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ORIGINAL PAPER

Microbial production of a biofuel (acetone–butanol–ethanol) in a continuous bioreactor: impact of bleed and simultaneous product removal

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Abstract Acetone butanol ethanol (ABE) was produced in an integrated continuous one-stage fermentation and gas stripping product recovery system using *Clostridium beijerinckii* BA101 and fermentation gases (CO_2 and H_2). In this system, the bioreactor was fed with a concentrated sugar solution (250–500 g L^{-1} glucose). The bioreactor was bled semi-continuously to avoid accumulation of inhibitory chemicals and products. The continuous system was operated for 504 h (21 days) after which the fermentation was intentionally terminated. The bioreactor produced 461.3 g ABE from 1,125.0 g total sugar in 1 L culture volume as compared to a control batch process in which 18.4 g ABE was produced from 47.3 g sugar. These results demonstrate that ABE fermentation can be operated in an integrated continuous one-stage fermentation and product recovery system for a long period of time, if butanol and other microbial metabolites in the bioreactor are kept below threshold of toxicity.

Keywords Butanol · *Clostridium beijerinckii* · Simultaneous product removal · Gas stripping · Acetone

Introduction

Production of liquid biofuels such as ethanol and butanol by fermentation has drawn worldwide attention for two important reasons: (1) energy security (producing biofuels from renewable agricultural residues at an economical price), and (2) climate change [1, 2]. Strategic development of the biofuel industry has great potential for benefiting nations with agriculturally based economies. Butanol, currently manufactured from petroleum feedstocks, is an important chemical with many applications in the production of solvents, plasticizers, butylamines, amino resins, butyl acetates, etc. [3]. Butanol is not as widely promoted as a biofuel as ethanol; however, butanol has a number of advantages over ethanol having a greater energy content and is a cleaner burning fuel [4]. Butanol is also highly toxic to the microorganisms involved in its production [5] and this reduces the productivity of classical butanol fermentation.

Economical production of bio-butanol via the acetone butanol ethanol or ABE fermentation can be improved by the application of "cutting edge" science and technology. The "cutting edge" fermentation technology includes developing more productive reactor systems, using concentrated sugar solutions, recovering product employing various energy efficient separation techniques, and process integration where fermentation and product recovery are

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combined. Integrated fermentation processes also enhance water and nutrients recycle and reduce process cost. Reactor productivity can be improved either by employing cell recycle techniques [6–9] or using cell immobilization methods [10, 11].

For simultaneous fermentation and product recovery from fermentation broth, a number of techniques can be used including adsorption, gas stripping, pervaporation, liquid–liquid extraction, perstraction, reverse osmosis, and other technologies [12]. All these techniques have advantages and disadvantages. Gas stripping, e.g., is a simple technique that requires no membrane and expensive chemicals, is free of emulsion formation, and can be performed in the bioreactor with or without an external stripper [13–16]. In addition, fermentation gases (CO_2 and H_2) can be used for product separation [17]. We have previously used gas stripping technology to significantly reduce butanol toxicity to the culture and improve productivities in batch and fed-batch reactor systems [5, 18, 19].

We demonstrated that production of butanol in an integrated fed-batch fermentation system coupled with gas stripping for product removal is more efficient than a batch fermentation product recovery system [5]. In this type of fed-batch reactor, 232.8 g L^{-1} ABE was produced from 500.1 g L^{-1} sugar as compared to a control batch reactor, where only 17.6 g L^{-1} ABE was produced (60 h batch fermentation). The fed-batch fermentation ceased after a period of 201 h due to the accumulation of salts, dead cells, and other unknown bioproducts. This type of reactor system would be advantageous for ABE fermentation if accumulation of toxic salts and bioproducts is prevented by application of a continuous/semi-continuous bleed to the reactor and removal of toxic ABE by gas stripping.

Because the integrated fed-batch fermentation product recovery system ceased operation at 201 h, there is a need to design a simple one-stage continuous system with bleed and simultaneous product recovery. Hence, the objective of the present research was to design and operate a simple one-stage continuous butanol production system with semi-continuous bleed to prevent salts and inhibitors accumulation in the reactor, thereby extending the functional life of the reactor.

