IChemE
www.icheme.org/journals
doi: 10.1205/fbp.04163

0960–3085/05/$30.00+0.00
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Trans IChemE, Part C, March 2005
Food and Bioproducts Processing, 83(C1): 43–52

REDUCTION IN BUTANOL INHIBITION BY PERSTRACTION: Utilization of Concentrated Lactose/Whey Permeate by *Clostridium acetobutylicum* to Enhance Butanol Fermentation Economics†

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Acetone-butanol-ethanol (ABE) were produced from whey permeate medium, supplemented with lactose, in a batch reactor using *Clostridium acetobutylicum* P262, coupled with ABE removal by perstraction. ABE (98.97 gL^{-1}) were produced from lactose (227 gL^{-1}) at a yield of 0.44 and productivity of 0.21 $gL^{-1}h^{-1}$. It should be noted that the ratio of acids to solvents was significantly lower in the perstraction experiment compared to the control batch process suggesting that acids were converted to solvents. The perstraction experiment results are superior to the control batch fermentation where 9.34 gL^{-1} ABE was produced. It was determined that lactose at 250 gL^{-1} was a strong inhibitor to the cell growth of *C. acetobutylicum* and fermentation. A membrane with an area of 0.1130 m^2 was used as the perstraction membrane while oleyl alcohol as the perstraction solvent. Removal of ABE by perstraction was faster than their production in the reactor, and the maximum concentration of ABE in the oleyl alcohol was 9.75 gL^{-1}. It is viewed that recovery of ABE from oleyl alcohol (at this concentration) would be more economical than recovery from the fermentation broth. It is suggested that a new membrane be developed which can offer a higher ABE flux. Alternately, silicalite membranes that were successfully developed for pervaporation could be used for perstraction. Using such an integrated system would reduce process streams and save significant processing costs. It is also viewed that the process of concentrated lactose-whey permeate fermentation to butanol can be adapted in the existing solvent fermentation industries without making significant changes.

Keywords: butanol; whey permeate; lactose; Clostridium acetobutylicum; perstraction.

INTRODUCTION

Butanol fermentation [also called acetone butanol ethanol (ABE) or solvent fermentation], a historical process, is second to ethanol in importance. The production of butanol was discovered by Pasteur in 1861 (Jones and Woods, 1986). From the early part of 20th century until WWII, this fermentation was used commercially. Unfortunately ABE fermentation ceased to be operational after WWII due to development of petrochemical industry. Due to availability of cheaper butanol from petrochemical sources, the fermentation route for butanol production could not compete with the chemical process. Due to butanol fermentation being noncompetitive, scarce research activity continued. The only operational plant was in South Africa and it was forced to cease in 1983 when the price of molasses rose dramatically caused by a severe draught.

Although petrochemicals are available at cheaper prices, their supply has not been without problems including constant conflicts in the Middle Eastern region of the world. When oil price suddenly increased in 1973, intensive research interests returned on the conversion of agricultural products into fuels and chemicals. During the early 1980s, a number of problems that have prevented butanol fermentation from being commercially viable, were identified. These obstacles can be enumerated as: (1) use of dilute sugar solutions due to butanol inhibition; (2) low product concentration in fermentation broth which is attributed to butanol inhibition and results in cost intensive recovery; (3) low product yield, usually of the order of 0.3 due to conversion of approximately 53% of substrate into CO_2 and H_2; and (4) low reactor productivity. In spite of these problems, it has been stressed

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that butanol can be economically produced if cheaper substrates are used. Substrates that require minimum processing and fall in this category are by-products or waste products of agricultural industries such as molasses, and whey permeate.

Traditionally, butanol is produced in batch reactors using the anaerobic bacteria *Clostridium acetobutylicum* or *C. beijerinckii.* The reactor is charged with a sugar concentration of 50–60 gL^{-1}, and ABE up to 20 gL^{-1} is produced in the ratio of 3 : 6 : 1, accompanied by some acetic and butyric acids (1–3 gL^{-1}). The ABE yield approximates 0.3 (wt ABE per wt sugar used) and occasionally some residual sugar remains. Normally, higher initial sugar concentrations cannot be used because of butanol toxicity. However, use of concentrated substrate would benefit the process economics. In order to be able to use concentrated sugar solution, toxic butanol should be removed from the reactor as it is produced (Qureshi and Maddox, 1991). This would prevent reaching the tolerance level of the culture. In order to achieve this, a number of product removal techniques have been investigated including adsorption (Ennis *et al.*, 1987; Yang *et al.*, 1994; Holtzapple and Brown, 1994; Milestone and Bibby, 1981; Groot and Luyben, 1986; Nielsen *et al.*, 1988; Yang and Tsao, 1995), gas stripping (Ennis *et al.*, 1986b; Qureshi and Blaschek, 2001a; Qureshi *et al.*, 1992; Groot *et al.*, 1989; Maddox *et al.*, 1995), pervaporation (Larrayoz and Puigjaner, 1987; Qureshi *et al.*, 2001; Friedl *et al.*, 1991; Matsumura *et al.*, 1992; Groot *et al.*, 1984), reverse osmosis (Garcia *et al.*, 1986), and liquid–liquid extraction (Roffler *et al.*, 1987a, b, 1988; Shah and Lee, 1994; Dadgar and Foutch, 1985; Qureshi and Maddox, 1995; Compere *et al.*, 1984; Traxler and Wood, 1985). The details of these techniques have been documented elsewhere (Maddox, 1989; Ennis *et al.*, 1986a). Increased sugar utilization has been achieved using these techniques in combination with fermentation.

