g/L. From the point of view of degermed corn fermentation, these results are encouraging, and it is likely that the concentration of ABE can be increased by further optimizing the nutrient requirement of the culture in combination with CSL. At this stage, it is anticipated that 26.5 g/L of total ABE can be produced in a degermed corn–optimized fermentation. With the anticipated ABE concentration of 26.5 g/L, derived from degermed corn fermentation, a byproduct credit of $\$8 \times 10^6$ would become a reality. This is based on a plant capacity of 121.6×10^6 kg/yr of butanol.

In conclusion, degermed corn (boiled and nutrient supplemented) can be used to produce butanol. P2 medium is a good source of nutrients for this fermentation using *C. beijerinckii* BA101. Fermentation of degermed corn (61.1 g/L of starch) supplemented with P2 medium stock solutions resulted in the production of 24.80 g/L of ABE with an ABE yield of 0.42. Fermentation with CSL resulted in the production of 19.28 g/L of ABE, which requires further optimization. At this stage, it appears that the use of degermed corn and CSL has great potential for bioconversion of corn to butanol.

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