Materials and methods

Microorganism, culture maintenance, and inocula development

The microorganism used in this study was *Clostridium beijerinckii* BA101. This strain was generated using *N*-methyl-*N*-nitrosoguanidine (NTG) together with selective enrichment on the non-metabolizable glucose analog 2-deoxyglucose [20]. The wild type strain, from which culture BA101 was developed, was *C. beijerinckii* NCIMB 8052 (ATCC 51743). *C. beijerinckii* BA101 spores were maintained in sterile distilled water at 4 °C. For *C. beijerinckii* BA101 spore activation, 200–300 μL of spores was heat shocked at 80 °C for 10 min and 100 μL of heat shocked spores was transferred into 25 mL anoxic pre-sterilized cooked meat medium (CMM; Difco™; Becton, Dickinson, and Company, Sparks, MD, USA) followed by anaerobic incubation at 35 °C for 14–16 h [5]. The culture with cell growth in CMM was used as the first-stage inoculum. Active inoculum development is a key factor for a successful ABE fermentation. Inocula for the reactor were developed in 125 mL screw capped bottles (100 mL working volume) containing Tryptone glucose yeast extract (TGY) medium (g L^{-1}: tryptone, 30; glucose, 20; yeast extract, 10; L-cysteine, 1). Glucose and L-cysteine were purchased from Sigma Chemicals (St. Louis, MO, USA) and tryptone and yeast extract were procured from Difco Laboratories (Detroit, MI, USA) The media in loosely capped bottles was autoclaved at 121 °C for 15 min and cooled to 37 °C. To create anaerobic conditions, loosely capped bottles with sterilized TGY medium were placed in the anaerobic chamber for 48 h to facilitate exchange of gases between the medium and the gases in the anaerobic chamber. This was followed by transferring 5 mL of first-stage inoculum into 95 mL of TGY medium in 125 mL bottles and subsequently incubated at 35 °C for 3–4 h until the *C. beijerinckii* BA101 culture reached an optical density of 0.8–1.0 at 540 nm λ. This was used as the second-stage inoculum.

Production of ABE in a batch reactor

To evaluate the performance of the continuous fermentation and recovery system, batch fermentations were performed in a 2 L glass bioreactor (New Brunswick Scientific Co., New Brunswick, NJ, USA) as previously described [5]. The bioreactor containing 1 L medium (glucose 60 g L^{-1} and yeast extract 1 g L^{-1}) was autoclaved at 121 °C for 15 min followed by cooling to 35 °C under an O_2 free N_2 atmosphere. Ten mL each of filter sterilized buffer, mineral, and vitamin solutions [5, 21] (P2 solution) was added followed by inoculation of the bioreactor with 50 mL of stage II inocula (cell broth). The bioreactor was maintained at 35 °C and pH 5.0 (adjusted with 4 M NaOH). To maintain anaerobic conditions in the bioreactor during the early stages of cell growth, O_2 free N_2 gas was used to flush the headspace of the bioreactor for 18 h during which the *C. beijerinckii* BA101 culture produced enough fermentation gases (CO_2 and H_2) to keep the bioreactor anaerobic. Two mL culture samples were taken at intervals to measure cell, glucose, and ABE concentrations.

The samples were centrifuged at 10,000 rpm for 2 min in a refrigerated microcentrifuge. Clear supernatant was transferred into a fresh tube and stored at −18 °C until analyzed for residual glucose and ABE concentrations. Results from this fermentation served as the control for comparison to the continuous process.

Continuous fermentation and ABE recovery by gas stripping

A 2 L bioreactor (New Brunswick Scientific Co.) containing 1 L medium (100 g L^{-1} glucose and 1 g L^{-1} yeast extract) was autoclaved at 121 °C for 15 min. Following cooling to 35 °C (under O_2 free N_2 gas), 10 mL of each P2 stock solutions [5, 21] were added. The bioreactor was inoculated with 50 mL of stage II inoculum and fermentation was initiated as previously described [5]. The fermentation proceeded for 20 h followed by ABE recovery by gas stripping using CO_2 and H_2 as carrier gasses. Before initiating ABE recovery, connecting lines and condenser environments were made anaerobic by flushing with an adequate amount of O_2 free N_2 gas. A twin head peristaltic Masterflex™ pump (Cole-Parmer, Vernon Hills, IL, USA) and 18 size norprene tubing were used to recycle CO_2 and H_2 gases (4,000–6,000 mL min^{-1}) through the sparger holes in the bioreactor. Gas bubbles produced near the bottom of the bioreactor during the recycling process became saturated or at least partially saturated with ABE vapors as migration towards the bioreactor headspace occurs. The gases were cooled in the glass condenser (62 × 600 mm, cooling coil external surface area 1,292 cm^2) at 1 °C and ABE was condensed. The stripped ABE was pumped into the solvent collector using a peristaltic pump. To cool the condenser, a cooling machine (GeneLine) with circulator was utilized (Beckman Instruments, Palo Alto, CA, USA). Cooling medium was 50 % (v/v) ethylene glycol and the circulation rate through the condenser was 600 mL min^{-1}. A schematic diagram of the process is shown in Fig. 1.