Among butanol removal techniques, liquid–liquid extraction has been reported to be one of the economical techniques (Groot *et al.*, 1992; Qureshi *et al.*, 2003). Liquid–liquid extraction has been used to remove ABE from the fermentation broth and numerous extractants have been tested (Roffler *et al.*, 1987a, b, 1988; Taya *et al.*, 1985; Shah and Lee, 1994; Dadgar and Foutch, 1985; Qureshi *et al.*, 1992; Davison and Thompson, 1993; Qureshi and Maddox, 1995; Compere *et al.*, 1984; Traxler and Wood, 1985; Parekh *et al.*, 1988). However, many of these extractants are toxic to the bacterial cells, and hence continuous or long-term fermentations cannot be run using these chemicals (Friedl, 1990; Qureshi *et al.*, 1992). In addition, extractive fermentations may result in accumulation and inactivation of cells at the interface, loss of extractant due to incomplete phase separation, extraction of reaction intermediates, formation of emulsion which may be difficult to separate, and cell inhibition due to prolonged exposure of cells to extraction solvent/s (Maddox, 1989; Qureshi *et al.*, 1992). These problems can be solved by placing a butanol permeable membrane in between fermentation broth and the extraction solvents. Such a process is termed as 'perstraction'. In this process, diffusion of fermentation products through the membrane is the rate-controlling process, and is due to the vapour pressure difference of the diffusing components between the feed side and the extractant side. Having diffused through the membrane, the products are instantaneously dissolved in the extractant. In such a process, it is desirable that the diffusion rate of the fermentation products exceeds their production rate in the reactor. Authors who have investigated perstraction to remove ABE from fermentation broth include Qureshi *et al.* (1992), Jeon and Lee (1989) and Groot *et al.* (1987).

Having emphasized the importance of use of concentrated sugar solution in butanol fermentation and perstraction, it became our objective to produce butanol from concentrated lactose/whey permeate solution in batch reactor and recover it by perstraction. Whey permeate is a dairy industry by-product and can be used as a successful substrate. Fermentation of concentrated sugar solution in batch reactors is considered to be an economic option as compared to the use of dilute sugar solutions which increase process stream volume significantly (Qureshi and Blaschek, 2001b). It is anticipated that simultaneous removal of butanol from concentrated lactose/whey permeate medium by perstraction would reduce product inhibition, enhance cell growth, improve productivity, and recover butanol economically. For the present studies, oleyl alcohol was chosen as the extractant. Use of a commercially available concentrated substrate, such as whey permeate, for butanol production in batch reactor in combination with perstraction has not been reported. Hence, this work is considered 'novel'.

MATERIALS AND METHODS

Organism and Media

Clostridium acetobutylicum P262 was maintained as a spore suspension in distilled water at 4°C. Spray-dried sulfuric acid casein whey permeate, obtained from the New Zealand Dairy Research Institute (Palmerston North, New Zealand) was reconstituted to the appropriate concentration using distilled water and supplemented with yeast extract (5 gL^{-1}, Difco Laboratories, Detroit, MI, USA). Additional lactose was added as required. The medium was adjusted to pH 5.0 using 1 M NaOH, autoclaved at 121°C, and was cooled under a nitrogen atmosphere to maintain anaerobiosis.

Oleyl alcohol was obtained from BDH Chemicals Ltd. (Poole, England) while the silicone tubing which was used as the membrane was from Elastomer Products Ltd. (Auckland, New Zealand). A schematic diagram of the process and details of the membrane and perstractor have been given elsewhere (Qureshi *et al.*, 1992). The membrane area based on internal diameter (inside diameter 3.92 mm, outside diameter 4.72 mm) of the tubing was 0.1130 m^2. Neither the oleyl alcohol nor the extractor were sterilized, while the tubing was autoclaved at 121°C for 20 min.

Cell Cultivation and Bioreactor

Inocula were prepared by adding spore suspension (0.1 mL) to cooked meat medium (20 mL, Difco Laboratories, Detroit, MI), supplemented with lactose (10 gL^{-1}), followed by heat-shocking at 75°C for 2 min and then cooling in ice cold water for 1 min. After cooling, the

culture was incubated for 16–18 h at 35°C in an anaerobic jar. Following this, 3–5 mL of actively growing culture was inoculated in 100 mL of whey permeate medium contained in a 125-mL screw-capped bottle. After incubation at 35°C for 18 h, the main fermenter was inoculated with the inoculum developed above [7% (v/v) inoculum].

Perstraction assisted fermentations were performed in a 2-litre glass reactor (New Brunswick, New Jersey, USA) using an initial culture volume of 1.38 L. After inoculation, oxygen-free nitrogen gas was swept across the medium surface until the culture started producing its own gas, while the pH was controlled at pH 5.0 using automatic addition of 50% (v/v) ammonia solution. The temperature was controlled at 35°C. A peristaltic pump was used to circulate the culture through the perstraction membrane at a flow rate of 25.5 Lh^{-1}. The oleyl alcohol and the extractor were kept anaerobic by means of oxygen-free nitrogen gas. In order to keep the level of reaction mixture constant in the reactor, sterile distilled water was added using a level controller. Results of experiments presented in Table 1 were conducted in 125 mL screw capped bottles.

At the end of 430 h when fermentation stopped, 100 mL of culture broth was removed aseptically from the fermentor to investigate the reason for cessation of fermentation. To this, sterile yeast extract solution was added to give a final yeast extract concentration of 2 gL^{-1}. The fermentation mixture was then transferred to a 125 mL screw capped bottle and inoculated with 7 mL of actively growing inoculum followed by incubation at 35°C for fermentation. The fermentation was vigorous and completed in 96 h. Following this, the remaining fermentation mixture (1.28 L in reactor) was inoculated with 100 mL of an inoculum developed with whey permeate containing 31.0 gL^{-1} lactose and 5 gL^{-1} yeast extract. The fermentation mixture was also added with concentrated sterile yeast extract solution (25 mL containing 2.76 g yeast extract) to give a final yeast extract concentration of 2 gL^{-1}. As soon as gassing was observed, perstraction was restarted and continued until all the lactose was used by the culture.

