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ORIGINAL PAPER

Production of acetone butanol (AB) from liquefied corn starch, a commercial substrate, using *Clostridium beijerinckii* coupled with product recovery by gas stripping

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Abstract A potential industrial substrate (liquefied corn starch; LCS) has been employed for successful acetone butanol ethanol (ABE) production. Fermentation of LCS (60 g l^{-1}) in a batch process resulted in the production of 18.4 g l^{-1} ABE, comparable to glucose: yeast extract based medium (control experiment, 18.6 g l^{-1} ABE). A batch fermentation of LCS integrated with product recovery resulted in 92% utilization of sugars present in the feed. When ABE was recovered by gas stripping (to relieve inhibition) from the fed-batch reactor fed with saccharified liquefied cornstarch (SLCS), 81.3 g l^{-1} ABE was produced compared to 18.6 g l^{-1} (control). In this integrated system, 225.8 g l^{-1} SLCS sugar (487 % of control) was consumed. In the absence of product removal, it is not possible for *C. beijerinckii* BA101 to utilize more than 46 g l^{-1} glucose. A combination of fermentation of this novel substrate (LCS) to butanol together with product recovery by gas stripping may economically benefit this fermentation.

Keywords ABE fermentation · Liquefied cornstarch (LCS) · Saccharified liquefied cornstarch (SLCS) · *Clostridium beijerinckii* BA101 · Gas stripping

Introduction

Butanol is an important chemical with many applications in the plastic industry and as a potential fuel extender. This chemical has many characteristics, which make it a better fuel extender than ethanol (presently used to boost octane number in gasoline). Butanol also has good potential to be used as an extractant in the food and flavor industry. Fermentation derived butanol is preferred over petrochemically obtained butanol because of the potential risk of carcinogen carryover [8]. Butanol production via clostridial fermentation has been widely studied. Before the 1950s, the clostridial AB [acetone butanol or acetone butanol ethanol (ABE)] fermentation ranked second only to the ethanol fermentation in its importance and scale of production, but declined due to increasing substrate costs and availability of much cheaper petrochemically derived butanol [4]. Additionally, the AB fermentation suffers from severe limitations such as low product yields, low productivity, low final concentrations of products in the fermentation broth due to butanol toxicity, and high energy requirements to recover AB/ABE from the fermentation broth [16].

Over the years, AB fermentation limitations made the production of fermentation-derived butanol less attractive when compared to the production of petroleum-based butanol. To solve the problem of butanol toxicity as it impacts the culture, in situ/or inline product removal using a number of alternative techniques have been proposed [13]. Among these techniques (pervaporation, gas stripping, reverse osmosis, and liquid–liquid extraction, etc.), gas

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stripping has been found to be particularly promising [6, 7]. This technique allows for the selective removal of volatiles from the fermentation broth and uses no membranes. With the application of this technology, the use of concentrated sugar solution/s and a reduction in butanol toxicity has been possible [6, 7]. In addition complete sugar utilization and improved reactor productivities have been achieved in the integrated fermentations where production and recovery are combined.

Clostridium beijerinckii BA101 is able to ferment a wide variety of substrates [11]. A reduction in substrate and nutrient costs is of particular interest because it directly affects the process economics [14, 17]. Development of a cost effective biomass-to-butanol process could be made more economical if cheaper commercial substrates such as liquefied corn starch (LCS) in combination with product removal by gas stripping are used. However, we are not ignorant of the fact that development of LCS market for butanol production may result in increased LCS costs which may lead to increase in the production cost of fermentation-derived butanol. The reader is advised that LCS is a viscous substrate, which may adversely impact AB recovery by gas stripping. Hence, the objective of this study was to examine the use of LCS as an inexpensive novel substrate for production of AB in combination with product recovery by gas stripping. These studies are considered novel as LCS (a product of corn processing industry) has not been previously used as a substrate for the ABE fermentation.