The bioreactor was fed continuously (average dilution rate 0.03 h^{-1}) with a sterile feed maintained under O_2 free N_2 gas and containing 250–500 g L^{-1} glucose solution supplemented with 1 g L^{-1} yeast extract. To keep the amount of liquid constant in the bioreactor, sterile anoxic water was added and the flow rate was varied with the rate of ABE removal from the reactor. The bioreactor was bled semi-continuously, as required, to remove undetermined inhibitors [5] and to keep the cell concentration in the reactor within 3–7 g L^{-1}. Feed, water, and cell broth were pumped using sterilized 13–14 size silicone tubing and peristaltic Masterflex™ pumps. Fermentation was routinely monitored by measuring the optical density of the growing *C. beijerinckii* BA101 cells and by visual

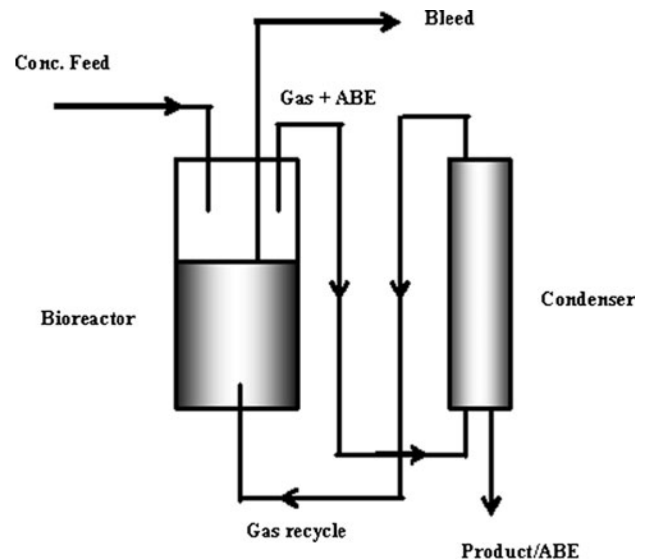

Fig. 1 A schematic diagram of continuous ABE production and simultaneous product removal using gas stripping

examination of gases (CO_2 and H_2) produced. When gas production was observed to be less than that produced during active stage of fermentation (such as during initial 24–36 h), P2 medium stock [5, 21] and yeast extract solutions were added at amounts presented in Table 1.

Analytical procedures

ABE and acid (acetic and butyric) concentrations were measured using a 6890 Hewlett-Packard gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame Ionization detector (FID) and 1,824 × 2 mm (6 ft × 2 mm) glass column (10 % CW–20 M, 0.01 % H_3PO_4, support 80/100 Chromosorb WAW) as described previously [2, 5]. ABE productivity, or rate of production, was calculated as total ABE produced (g L^{-1} culture volume) divided by fermentation time (h). Yield was defined as total grams of ABE produced per total grams of glucose utilized and is expressed as gg^{-1}. The rate of glucose utilization was defined as the grams of glucose utilized per liter culture volume for a given time interval. Selectivity (a measure of the preferential removal of ABE over other components present in the mixture such as water) was calculated as $\alpha = [y/(1-y)]/[x/(1-x)]$, where x and y are weight fractions of ABE in fermentation broth and condensate, respectively.