In order to calculate loss of ABE (diffused through the connecting silicone tubing), a model solution was made containing an average concentration of acetone, butanol, and ethanol of 3.78, 2.11 and 1.33 gL^{-1} in 113.5 gL^{-1} lactose (60 gL^{-1} whey permeate containing 50 gL^{-1} lactose and 63.5 gL^{-1} added lactose). The above ABE concentrations were the actual average concentrations that were present in the fermentor during the perstraction run. In addition to these components 5 gL^{-1} cells were added to the mixture. The volume of the mixture was 1.38 L. The length and diameter of the connecting tubing were 297 mm and 6.5 mm, respectively. In order to find losses the mixture was circulated through the connecting tubing only (perstraction membrane was removed). The mixture was circulated at the same rate as the fermentation broth in the perstraction experiment. The temperature of the mixture was controlled at 35°C. In order to keep ABE concentration constant in the mixture, these chemicals were added every six hours manually. Samples were taken every six hours and injected to GC to find the amount of diffused ABE. The experiment was run continuously for 96 hours.

Analyses

Cell concentration in the mixture was measured by optical density method. Acetone, butanol, ethanol, acetic acid and butyric acid were determined using gas chromatography, while lactose was determined by high performance liquid chromatography (Ennis and Maddox, 1985). Reactor productivity was determined as the total ABE produced, in gL^{-1}, divided by the fermentation time. Yield was calculated as g of ABE produced per g of lactose used. Lactose utilization rate was calculated as lactose utilized, in gL^{-1}, divided by the respective fermentation time. Acids to ABE ratios were calculated as total acids produced, in gL^{-1}, divided by the total ABE produced, in gL^{-1}, in the system. Butanol and ABE flux through the membrane was calculated as butanol amount, in g, dissolved in oleyl alcohol divided by the membrane area and the time period during which diffusion occurred. The rate of loss of ABE through the tubing was calculated as the amount of individual components (or total ABE) divided by the total time and it was expressed as gh^{-1}. The total loss of an individual component was calculated by multiplying rate of loss by perstraction time. The total ABE loss was calculated by adding acetone, butanol, and ethanol losses.

RESULTS AND DISCUSSION

In order to compare the performance of the perstraction experiment, a batch fermentation of 60 gL^{-1} whey permeate was run. The initial lactose concentration was 48.4 gL^{-1}. The fermentation was run for 120 h and it produced 9.34 gL^{-1} total ABE (Figure 1A) resulting in a productivity of 0.08 $gL^{-1}h^{-1}$ and a lactose utilization of 28.6 gL^{-1} leaving behind 19.8 gL^{-1} unused lactose. The reason for unused lactose was toxicity due to ABE, in particular butanol (Qureshi *et al.*, 1988). Using *C. acetobutylicum* a total concentration of ABE of 20 gL^{-1} is rarely reached in a batch reactor. At various stages of fermentation lactose utilization fluctuated which is shown in Figure 1B. A maximum lactose utilization rate of 0.47 $gL^{-1}h^{-1}$ was recorded between 56 and 75 h of fermentation. A yield of solvent of 0.33 was calculated from this run. During this fermentation 0.70 gL^{-1} acetic acid and 0.25 gL^{-1} butyric acid was also produced.

Table 1. Batch fermentation of whey permeate supplemented with lactose using *C. acetobutylicum*. Results are reported after 96 h of fermentation.

Whey permeate (gL^{-1})	Added lactose (gL^{-1})	Total lactose (gL^{-1})	Acetone (gL^{-1})	Butanol (gL^{-1})	Ethanol (gL^{-1})	Acetic acid (gL^{-1})	Butyric acid (gL^{-1})	Total ABE (gL^{-1})	Total acids (gL^{-1})
60	0	50	1.91	5.56	0.25	0.38	0.48	7.72	0.86
60	50	100	1.78	5.49	0.22	0.42	0.33	7.49	0.75
60	100	150	1.67	5.36	0.27	0.31	0.29	7.30	0.60
60	150	200	1.87	5.14	0.21	0.25	Traces	7.22	0.25
60	175	225	1.60	5.08	0.30	0.30	0.41	6.98	0.71
60	200	250	0.26	0.72	0.13	0.77	3.12	1.11	3.89

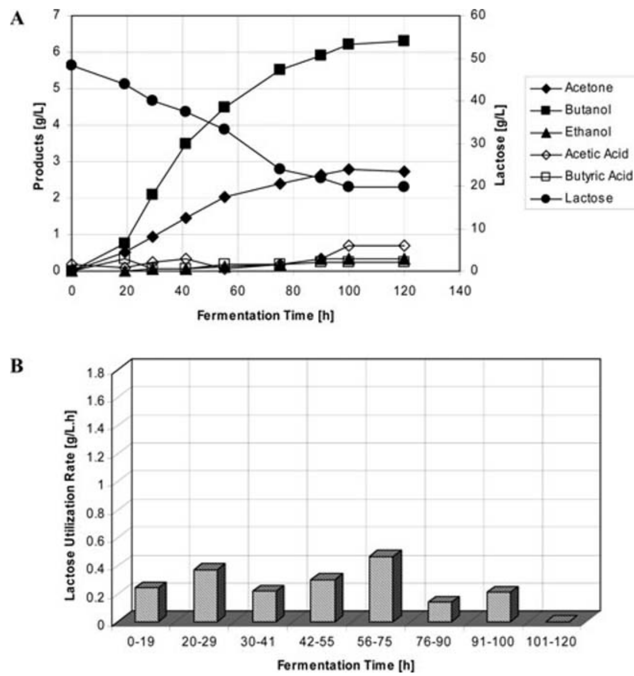

Figure 1. Production of ABE from 60 gL^{-1} whey permeate in a batch reactor of *C. acetobutylicum* P262. (A) Products; (B) lactose utilization rate during fermentation.