Materials and methods

Microorganism

A stock culture of *C. beijerinckii* BA101 was maintained as a spore suspension in distilled water at 4°C. Spores (0.3 ml) were heat shocked at 80°C for 10 min, and were grown anaerobically in cooked meat medium (CCM) (Difco Laboratories, Detroit, MI, USA) containing 30 g l^{-1} glucose at 36°C. This was followed by transferring 5 ml of actively growing culture (16–18h) to 100 ml of tryptone–glucose–yeast extract (TGY) medium. Vegetative cells were grown anaerobically for 3–6 h at 36°C before they were transferred into solvent production medium containing one of the carbon sources [glucose or LCS or saccharified liquefied corn starch (SLCS)].

Substrates and nutrient sources

LCS [12 DE (dextrose equivalent)] and corn steep liquor (CSL) were obtained from the Archer Daniels Midlands (ADM) Company located in Decatur, IL, USA. DE is expressed as follows: [Amount of reducing sugars expressed as glucose (g)/Total carbohydrate (g)] × 100.

LCS is composed of 35–40% dry solids as reducing sugars and dextrin. The concentrations of glucose, reducing sugars, and sodium metabisulfite ($Na_2S_2O_5$) in LCS were 5.58 ± 0.26, 337.5 ± 2.12, and 0.71 ± 0.10 g l^{-1}, respectively. The concentrations of these chemicals in CSL were 0.31 ± 0.04, 5.03 ± 0.22, and 1.31 ± 0.15 g l^{-1}, respectively. During liquefaction, usually carried out at temperatures above 100°C, the starch molecule is hydrolyzed by thermostable α-amylase enzyme to produce predominantly maltose and oligosaccharides. The breakdown of starch drastically reduces the viscosity of the gelatinized starch solution.

Saccharification of liquefied cornstarch

For rapid saccharification, the pH of LCS [1 l in a 2-l bioreactor (New Brunswick Scientific Co., New Brunswick, NJ, USA)] was adjusted to pH 4.5 with 1 M HCl, followed by raising the temperature of the bioreactor to 45°C. The reaction mixture was agitated at 200 rpm. One milliliter of glucoamylase (400 U ml^{-1}) (Archer Daniels Midland Co., Decatur, IL, USA) was added and saccharification of the LCS was completed in 5–6 h. This substrate was called saccharified liquefied cornstarch (SLCS). The SLCS contained glucose 460 ± 3 g l^{-1} and $Na_2S_2O_5$ 0.70 ± 0.11 g l^{-1}.

Preparation of corn steep liquor

To one liter raw CSL (pH 3.9), 0.6% cysteine–HCl was added followed by adjustment of pH to 6.8 with NaOH. This was stored at 4°C overnight, and centrifuged the following day at 27,500*g* for 20 min at 4°C. The clear supernatant obtained following centrifugation was filter sterilized using 0.45 μm sterile filters and stored at 4°C prior to use.

Medium preparation

LCS or SLCS (60 g l^{-1}) was sterilized at 121°C for 15 min. On cooling to 35°C under oxygen-free nitrogen atmosphere, 56 ml of CSL, and 10 ml filter-sterilized P2 buffer (KH_2PO_4, 50 g l^{-1}; K_2HPO_4, 50 g l^{-1}; ammonium acetate, 220 g l^{-1}) solutions were added to 934 ml (total volume 1 l) LCS or SLCS solution. To 1 l medium, filter sterilized $FeSO_4 \cdot 7H_2O$ solution was also added to give a final concentration of 12 mg l^{-1}. The medium (950 ml) was then inoculated with 50 ml (total volume 1 l) actively growing cells of *C. beijerinckii* BA101. For controls (Run I and Run IV) glucose-based P2 medium was used [7, 11, 15]. For Run II–III and V–VII CSL (56 ml l^{-1}) was used

as nutrient source [9] and LCS or SLCS were used as substrates.

Batch fermentation without product recovery

Batch fermentation studies (Run I–III) were conducted in 150 ml screw-capped bottles containing 100 ml medium. The bottles were inoculated with a 5 ml TGY-grown active culture, and incubated at 35°C for 120 h inside an anaerobic chamber. Samples were collected at various intervals for analysis. It is authors' experience that the levels of AB achieved in bottles were within ±5% of that achieved in 1–2 l bioreactors. Hence, Runs I–III was performed in bottles.