Glucose concentration was determined using a hexokinase and glucose-6-phosphate dehydrogenase (Sigma Chemicals, St. Louis, MO, USA) coupled enzymatic assay as described elsewhere [5]. Cell concentration was estimated by optical density and cell dry weight method using a predetermined correlation between optical density at

Table 1 Addition of P2 stock solutions and yeast extract to the bioreactor

Time (h) at which P2 stock solutions were added	Amount of added P2 stock solutions (mL)
48	2.0
223	2.5
258	5.3
270	2.5
293	5.0[a]
365	3.0
388	2.5
413	3.0
437	4.5

[a] In addition to P2 stock solution 10 ml of yeast extract solution containing 0.3 g yeast extract was added at 293 h

540 nm and cell dry weight [5, 18]. Dilution rate was defined as feed flow rate (mL h^{-1}) per liquid volume in the reactor (mL) and is expressed as h^{-1}. Usually, a continuous reactor is defined as a system where rate of feed flow into it and rate of product out flow are equal. In the present case, the bioreactor was fed continuously with a feed solution and the product was removed in a continuous manner using gas stripping where product was ABE solution. A bleed was also drawn in a semi-continuous manner to maintain liquid level constant (and undefined inhibitors level below toxicity). So this bioreactor had one feed stream and two product streams (ABE rich condensate stream and fermentation broth/bleed). Since the level inside the reactor was maintained constant, we called it a continuous system.

Results and discussion

To evaluate performance of the continuous fermentation coupled with ABE recovery system, batch fermentation in the absence of product recovery was conducted in a 2 L bioreactor. The batch fermentation produced 18.4 g L^{-1} total ABE in 65 h resulting in productivity and yield of 0.28 g L^{-1} h^{-1} and 0.39 gg^{-1}, respectively. At 0 h fermentation, the glucose concentration in the bioreactor was 60.4 g L^{-1} and the residual glucose concentration at the end of fermentation was 13.1 g L^{-1}. The culture was unable to use all the glucose for growth and ABE production due to butanol inhibition. We previously conducted batch [18] and fed-batch [5] fermentations with integrated ABE recovery by gas stripping which resulted in total glucose consumption of 161.7 and 500.1 g L^{-1}, respectively. A total of 75.9 and 232.8 g L^{-1} ABE were produced from these batch and fed-batch fermentations, respectively, before the fermentations ceased. Undetermined inhibitors and salts were found to be responsible for the termination of the fermentation [5].

To extend the fermentation time beyond the 201 h previously achieved using fed-batch fermentation and recovery by gas stripping [5], inhibitors, salts and ABE need to be removed concurrently from the bioreactor during fermentation. A batch ABE fermentation system was initiated with 100 g L^{-1} glucose in P2 medium. Following 20 h of fermentation, 1.49 g L^{-1} acetone, 2.71 g L^{-1} butanol, and 0.09 g L^{-1} ethanol were measured in the bioreactor, resulting in total ABE of 4.29 g L^{-1} (Fig. 2a). At this stage, continuous fermentation and product recovery by gas stripping were initiated. During the course of fermentation, fluctuations in the concentrations of acetone and butanol were observed (Fig. 2a). At 112, 150, 234, 270, 340, 437, and 504 h, greater concentrations of acetone and butanol were observed. Increased concentrations of acetone and butanol were accumulated due to increased fermentation activity which may be attributed to the accumulation of *C. beijerinckii* cells in the bioreactor. The greatest concentration of ABE (Fig. 2a; 12.2 g L^{-1}) in the bioreactor was obtained at 112 h. At 138, 199, 223, 305, 365, and 471 h fermentation, lower ABE concentrations were present in the bioreactor. At 223 h, the lowest ABE concentration (0.64 g L^{-1}) was measured in the bioreactor. Ethanol concentration was very low, ranging from 0.0 to 0.19 g L^{-1} throughout the fermentation.

The concentrations of acetic and butyric acids were also observed to fluctuate during the course of the fermentation (Fig. 2b). Acetic acid concentrations measured during the course of fermentation ranged from 0.26 to 6.12 g L^{-1} with maximum concentration (6.12 g L^{-1}) measured at 223 h. Butyric acid concentrations in the bioreactor varied from 0.10 to 1.69 g L^{-1}. When the fermentation was terminated after 504 h, acetic and butyric acid concentrations in the bioreactor were 1.64 and 0.53 g L^{-1}, respectively. The relatively final low butyric and acetic acid concentrations suggest that they were constantly converted to acetone and butanol.