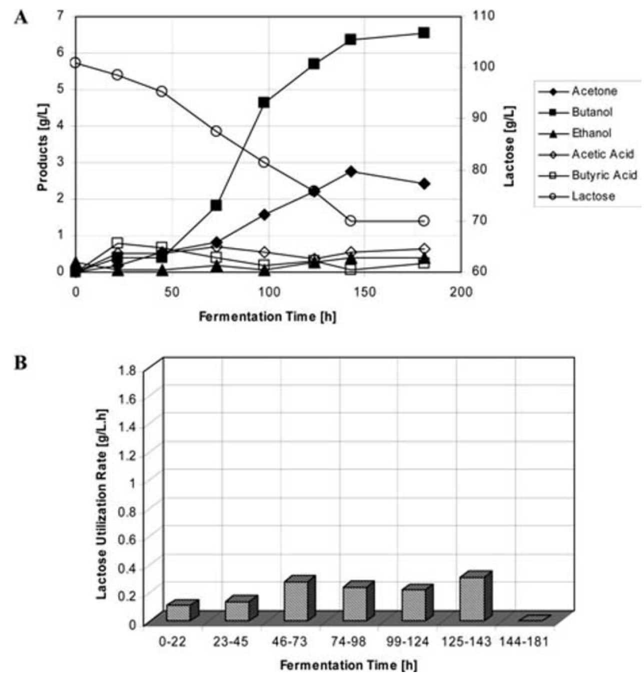

Figure 2. Production of ABE from 130 gL^{-1} whey permeate in a batch reactor using *C. acetobutylicum.* (A) Products and lactose; (B) lactose utilization rate during fermentation.

Since the economics of butanol fermentation could be improved by use of more concentrated sugar feedstocks, an experiment was run with whey permeate concentration of 130 gL^{-1}, corresponding to a lactose concentration of approximately 101 gL^{-1}. The resulting fermentation displayed a long lag phase (Figure 2A), and after 180 h of incubation the ABE concentration was 9.31 gL^{-1} with a residual lactose concentration of approximately 70 gL^{-1}. Previous work has suggested that this sluggish fermentation was due to the relatively high salt concentration of the medium rather than to the high lactose concentration (Maddox *et al.*, 1995). In this fermentation an ABE productivity and yield of 0.05 $gL^{-1}h^{-1}$ and 0.30 was observed, respectively. The rate of lactose utilization is plotted in Figure 2B which also shows that the fermentation was sluggish.

To investigate further if lactose at this level was inhibitory to fermentation, a series of experiments were performed using whey permeate at 60 gL^{-1}, supplemented with lactose at concentrations of 50, 100, 150, 175 and 200 gL^{-1}. It should be noted that inclusion of 60 gL^{-1} of whey permeate is beneficial for a successful butanol fermentation. This is because whey permeate contains essential minerals that are required for cell growth and fermentation. The productivities obtained in these fermentations are plotted against initial lactose concentration (Figure 3). It shows that a lactose concentration up to 225 gL^{-1} was not so inhibitory. However, a lactose concentration of 250 gL^{-1} was strongly inhibitory and resulted in a productivity of 0.01 $gL^{-1}h^{-1}$. Various ABE amounts that were produced in these fermentations are shown in Table 1.

Since a lactose concentration of 225 gL^{-1} was not inhibitory to ABE production, another batch fermentation was started at this value (whey permeate, 60 gL^{-1}, supplemented with lactose at 175 gL^{-1}), with the objective of removing ABE as they were produced. After 69 h of fermentation, when product concentrations were: acetone 1.41 gL^{-1}, butanol 2.26 gL^{-1}, ethanol 0.63 gL^{-1}, acetic acid 0.28 gL^{-1} and butyric acid 0.62 gL^{-1}, continuous product removal by perstraction was initiated (Figure 4A). As perstraction was started, butanol concentration in the fermentation broth decreased rapidly, suggesting that rate of diffusion of butanol through the tubing was greater than the rate of butanol production in the reactor. This resulted in an increased rate of lactose utilization. Figure 4A shows that butanol concentration in the broth never exceeded 3 gL^{-1}. At 72 h, the lactose concentration in the broth was 194 gL^{-1}. After 142 h of fermentation, when the butanol concentration in the broth was 2.50 gL^{-1}, the oleyl alcohol in the extractor was replaced with fresh oleyl alcohol. At that time butanol concentration in the oleyl alcohol was 6.70 gL^{-1}. Acetone was not detected in the oleyl alcohol while ethanol concentration was

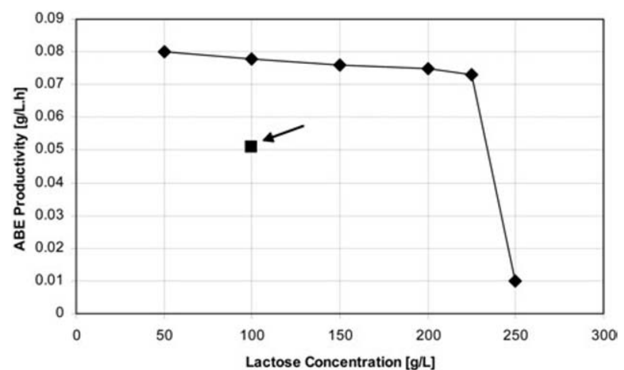

Figure 3. ABE productivities during fermentation at various initial lactose concentrations in the fermentation broth. Arrow shows ABE productivity of 130 gL^{-1} whey permeate fermentation.