Batch/fed-batch fermentation with product recovery

Batch process

A 2-l bioreactor (New Brunswick Scientific Co., New Brunswick, NJ, USA) with 1 l reaction volume was used throughout these studies (Run IV–VI). In these experiments, the temperature was maintained at 35°C in the absence of agitation or pH control. The bioreactor containing medium was autoclaved at 121°C for 15 min. On cooling to 35°C under oxygen free nitrogen atmosphere, CSL and buffer solutions were added to the specified level. After inoculation, oxygen-free nitrogen gas was swept over the surface of the medium until the culture started producing its own gases (CO_2 and H_2). Static fermentation was allowed to proceed for 18–24 h when the ABE concentration approached approximately 3–4 g l^{-1}, after which gas-stripping was applied according to the method previously described [7].

Fed-batch process

Fed-batch fermentation with 60–70 g l^{-1} initial SLCS was initiated as a batch process. Samples were aseptically withdrawn at various intervals for sugar and ABE analysis. Based on the sugar and ABE analysis results, concentrated SLCS solution (containing 460 g l^{-1} sugar), P2 buffer solution, and CSL were added to the reactor at intervals, to replace the utilized SLCS and nutrients. Antifoam 204 (Sigma Chemicals, St. Louis, MO, USA) was added manually as required for foam control. The antifoam was diluted five times and autoclaved at 121°C before use. Each time foam developed, approximately 0.2 ml antifoam solution was added to collapse the foam. The feed medium was kept anaerobic by sweeping O_2-free N_2 gas across the medium surface. During fermentation, the pH was not controlled as the culture controlled its own pH during solventogenesis. Schematic diagrams of ABE recovery by gas stripping in batch and fed-batch fermentations have been published elsewhere [7].

Analytical procedures

Cell concentration [g cell dry weight (CDW) per l fermentation broth] was estimated by optical density method using a predetermined correlation between optical density at 540 nm and CDW. $Na_2S_2O_5$ concentration was determined using the Joint Expert Committee on Food Additives method [12]. ABE and acids (acetic and butyric) were measured using a 6890 Hewlett-Packard gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector (FID) and 6 ft × 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). Yield was defined as total grams of ABE produced per total grams of glucose utilized. Fermentation time was defined as the time period when a maximum concentration of ABE was achieved or when the fermentation stopped whichever was shorter. The reducing sugars in the LCS and CSL were measured using the 3, 5-dinitrosalicylic acid (DNSA) method [2]. Glucose concentration was determined using a hexokinase and glucose-6-phosphate dehydrogenase (Sigma, St. Louis, MO, USA) coupled enzymatic assay [5].

Results and discussion

In order to compare performance of the culture with LCS and SLCS as substrates, a control batch fermentation (Run I) was carried out using glucose as the carbon source. The fermentation was started with 59.7 g l^{-1} initial glucose (Table 1). The fermentation lasted for 68 h and during that time 18.6 g l^{-1} ABE [acetone (A), 4.4; butanol (B), 13.7; and ethanol (E), 0.5 g l^{-1}) was produced thus resulting in a productivity of 0.27 g l^{-1} h^{-1}. At the end of fermentation, 13.3 g l^{-1} residual glucose was measured. The culture could not utilize all the glucose present in the system due to ABE toxicity, in particular toxicity due to butanol [7].

Batch fermentation was also run with LCS (60.1 g l^{-1} reducing sugars) as the substrate. It was important to run this fermentation for two reasons: (1) LCS is an industrially important and economic substrate and hence should be used in value-added fermentations such as bio-butanol production, and (2) it contained $Na_2S_2O_5$. The latter is used as a preservative or bactericidal agent. It is likely that $Na_2S_2O_5$ may inhibit growth of *C. beijerinckii* BA101. Hence, experiments were conducted to study the effect of $Na_2S_2O_5$ on *C. beijerinckii* BA101 growth and AB production. The results suggested that $Na_2S_2O_5$ begins to inhibit *C. beijerinckii* BA101 growth and AB production at a concentration as low as 0.20 g l^{-1} (Fig. 1). At a concentration of 0.6 g l^{-1} *C. beijerinckii* BA101 stops growing and producing AB.