Potential reasons for ABE fluctuations in ABE and acid concentrations may have been due to depletion of nutrients in the bioreactor and diffusion of O_2 across tubing connectors [5]. Another possible reason for these fluctuations involves morphological changes of the *C. beijerinckii* BA101 cells over the course of the fermentation [22, 23]. Four different physiological stages of *C. acetobutylicum* cells have been reported to occur in a continuous fermentation system [22]. These cell types include actively growing, ABE producing, sporulated, and dead cells. Presence of spores was observed when culture broth was examined using a microscope, however, actively growing and butanol producing *C. beijerinckii* could not be differentiated. Similarly, dead cells could not be clearly identified

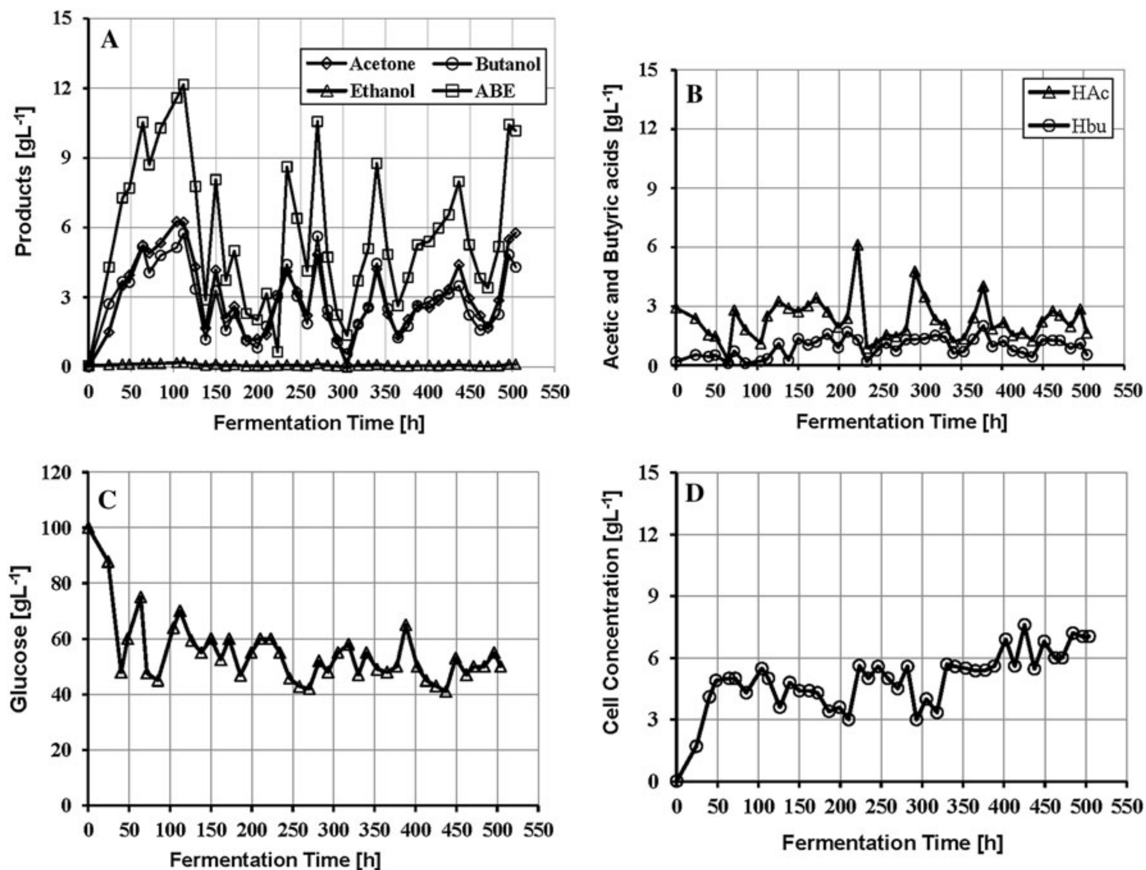

Fig. 2 Continuous production of ABE from glucose using *C. beijerinckii* BA101 coupled with product recovery by gas stripping. **a** Acetone, butanol, ethanol, and total ABE, **b** acetic acid (HAc) and butyric acid (HBu), **c** glucose level in the reactor, and **d** cell concentration

when observed under microscope, although numerous non-motile stationary growth phase cells were observed. Continuous production of ABE is known to fluctuate and has been previously reported [11, 24]. Although there were fluctuations in ABE concentrations in the bioreactor, no complexity is expected to be seen during scale up of this process other than adjusting the amount of gas recycles which would benefit the process. At lower ABE concentrations in the broth, lower amount of gas recycle would be required. During recovery, gas stripping does not remove acid from the bioreactor until these are converted to ABE [5, 18], suggesting that the fluctuations (in acid concentrations) were not the result of gas stripping. It is important, therefore, to note that in ABE fermentation, acetic and butyric acids are metabolic intermediates and are produced and then re-assimilated by *C. beijerinckii* BA101 cells and converted to acetone and butanol [5].