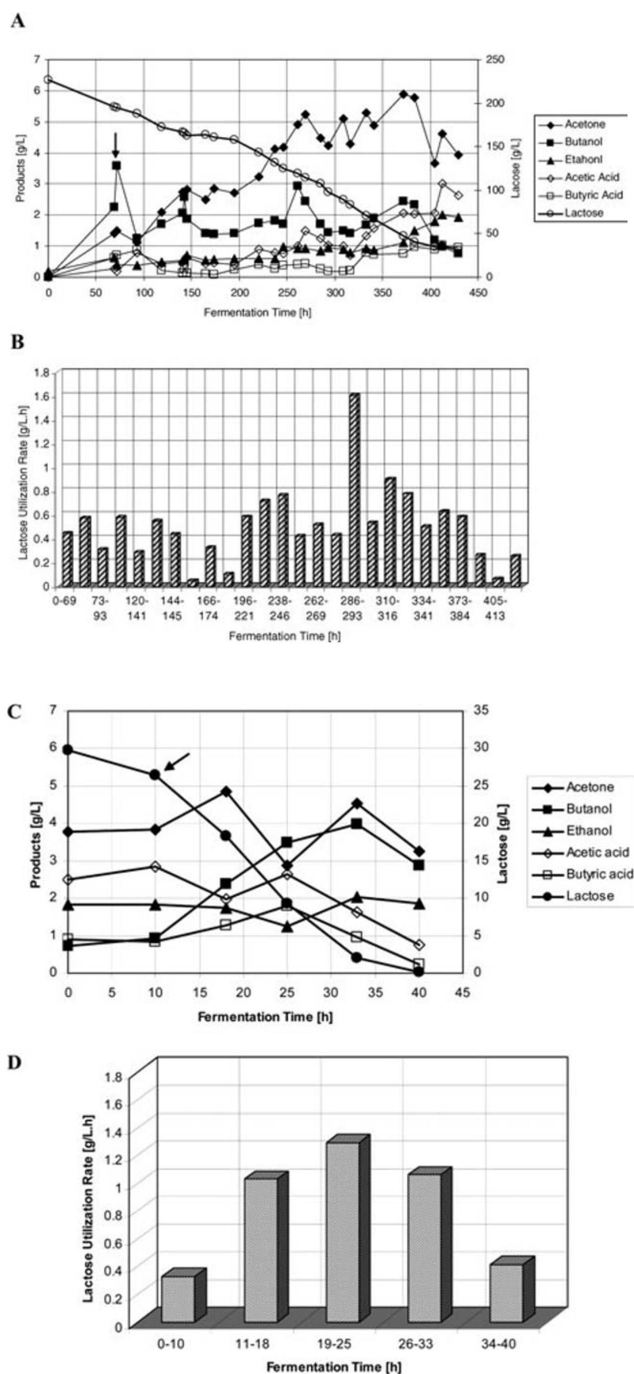

Figure 4. Production of ABE from concentrated substrate (60 gL^{-1} whey permeate supplemented with 175 gL^{-1} lactose) using *C. acetobutylicum* in a batch reactor coupled with product removal by perstraction. (A) Products and lactose: arrow shows the time when perstraction into fresh oleyl alcohol was commenced; (B) lactose utilization rate; (C) fermentation of residual lactose in perstraction assisted fermentation: Products and lactose: arrow shows the time when perstraction was started; (D) lactose utilization rate during fermentation of residual lactose.

1.07 gL^{-1}. Figure 4A shows that acetone accumulated in the reactor. Traces of acetic acid and 0.14 gL^{-1} of butyric acid were detected in the oleyl alcohol. In order to keep butanol concentration low in the reactor, oleyl alcohol was replaced as its concentration reached 6.65 to 7.50 gL^{-1} in the organic phase. Further replacements were done after 269, 348, and 413 h of fermentation, and the results are summarized in Table 2. During the fermentation lactose utilization continued until fermentation slowed significantly at 413 h (Figure 4B). Maximum lactose utilization rate had reached a value of 1.60 $gL^{-1}h^{-1}$, a sign of vigorous fermentation (Figure 4B). This was possible due to simultaneous removal of butanol from the fermentation broth.

After 405 h of fermentation lactose concentration in the reactor was 34.9 gL^{-1} while at 413 h it was measured at 34.2 gL^{-1} suggesting that lactose utilization had decreased significantly and fermentation started becoming acidogenic (Figure 4A). It should be noted that during 413 h of fermentation additional nutrients were not added to the reactor. The possible reasons for cessation of fermentation were: depletion of nutrients in the bioreactor, back diffusion of oleyl alcohol into the aqueous phase, product inhibition, and diffusion of oxygen through the connecting tubing thus preventing the culture from growing and fermenting. Diffusion of oxygen was disregarded as our previous experiment with integrated product recovery was successful (Maddox *et al.*, 1995). Product concentrations in the culture were not sufficiently high to cause inhibition. Back diffusion of oleyl alcohol was also disregarded.

In order to determine if depletion of nutrient was the cause, nutrient supplementation was investigated. Hence, at 430 h, 100 mL of culture broth containing 31.2 gL^{-1} lactose was withdrawn from the reactor and fermented after supplementing with 2 gL^{-1} yeast extract as described in Materials and Methods. As expected, the fermentation was vigorous with the supplemented nutrient. The fermentation stopped after 96 h leaving behind 8.7 gL^{-1} residual lactose. Before fermentation, the concentrations of products were: acetone 3.94, butanol 0.75, ethanol 1.74, acetic acid 2.60 and butyric acid 0.95 gL^{-1}. After fermentation the concentrations of these products were: acetone 5.58, butanol 6.38, ethanol 1.90, acetic acid 0.45 and butyric acid 0.68 gL^{-1}. The amount of ABE that was produced was 7.43 gL^{-1}. During the fermentation acids were used as their concentration decreased from 3.55 gL^{-1} to 1.13 gL^{-1}. This experiment suggested that nutrient deficiency was the cause of cessation of the fermentation.

Since the fermentation–perstraction experiment stopped due to deficiency of nutrients, the fermentor containing 1.28 L fermentation broth with lactose at 31.2 gL^{-1} was re-inoculated with actively growing inoculum and supplemented with 2 gL^{-1} yeast extract. After 10 h of stationary fermentation when significant gassing was observed in the reactor, perstraction was re-started and continued until 40 h when all the lactose was utilized (final concentration 0.1 gL^{-1}). At that time the concentrations of various products in the reactor were acetone 3.25, butanol 2.89, ethanol 1.87, acetic acid 0.75, and butyric acid 0.23 gL^{-1} (Figure 4C). Lactose utilization was rapid as butanol concentration was below inhibitory level (Figure 4D). The maximum butanol concentration in the reactor during the second fermentation was 3.97 gL^{-1}. During this fermentation–perstraction experiment a maximum lactose utilization rate was 1.29 $gL^{-1}h^{-1}$ (Figure 4D).