Table 1 ABE production in batch and fed-batch reactors from LCS and SLCS using *C. beijerinckii* BA101

Parameters	Without product recovery			With product recovery by stripping			
	Control[b] (Run I)	LCS (Run II)	SLCS (Run III)	Control[b] (Run IV)	LCS[a] (Run V)	SLCS	
						BF–GS (Run VI)	FBF–GS[a] (Run VII)
Cell concentration (g l^{-1})	3.41	–	3.05	4.50	–	4.10	5.20
Acetone (g l^{-1})	4.4	4.3	4.0	7.2	7.7	8.3	24.1
Butanol (g l^{-1})	13.7	13.4	13.4	16.8	15.1	17.6	56.2
Ethanol (g l^{-1})	0.5	0.7	0.8	0.5	1.1	0.6	1.0
Total ABE (g l^{-1})	18.6	18.4	18.2	24.5(14.7)[c]	23.9 (15.1)[d]	26.5 (17.3)[e]	81.3 (76.8)[f]
Initial glucose (g l^{-1})	59.7	60.1[g]	59.7	60.5	59.8[g]	64.3	70.9
Residual glucose (g l^{-1})	13.3	15.2[g]	14.0	0.0	4.7[g]	0.0	40.6
Glucose used (g l^{-1})	46.4	44.9[g]	45.7	60.5	55.1[g]	64.3	225.8
Fermentation time (h)	68	120	78	42	78	67	137
ABE productivity (g l^{-1} h^{-1})	0.27	0.15	0.23	0.58	0.31	0.40	0.59
ABE yield (g g^{-1})	0.4	0.41	0.40	0.40	0.43	0.41	0.36

The data represent the average of duplicate determinations

– Not measured, *LCS* liquefied cornstarch, *SLCS* saccharified liquefied cornstarch

[a] At 48 h nutrient solutions were added to the bioreactor (28 ml CSL, 12 mg l^{-1} $FeSO_4 \cdot 7H_2O$ and 5 ml P2 buffer)

[b] Glucose used as a substrate

BF–GS Batch fermentation integrated with product recovery by gas stripping

FBF–GS fed-batch fermentation integrated with product recovery by gas stripping

Values in brackets are ABE amounts recovered in the condensate and expressed in grams

[c, d, e, f] Represent amount of condensate removed: 182, 265, 250, and 754 ml, respectively

[g] Reducing sugars

Concentrated LCS contained $Na_2S_2O_5$ at a concentration of 0.71 ± 0.10 g l^{-1}. Prior to batch fermentation, LCS was diluted seven times which resulted in a $Na_2S_2O_5$ concentration of 0.10 ± 0.01 g l^{-1} and provided adequate reducing sugar concentration (60 g l^{-1}) for AB batch fermentation. Supplementation with diluted CSL resulted in an additional 0.08 ± 0.01 g l^{-1} of $Na_2S_2O_5$. Therefore the total concentration of $Na_2S_2O_5$ in the fermentation medium was in the range of 0.20 g l^{-1}. For this reason the fermentation lasted for 120 h (Run II) as opposed to 68 h (Run I) as shown in Table 1. The culture produced 18.4 g l^{-1} ABE (A, 4.3; B, 13.4; and E, 0.7 g l^{-1}). The longer fermentation time resulted in a lower productivity of 0.15 g l^{-1} h^{-1} (control, Run I, 0.27 g l^{-1} h^{-1}). The possible reasons for the low productivity include: (1) slow secretion of amylolytic enzymes thereby resulting in poor hydrolysis of LCS and consequently resulting in a longer ABE fermentation time; and (2) cell growth inhibition caused by $Na_2S_2O_5$ at a level approaching 0.20 g l^{-1}. *C. beijerinckii* BA101 is a hyper-amylolytic strain [1] which secrets the required amylolytic enzymes necessary for efficient starch and oligosaccharides hydrolysis. In actual practice this fermentation took longer than the usual batch fermentation (68–72 h). After the fermentation was complete, 15.2 g l^{-1} residual sugars were measured in the broth and an ABE yield of 0.41 g g^{-1} was calculated.