A glucose concentration of 40–70 g L^{-1} was maintained (Fig. 2c) in the bioreactor by continuous feeding with a medium containing 250–500 g L^{-1} glucose solution. The sugar concentration fluctuated in the bioreactor because of varied sugar consumption due to fluctuations in cell concentration, bleed and continuous sugar feed to the bioreactor. The sugar concentration in the bioreactor, however, was within the range that supports adequate clostridial growth and ABE production. Addition of sugar medium and water varied with time and was dependent on rates of sugar utilization, ABE production, and recovery.

C. beijerinckii BA101 cell concentrations were maintained below 6.0 g L^{-1} (Fig. 2d) except during the latter stages of the fermentation when the cell concentration was allowed to increase slowly up to 7.04 g L^{-1}. To maintain the cell concentrations below 6.0 g L^{-1}, constant bioreactor bleed and nutrient limitation were applied. An average rate of bleed was 13.9 mL h^{-1} which is a fraction of 0.014 of total liquid volume in the reactor. The bleed contained microbial cells, residual sugar and left over nutrients. During the course of fermentation (504 h) 6,990 mL of fermentation broth was removed. For calculations, ABE and glucose concentrations present in the bleed were determined and accounted for productivity and yield measurements. The reader is informed that the collected fermentation broth was not recycled and was fermented in a separate vessel until all the sugar was utilized. However, it is possible to recycle such streams for economic reasons. ABE productivities were calculated at various times, and

Fig. 3 Continuous production of ABE in a reactor assisted with bleed and simultaneous product recovery by gas stripping. **a** ABE productivities at various fermentation times, and **b** sugar utilization rate