Perstraction has a number of advantages over liquid–liquid extraction using oleyl alcohol including no extractant (oleyl alcohol) toxicity to the culture (Qureshi *et al.*, 1992). In addition there was no emulsion formation, loss of cells at the interphase, and difficulty in separating the aqueous and organic phases. Our direct liquid–liquid extraction was run for approximately 170 h and it produced 23.81 g ABE from

Table 2. Equilibrium concentrations of acetone, butanol, ethanol, acetic acid, and butyric acid in fermentation broth and oleyl alcohol during perstraction.

	Oleyl alc. vol. (mL)									
	1290		1641		1641		1585		1000	
	Extraction I (gL^{-1})		Extraction II (gL^{-1})		Extraction III (gL^{-1})		Extraction IV (gL^{-1})		Extraction V (gL^{-1})	
Products	C_R	C_E	C_R	C_E	C_R	C_E	C_R	C_E	C_R	C_E
Acetone	2.73	N_D	5.24	N_D	4.88	N_D	3.94	N_D	3.25	N_D
Butanol	2.50	6.70	2.45	6.65	1.89	7.50	0.75	3.20	2.89	9.75
Ethanol	0.54	1.07	0.91	1.50	0.87	1.60	1.93	4.25	1.87	1.63
Acetic acid	0.50	T_R	1.48	T_R	1.58	T_R	2.63	T_R	0.75	T_R
Butyric acid	0.13	0.14	0.43	0.12	0.72	0.23	0.95	0.29	0.23	0.18

C_R – concentration in reactor; C_E – concentration in oleyl alcohol (extractant); N_D – not detected; T_R – traces.

68.6 g lactose (Qureshi *et al.*, 1992). After 170 h fermentation stopped suggesting that prolonged exposure of cells to oleyl alcohol is inhibitory. The silicone membrane was chosen for perstraction for the following reasons: it was easily available at a cheap price; it had a large diffusion area which could be fitted into a vessel conveniently; it did not have fouling problems; and it did not have any dead space for bacterial growth as in case of a flat-sheet membrane (along edges and in module corners).

Acetone was not detected in the oleyl alcohol indicating that either it did not diffuse through the membrane or was not extracted. Since model solutions and pervaporation experiments (unpublished results) have shown that diffusion does occur through this membrane, the reason for this discrepancy is unclear. Acetone is also extracted into oleyl alcohol with a low distribution coefficient of 0.34 (Shah and Lee, 1992). In addition to butanol, ethanol diffused through the membrane (Figure 5). The concentrations of butanol and ethanol in the organic phase at various concentrations in the aqueous phase are shown in Figure 5. Diffusion of acetic acid was insignificant as only traces of acetic acid were detected. Butyric acid diffused through the membrane however, in low concentrations (Figure 5). It should be noted that butanol concentration in the organic phase was low, however, it would be economical to recover butanol from organic phase as compared to the aqueous phase. The boiling point of butanol is 118°C as compared to water (100°C).

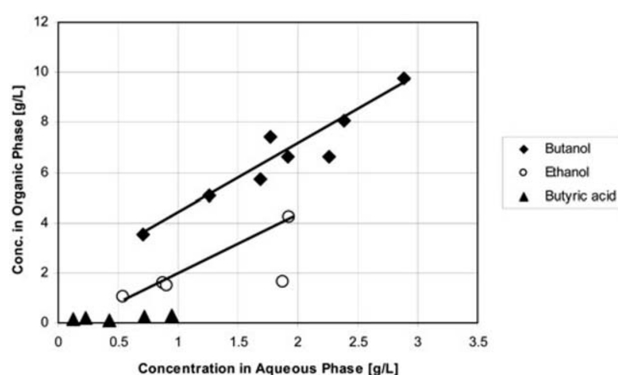

Figure 5. Butanol, ethanol, and butyric acid concentrations in oleyl alcohol at various levels of these chemicals in aqueous phase during fermentation perstraction experiment.

The higher boiling point of butanol than water makes recovery by distillation energy intensive as significant amount of water is evaporated during recovery thus taking significant amount of latent heat. In contrast, butanol extracted into oleyl alcohol (boiling point 170°C) does not require such a large amount of energy (Qureshi *et al.*, 2003).

The amounts of oleyl alcohol used during the five perstractions and the corresponding ethanol, butanol concentrations that were achieved in the extraction phase (and aqueous phase) are presented in Table 2. The maximum concentration of butanol that was achieved in oleyl alcohol was 9.75 gL^{-1} with a corresponding butanol concentration in fermentation broth of 2.89 gL^{-1}. The maximum concentration of ethanol in the organic phase was 4.25 gL^{-1} with a corresponding ethanol concentration of 1.93 gL^{-1} in the aqueous phase.

During the fermentation–perstraction experiment a total of 313.3 g lactose (227.0 gL^{-1} and volume 1.38 L) was used in 470 h (430 + 40) of fermentation (Table 3). Perstraction was run for 391 h. This lactose was converted to 136.58 g ABE resulting in a superior yield of 0.44 and a productivity of 0.21 $gL^{-1}h^{-1}$ which is based on the total fermentation time. The material balance is given in Table 3. The total amount of ABE that was produced is the sum of removed with the fermentation broth (100 mL; to check as to why fermentation stopped), ABE remaining in the broth at the end of fermentation, BE (butanol, ethanol) extracted into oleyl alcohol and losses that occurred through the connecting tubing. The losses were measured as described in the Materials and Methods. It was identified that acetone diffused at a rate of 0.11 gh^{-1}, while butanol and ethanol diffused at the rates of 0.04, and 0.012 gh^{-1}. During 96 h of an ABE loss experiment 10.56 g acetone, 3.84 g butanol, and 1.15 g ethanol were added to the reactor to keep average concentration constant in the reactor (loss = added amount). The reader is advised that a loss of 63.34 g total ABE is significant, however, it was not our objective to prevent those losses. The objective was to utilize concentrated whey permeate-lactose solution. The rate of loss of acetone was high at 0.11 gh^{-1} as opposed to 0.04 gh^{-1} of butanol and 0.012 gh^{-1} of ethanol. The significant loss of acetone can be explained by the high concentration of acetone in the fermentation broth as compared to ethanol and butanol. These losses can be prevented by using a metal connecting tubing rather than silicone

Table 3. Production of total ABE during the fermentation–perstraction experiment (material balance).