In order to study as to why the fermentation time in the above experiment (Run II) was longer than the control (Run I), the LCS was saccharified prior to fermentation. Hence, the SLCS was used as the fermentation substrate for Run III. The initial sugar was 59.7 g l^{-1} and the residual sugar was 14.0 g l^{-1}. It was observed that the fermentation time was reduced to 78 h as compared to 120 h in Run II. This suggested that the presence of $Na_2S_2O_5$ reduced the rate of secretion of amylolytic enzymes in *C. beijerinckii* BA101 as the sugar and CSL concentrations in run III were in the same range as Run II and also contained same concentration of $Na_2S_2O_5$ in the range of 0.20 g l^{-1}. The longer fermentation time by 10 h (78 vs. 68 h in the control Run I) may have been as a result of growth inhibition caused by $Na_2S_2O_5$ at a level of 0.20 g l^{-1}. During this fermentation, 18.2 g l^{-1} ABE (A, 4.0; B, 13.4; and E, 0.8 g l^{-1}) was produced with a productivity of 0.23 g l^{-1} h^{-1}. In this run, ABE yield of 0.40 g g^{-1} was achieved.

Glucose-based ABE fermentations, where product recovery techniques are applied, result in higher productivities and complete sugar utilization as shown in Run IV (control experiment with product recovery). The reader is advised that LCS and SLCS were combined to produce fermentation derived butanol for the first time, and hence Runs I-III were considered to be essential for comparison pur-

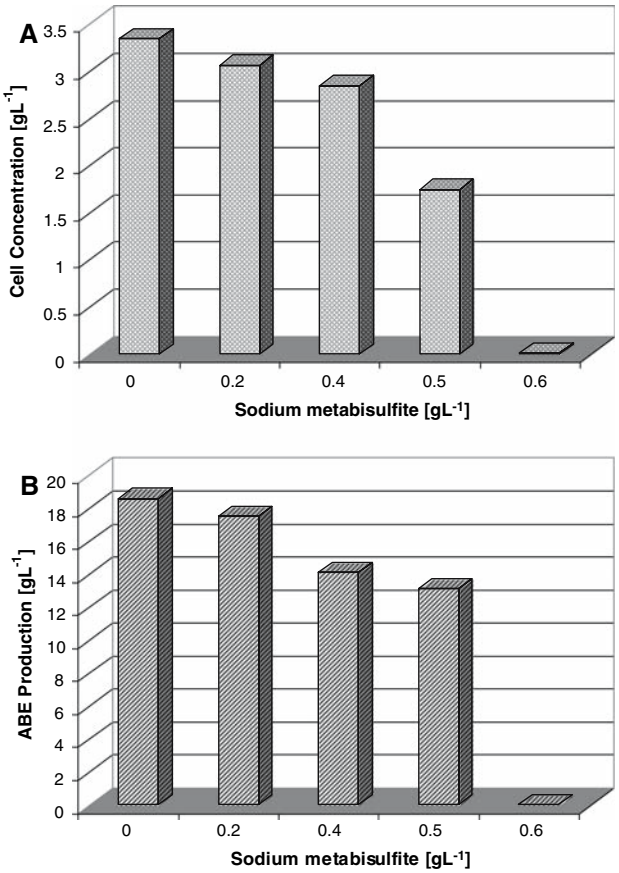

Fig. 1 Production of AB/ABE from glucose in presence of sodium metabisulfite ($Na_2S_2O_5$) using *C. beijerinckii* BA101. **a** Cell growth of *C. beijerinckii* BA101, **b** production of total ABE in presence of $Na_2S_2O_5$. The data represent an average of duplicate determinations