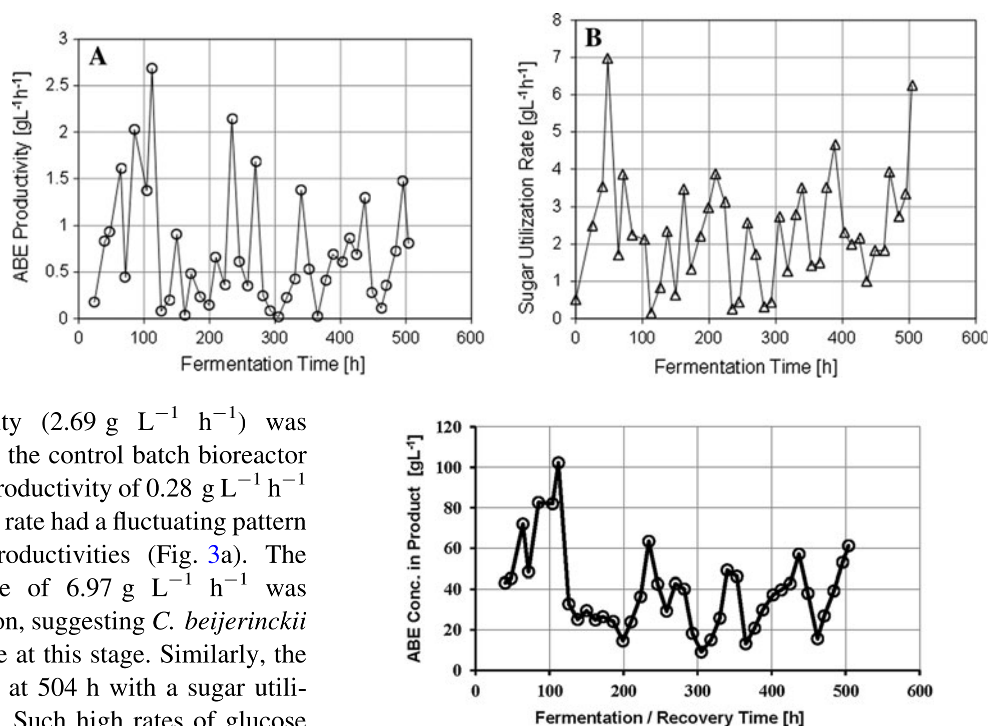

Fig. 4 ABE concentration in the product stream at various fermentation times

the highest ABE productivity (2.69 g L^{-1} h^{-1}) was obtained at 112 h (Fig. 3a). In the control batch bioreactor without ABE recovery, ABE productivity of 0.28 g L^{-1} h^{-1} was obtained. Sugar utilization rate had a fluctuating pattern (Fig. 3b) similar to ABE productivities (Fig. 3a). The greatest sugar utilization rate of 6.97 g L^{-1} h^{-1} was achieved at 48 h of fermentation, suggesting *C. beijerinckii* BA101 culture was very active at this stage. Similarly, the culture continued to be active at 504 h with a sugar utilization rate of 6.25 g L^{-1} h^{-1}. Such high rates of glucose utilization attest that high rates of ABE production can be achieved in suspended cell continuous fermentation.

Accumulation of increased cell concentrations (greater than 10–12 g L^{-1}) in the bioreactor resulted in production of ABE at a faster rate than the ability to recover ABE, leading to a net accumulation of ABE in the bioreactor (data not shown). Increasing the ABE recovery rate will require increasing the rate of gas recycling, which, in turn, will lead to increased foaming in the bioreactor and a requirement for increased anti-foam addition [25]. Increased cell concentrations in the bioreactor, in addition, result in an increase in the viscosity of fermentation broth which may reduce the rate of ABE removal by gas stripping. Hence, it is one of the basic requirements of the system to keep the cell mass within certain range, preferably less than 10–12 g L^{-1} for *C. beijerinckii* BA101.

ABE concentrations in the recovered stream were determined and a maximum concentration of 102.2 g L^{-1} was obtained at 112 h (Fig. 4). An average concentration of ABE in the recovered stream was 36.5 g L^{-1}, which is greater than ABE concentration in a typical ABE fermentation bioreactor [8, 13, 18, 26]. There was little acid (acetic and butyric) accumulation, and at the end of fermentation, only 2.17 g L^{-1} total acid was measured in the bioreactor. In addition, acetic and butyric acids were not detected in the recovered stream. A total of 461.3 g of ABE was produced by *C. beijerinckii* BA101 from 1,125.0 g of sugar resulting in an ABE yield and productivity of 0.41 g g^{-1} and 0.92 g L^{-1} h^{-1}, respectively (Table 2). ABE selectivities ranged from 3.36 to 19.79 while individual selectivity ranges for acetone, butanol, and ethanol were 3.08–9.02, 3.86–11.69, and 2.65–14.67, respectively. Selectivity depends upon weight fraction of ABE in fermentation broth and the recovered stream. A high weight fraction (x) in broth results in a low selectivity while a low x results in high selectivity. A selectivity of greater than 1 indicates that enrichment occurred which would require less energy for further separation preferably by pervaporation membrane or distillation. The recovered stream (a total of 6,903 mL) still contained a significant amount of water. Fermentation intermediates such as acetic and butyric acids were not detected in the condensate during gas stripping suggesting that either gas stripping does not remove acid from the bioreactor or their concentration levels (in the condensate) were too low to be detected by GC. Possible reasons for not removing these acids may be their low vapor pressure under fermentation conditions such as low concentrations, broth pH, and boiling points. Removal of acetic and butyric acids from the reactor by any recovery system is not desirable as these are fermentation intermediates and precursors of acetone and butanol [18].

Fluctuations in ABE concentrations in the recovered stream during simultaneous butanol fermentation and recovery by gas stripping indicated changes in ABE productivities with time (Fig. 3a), which is typical of ABE continuous fermentation systems [11, 26, 27]. Similar fluctuations in sugar utilization by *C. beijerinckii* BA101 were obtained during the same period (Fig. 3b). Examination of Fig. 3a, b suggests that ABE productivity is related

Table 2 A comparison of various fermentation systems coupled with product recovery by gas stripping