	Volume						
	100 mL broth (bottle)		1.38 L broth (fermentor)				
Products	(gL^{-1})	(g) a	(gL^{-1})	(g) b	Extracted (g) c	Losses (g) d	Total (g) (a + b + c + d)
Acetone	3.94	0.39	3.25	4.49	0.00	43.01	47.89
Butanol	0.75	0.08	2.89	3.99	46.69	15.64	66.40
Ethanol	1.93	0.19	1.87	2.58	14.83	4.69	22.29
Acetic acid	2.63	0.26	0.75	1.04	T_R	N_M	1.30
Butyric acid	0.95	0.10	0.23	0.32	1.39	N_M	1.81
Total ABE	6.62	0.66	8.01	11.06	61.52	63.34	136.58
Total acids	3.58	0.36	0.98	1.35	1.39	N_M	3.10
Kinetic parameters					*Values*		
Productivity ($gL^{-1}h^{-1}$)					0.21		
Yield (gg^{-1})					0.44		
Lactose used (g)					313.3		
Ferment. time (h)					470		
Perstraction time (h)					391		

N_M – not measured.

tubing as was used in these experiments. The total amount of acids that were produced in the system was 3.10 g. The results in fermentation-perstraction experiment are superior to those obtained in a similar system, but using gas stripping rather than perstraction as the product removal technique (Maddox *et al.*, 1995). In this system a lactose solution containing 199 gL^{-1} lactose was converted to 70 gL^{-1} ABE. Table 4 compares results of the present fermentation–perstraction system with liquid–liquid extraction, gas stripping, and pervaporation.

It should be noted that a lactose concentration of 227 gL^{-1} was successfully fermented to ABE in the fermentation-perstraction experiment. A non-integrated batch reactor is usually started with approximately 50 gL^{-1} lactose of which approximately 30 gL^{-1} is used leaving behind 20 gL^{-1} as residual lactose which is a significant loss. In order to utilize all the lactose fermentations should be integrated with an efficient recovery process. In the present experiment 7.57 times more lactose was used compared to the batch reactor thus reducing the process stream by this factor. This would save a significant amount of energy. In this system 10.6 times more solvents were produced compared to the control batch process. In addition the reactor productivity was increased from 0.08–0.21 $gL^{-1}h^{-1}$. This was due to the reduction in product inhibition. During the perstraction, butanol was removed efficiently. In order to be able to commercialize the process of butanol production, the fermentation process should be adaptable with a relative ease (requiring minimum modifications in the existing plant). Since batch fermentation is commonly used in the fermentation industry (such as in a distillery), this process/approach can be easily adapted thus saving significant capital cost which may arise due to new construction.

In a biological process where reaction intermediates are produced prior to their conversion to the final products it is desirable that minimum amount (if not 0) of intermediates are produced. This would enhance product yield thus benefiting the economics of the process. In our control experiment the ratio of acids to solvents was 0.10 (0.95 gL^{-1} acids and 9.34 gL^{-1} ABE) as shown in Figure 6. During the fermentation of 130 gL^{-1} whey permeate this ratio was 0.097 (0.90 gL^{-1} acids and 9.31 gL^{-1} ABE). Interestingly, in the fermentation product recovery experiment this ratio was 0.023 (2.25 gL^{-1} acids and 98.97 gL^{-1} ABE) which is significantly lower than the control experiment suggesting that acids were converted to solvents. This is the reason for high ABE yield (0.44) in the integrated experiment. This demonstrated that in this system acids were beneficially used by the culture. This system is also compared in terms of lactose utilization (Figure 7). In the control experiment 28.6 gL^{-1} lactose was utilized while in the fermentation perstraction experiment 227 gL^{-1} lactose was used. The fermentation of 130 gL^{-1} whey permeate used 31.0 gL^{-1} lactose.

Table 4. A comparison of ABE production from whey permeate in fermentation–product recovery systems.

	Processes				
Kinetic parameters	Liquid–liquid extraction[1]	Perstraction[2]	Pervaporation[2]	Gas stripping[3]	Batch ferm. perstraction
Total ABE (g)	23.81	57.80	42.00	70.00	136.58
Total lactose used (g)	68.6	157.5	123.4	199.0	313.3
Fermentation time (h)	170	288	310	220	470
Productivity ($gL^{-1}h^{-1}$)	0.14	0.20	0.14	0.32	0.21
Yield (gg^{-1})	0.35	0.37	0.34	0.35	0.44
Reference	4	4	4	5	This work

[1]Repeated batch fermentation; [2]continuous feeding; [3]concentrated feed batch fermentation; [4]Qureshi *et al.* (1992); [5]Maddox *et al.* (1995).

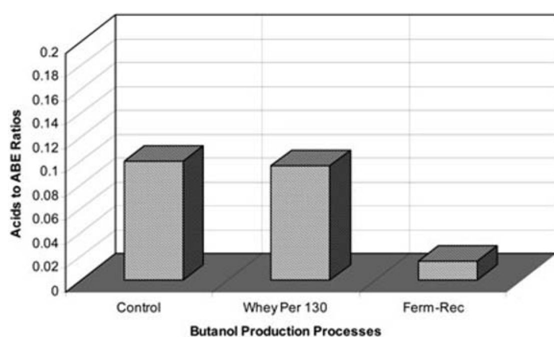

Figure 6. Ratios of acids to ABE during fermentation of 60 gL^{-1} whey permeate (control), 130 gL^{-1} whey permeate, and 227 gL^{-1} whey permeate–lactose (fermentation–recovery) fermentation.