poses. To compare performance of product removal by gas stripping, batch fermentation with gas stripping was run (Run IV). In this run, to allow for cell growth, the fermentation (Run IV) was allowed to proceed for 18 h, at which time the total ABE concentration was 4.7 g l^{-1}. At that stage ABE recovery by gas stripping was initiated and the fermentation was allowed to proceed for 42 h at which time the glucose concentration was reduced to 0 g l^{-1}. In this run (IV), a productivity of 0.58 g l^{-1} h^{-1} was achieved which is 215% of that obtained in the control experiment without product recovery (Run I). In order to increase productivities and achieve more sugar utilization, batch fermentations of LCS (Run V) and SLCS (Run VI) were conducted with product recovery. As above, cell growth and fermentation were allowed to proceed for 18 h, at which time the total ABE concentrations in the two runs (Run V and VI) with LCS and SLCS as substrates were 2.6, and 3.1 g l^{-1}, respectively. At that stage, ABE recovery by gas stripping was initiated. Following 48 h of fermentation, nutrient solutions [28 ml CSL, 12 mg l^{-1} $FeSO_4{\cdot}7H_2O$ and 5 ml P2 buffer] were added to the LCS and SLCS fermentations. The two fermentations were allowed to proceed for 78 and 67 h at which time the sugar concentrations were reduced to 4.7 (reducing sugars) and 0 g l^{-1}. In Run V a sugar utilization of 92% of that available in feed (59.8 g l^{-1}) was achieved. The residual sugars (in Run V) were reducing sugars containing oligosaccharides and limit dextrins that culture could not hydrolyze under the present conditions (pH, temperature, and presence of $Na_2S_2O_5$). As a result of product recovery, LCS fermentation time was reduced from 120 to 78 h. In this fermentation a productivity of 0.31 g l^{-1} h^{-1} was achieved. The Run VI (SLCS), also called BF–GS (batch fermentation: gas stripping), resulted in complete utilization of sugar (64.3 g l^{-1}), and an improved productivity of 0.40 g l^{-1} h^{-1}. Thus productivities were increased by 14.8 and 48.1% for LCS and SLCS, respectively (compared with the non-integrated control, Table 1). In batch fermentations with product recovery (Run IV–VI) more ABE was produced than in batch experiments (Run I–III) without product recovery. The total amount of ABE produced by *C. beijerinckii* BA101 when using LCS (Run V) and SLCS (Run VI) were 23.9 and 26.5 g l^{-1}. During simultaneous ABE fermentation and product recovery by gas stripping the ABE concentrations in the condensate ranged from 38.3–99.6 g l^{-1}. The total amount of condensed ABE recovered during fermentation of LCS and SLCS were 15.1 and 17.3 g, respectively.

In order to be able to use concentrated SLCS and improve the economics of ABE fermentation, a fed-batch fermentation employing gas stripping (FBF–GS) experiment (Run VII) was carried out. In this fermentation 24.1 g l^{-1} acetone, 56.2 g l^{-1} butanol and 0.1 g l^{-1} ethanol was produced thus totaling to 81.3 g l^{-1} ABE (Table 1). During the experiment an initial concentration of sugar was 70.9 g l^{-1} (Fig. 2a). When sugar level was below 25 g l^{-1}, additional sugar (as solution) was added to the reactor. SLCS (460 g l^{-1} sugar) was added to the reactor at 43, 52, 78, 104 and 125 h while nutrients (CSL, P2 buffer, and $FeSO_4.7H_2O$) [each time: equal to half of that added to the fresh medium] were added at 43, 56, 80, 104, and 120 h (Fig. 2a). The addition of substrate and nutrient solutions (mainly CSL) depended upon the residual sugar and cell concentration in the bioreactor. Addition of CSL to the culture broth provided nutrients for cell proliferation/maintenance and fermentation. CSL was added when fermentation appeared to be weak. The maximum concentration of sugar in the bioreactor did not exceed 73 g l^{-1}. Figure 2b shows the concentration of ABE present in the bioreactor during fermentation and recovery by gas stripping. The maximum amount of butanol present in the reactor was 5.5 g l^{-1}, which is within the tolerance level of the culture. In this fed batch product-recovery system, the maximum cell concentration reached, maximum ABE produced, and overall yield were 5.2 g l^{-1}, 81.3 g l^{-1}, and 0.36 g/g, respectively, as