Fermentation parameters	Control (NPR)	With product recovery by gas stripping		
		Batch	Fed-batch	Continuous
ABE produced (g L^{-1})	18.4 ± 0.8	75.9 ± 4.55	232.2 ± 11.61	461.3 ± 20.76
Butanol in broth (g L^{-1})	11.0 ± 0.5	8.5 ± 0.05	3.7 ± 0.04	4.3 ± 0.04
Total sugar utilized (g L^{-1})	47.3 ± 2.05	161.7 ± 11.39	500.1 ± 29.03	1,125.0 ± 53.22
Sugar conversion (%)	78.3 ± 3.0	100.0	95.1 ± 5.0	93.7 ± 2.2
Fermentation time (h)	65[a]	127[a]	201[a]	504[b]
Productivity (g L^{-1} h^{-1})	0.28 ± 0.01	0.60 ± 0.04	1.16 ± 0.06	0.92 ± 0.04
Yield (gg^{-1})	0.39	0.47	0.46	0.41
Dilution rate (h^{-1})	NA	NA	NA	0.03[c]
References	Batch; this work	[18]	[5]	This work

NPR no product removal, *NA* not applicable

[a] Fermentation ceased due to butanol or other unknown toxic chemicals

[b] Fermentation was stopped intentionally by the operator

[c] Average dilution rate

to the sugar utilization rate as productivity can also be calculated by multiplying sugar utilization rate and ABE yield (yield is an ABE conversion factor). The rate of gas circulation was not a factor contributing to fluctuations in ABE production, as it was maintained constant throughout the fermentation.

This investigation demonstrated that a continuous fermentation and product recovery employing gas stripping with bleeding can be operated for a much longer period of time than a fed-batch system. Use of a concentrated sugar solution as feed during continuous ABE fermentation and recovery will reduce the volume of process streams, effluents, and increase ABE concentrations in the recovered stream (Fig. 4). Further distillation and concentration of ABE from the recovered stream will require less energy than that needed to distill off ABE from bioreactor effluent. Calculation of energy requirement for ABE recovery requires economic modeling which is beyond the scope of these studies. To our knowledge, this is the first report on single-stage continuous butanol fermentation and simultaneous product recovery using gas stripping. In this system cell growth, fermentation, and product recovery was performed in the same reactor. The results on the length of operation of the bioreactor, total sugar utilized, and total ABE produced per liter broth presented in this work employing continuous fermentation and simultaneous in situ ABE recovery by gas stripping are superior to that reported by Groot et al. [13], where a separate stripper was used. In the present studies, *C. beijerinckii* BA101 strain that was genetically developed from *C. beijerinckii* NCIMB 8052 was used. *C. beijerinckii* BA101 is one of the best strains for ABE production and produces a significant amount of more (33.3–46.0 %) ABE than the parental *C. beijerinckii* NCIMB 8052.

In ABE fermentations, the maximum theoretical yield based on utilization of sugar is 0.40 gg^{-1}. In the present investigations, a yield of 0.41 gg^{-1} was obtained which is higher than the theoretical value. The possible reason for higher yield is the presence of other carbon sources in the medium components such as yeast extract and P2 buffer components (e.g. CH_3COONH_4). Another possibility for higher yield may be nearly complete utilization of reaction intermediates such as acetic and butyric acids.

Conclusions

A batch fermentation system (without product recovery) resulted in the production of 18.4 g L^{-1} ABE from 47.3 g L^{-1} glucose. This non-integrated fermentation system resulted in a productivity and yield of 0.28 g L^{-1} h^{-1} and 0.39 gg^{-1}, respectively. In comparison to this system, a continuous fermentation system operated with a bleed and simultaneous product recovery resulted in a total production of 461.3 g L^{-1} ABE from 1,125.0 g L^{-1} glucose (in 1 L of culture broth). This resulted in an ABE productivity of 0.92 g L^{-1} h^{-1} and yield of 0.41 g g^{-1}. In this study, productivity was improved by over threefold (328 %). It should be noted that yield did not increase as 0.41 gg^{-1} is higher than the theoretical yield due the presence of non-glucose carbons (e.g. CH_3COONH_4). Compared to a fed-batch reactor where fermentation failed after 201 h (over 8 days) due to accumulation of unknown inhibitors [5], continuous fermentation and in situ ABE recovery by gas stripping was operated for 504 h (21 days) after which it was intentionally terminated. It is concluded that a continuous ABE fermentation system with bleed and simultaneous ABE recovery by gas stripping can be operated for a prolonged period of time with good kinetic parameters

such as productivity and yield. Hence, the objective stated in the introduction section of this article was accomplished.

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