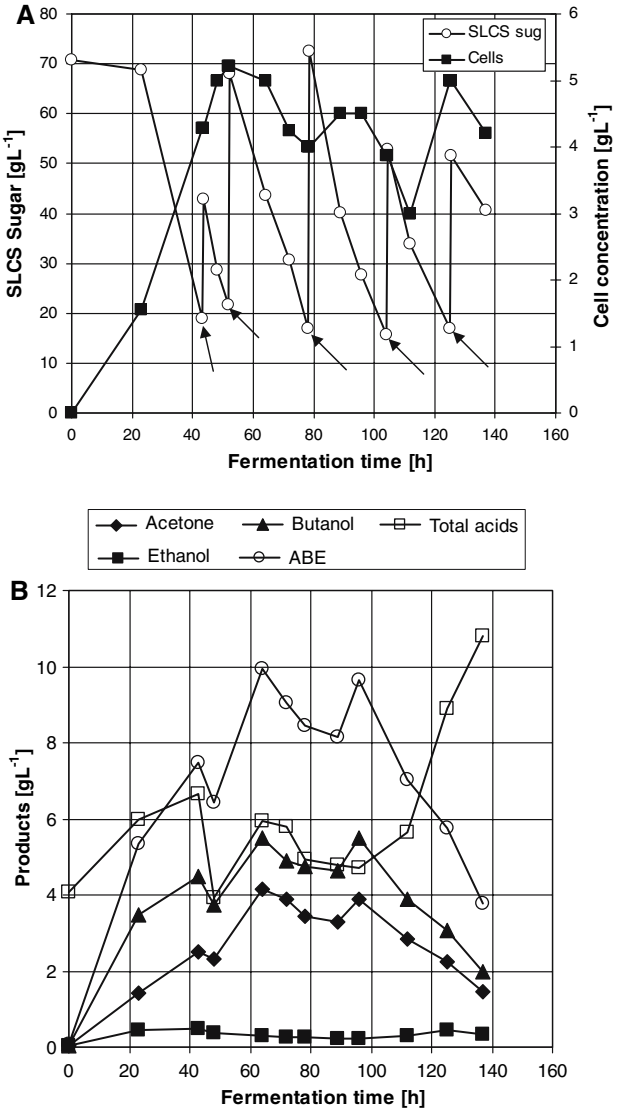

Fig. 2 Production of AB/ABE from SLCS in a fed-batch reactor of *C. beijerinckii* BA101 coupled with product recovery by gas stripping. **a** SLCS sugar and cell concentrations at various fermentation times; *arrows* show the points where additional sugar and nutrient solutions were added to the reactor. **b** ABE and acids at various fermentation times. The data represent an average of duplicate determinations. The ABE concentration in the condensate ranged from 38 to 100 g l^{-1}

shown in Table 1. Condensate was removed from the receiver flask at 26, 36, 60, 82, 100, 118 and 137 h. The amounts of condensates were 57, 80, 166, 103, 130, 106 and 112 ml, respectively.

In the FBF–GS system 225.8 g l^{-1} sugar was used before the fermentation completely turned to acidogenic as opposed to 46.4 g l^{-1} in Run I which is 487% of the control. It should be noted that this amount of sugar is less than half of that utilized in the previous fed-batch fermentation product recovery system [6]. Based on the amount of feed and CSL used in the system, an accumulation of 0.79 g l^{-1} of $Na_2S_2O_5$ in the reactor was calculated. Approximately 0.47 g l^{-1} $Na_2S_2O_5$ was fed with the SLCS and 0.32 g l^{-1} was fed with CSL. At a concentration of 0.60 g l^{-1} $Na_2S_2O_5$, *C. beijerinckii* BA101 stops growing and producing ABE (Fig. 1). Hence, it is clear that the butanol production stopped and the culture became acidogenic due to accumulation of 0.79 g l^{-1} $Na_2S_2O_5$. This concentration is above the maximum tolerable limit for *C. beijerinckii* BA101. In the fed-batch system a productivity of 0.59 g l^{-1} h^{-1} was achieved. The ABE productivity was improved by 119% of the control for FBF–GS experiment. The fermentation stopped after 137 h of fermentation (Fig. 2). After 56 h of fermentation, excessive foaming in the bioreactor became more frequent and hence antifoam was added. After 96 h of fermentation, the culture started experiencing difficulties in switching from acidogenesis to solventogenesis resulting in a sharp increase in total acids production after 112 h of fermentation (Fig. 2b). At the end of fermentation 10.8 g l^{-1} acids were measured. It should be noted that in LCS fermentation, rate of ABE production was lower than in SLCS fermentation. Also, the rate of removal of ABE in LCS fermentation was lower than in SLCS fermentation due to viscous nature of LCS (discussed later).

In Run VII the culture produced more than 400% ABE as compared to the control, when integrated with product recovery by gas stripping. Without product recovery, utilization of more than 46 g l^{-1} glucose and production of more than 18.6 g l^{-1} ABE were not possible. It is viewed that utilization of such a substrate and nutrient source in combination with product removal by gas stripping would bring this fermentation closer to commercialization. In this investigation, calculation of ABE selectivities was not considered important as they have been reported in our previous work [6, 7]. The rates of removal of ABE from the fermentation broth of control (Run IV) and SLCS (Run VII) were 0.61 g h^{-1} [Table 1; 14.7 g/(42–18) h] and 0.65 g h^{-1} [Table 1; 76.8 g/(137–18) h]. This comparison suggested that ABE removal efficiency was the same for the two fermentations and there was no negative effect due to the use of SLCS on the rate of recovery. However, the rate of removal of ABE from LCS (Run V) was only 0.25 g h^{-1} [Table 1; 15.1 g/(78–18) h], which is less than half of the control experiment. This suggested that removal of ABE from LCS was hampered due to the viscosity of the substrate. Hence, it is suggested that LCS be saccharified prior to fermentation and recovery.

Economic analysis demonstrated that the fermentation substrate is one of the most important factors that influenced the price of butanol [14, 17]. Since the cost of the fermentation substrate has the greatest influence on the price of butanol, we chose commercially feasible substrates such as LCS and SLCS. For the batch fermentations, optimum concentration of substrates of 60 g l^{-1} is normally used

because both substrate inhibition [7] and substrate limitation [3] negatively affect ABE production.

As shown in Table 1, incorporation of product recovery (Run IV–VII) resulted in an increased utilization of sugar, a decrease in the fermentation times and an increase in productivities due to elevated cell concentration in the bioreactor. As a result of product recovery cell growth inhibition was reduced thus resulting in cell proliferation. The results of the FBF–GS system are superior to the other 6 runs, demonstrating that use of SLCS for ABE fermentation is possible. As outlined in the introduction section of this article, the objective of these studies was to use LCS for ABE fermentation. We have demonstrated that LCS can be used for ABE fermentation in combination with product recovery. However, the rate of fermentation was lower than in glucose or SLCS. Also the rate of product removal was lower in LCS fermentation. Alternately SLCS fermentation and recovery was successful. It appears that the use of SLCS has great potential for bioconversion of corn to ABE. It is suggested that removal of $Na_2S_2O_5$ inhibition would make this fermentation more attractive. Bio-butanol research and commercialization, as recently announced by DuPont Chemicals and British Petroleum [10], is an encouraging step toward employing vast potential of this fermentation.

Conclusions

Batch fermentation of 60 g l^{-1} LCS resulted in the production of 18.4 g l^{-1} ABE, comparable to the glucose based semi-synthetic medium based fermentation (control run, 18.6 g l^{-1} ABE). A fed-batch reactor fed with SLCS resulted in the production of 81.3 g l^{-1} ABE (>400%). In this integrated system, 225.8 g l^{-1} SLCS sugar (487% of control) was consumed. In the absence of product removal, it was not possible for *C. beijerinckii* BA101 to utilize more than 46 g l^{-1} glucose. The use of LCS combined with gas stripping may be economically viable. It is recommended that $Na_2S_2O_5$ (in LCS or SLCS) be eliminated from the substrate prior to fermentation to take full advantage of this novel substrate.

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