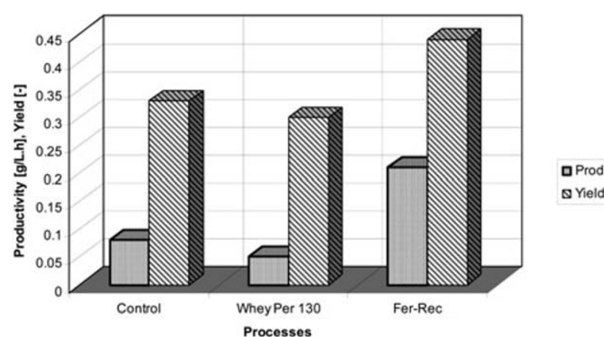

Figure 9. A comparison of productivities and yields in 60 gL^{-1} whey permeate (control), 130 gL^{-1} whey permeate, and 227 gL^{-1} whey permeate–lactose (fermentation–recovery) fermentation.

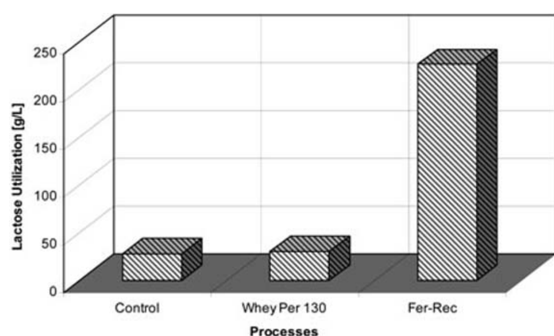

Figure 7. Lactose utilization during fermentation of 60 gL^{-1} whey permeate (control), 130 gL^{-1} whey permeate, and 227 gL^{-1} whey permeate–lactose (fermentation–recovery) fermentation.

The rates of lactose utilization in the three systems are presented in Figure 8. In the perstraction experiment the rate of lactose utilization was 0.48 $gL^{-1}h^{-1}$ as compared to 0.24 $gL^{-1}h^{-1}$ in the control run.

One of the methods to evaluate various systems is to compare their reactor productivities (Figure 9). In the control experiment a reactor productivity of 0.08 $gL^{-1}h^{-1}$ was obtained while in the integrated process a reactor productivity of 0.21 $gL^{-1}h^{-1}$ was obtained (Figure 9). The productivity in 130 gL^{-1} whey permeate fermentation was 0.05 $gL^{-1}h^{-1}$. The reduced productivity in this system was due to toxicity caused by excessive amount of salts present in 130 gL^{-1} whey permeate. ABE yields are also compared in the integrated experiment (Figure 9).

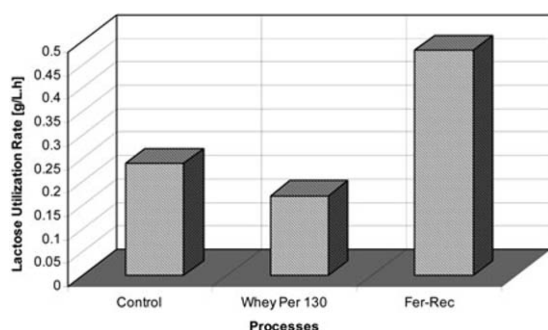

Figure 8. Lactose utilization rates during fermentation of 60 gL^{-1} whey permeate (control), 130 gL^{-1} whey permeate, and 227 gL^{-1} whey permeate–lactose (fermentation–recovery) fermentation.

During the fermentation BE diffused through the tubing which were extracted into oleyl alcohol. In order to evaluate the performance of the membrane, butanol and BE fluxes were calculated. Butanol flux through the membrane ranged from 0.55–2.05 $gm^{-2}h^{-1}$ while that of BE it ranged from 0.93–2.15 $gm^{-2}h^{-1}$ (Figure 10). It should be noted that these flux values are low. In order to improve fluxes of butanol or ABE an efficient membrane should be developed. It is also suggested that a silicalite membrane which adsorps butanol efficiently could be used for perstraction. A silicalite membrane was developed for butanol separation from fermentation broth using pervaporation (Qureshi *et al.*, 1999; Meagher *et al.*, 1998). Silicalite adsorps butanol instantaneously on contact with butanol aqueous solution.

CONCLUSIONS

In conclusion, ABE was successfully produced in the fermentation–perstraction batch experiment using concentrated lactose–whey permeate medium. Fermentation of 227 gL^{-1} lactose was possible due to significant reduction (if not elimination) of product inhibition. This is several times higher than used in a control batch reactor (28.6 gL^{-1}). In the integrated system 98.97 gL^{-1} (fermentation broth volume) (136.58 g) ABE was produced. After a period of 430 h, the culture stopped producing ABE due to deficiency of nutrients. At that time, the culture was spiked with additional nutrients (yeast extract) to ferment all the sugar that was remaining. It should be noted that such a

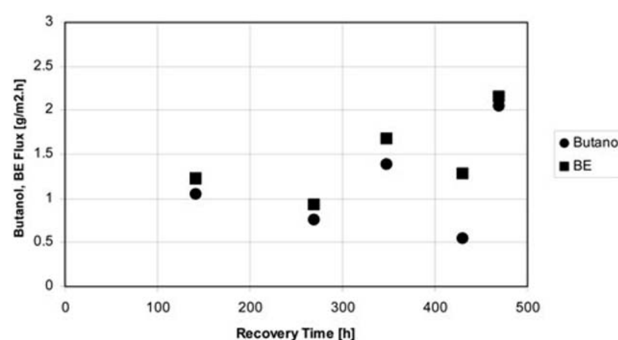

Figure 10. Butanol and butanol–ethanol flux through the membrane during perstraction experiment at various times of recovery.

high concentration of lactose was fermented in a batch reactor which can be applied to the existing fermentation industries without making significant changes. Lactose at $250\ gL^{-1}$ was found to be a strong inhibitor to the cell growth of *C. acetobutylicum* and fermentation. Using such an integrated system would reduce process streams and save significant processing costs. Using this system, the membrane was able to remove BE as fast as they were produced. Back-diffusion of oleyl alcohol (to aqueous phase saturation) through the membrane was not found to affect the fermentation negatively during the 391 h of perstraction. It should be noted that ratio of acids to solvents was significantly low in the perstraction experiment thus directing acids to solvents. During these experiments a couple of problems were identified which should be addressed to make the perstraction process more efficient. The number one problem was low ABE flux through the membrane. In order to improve ABE flux, a new membrane which can offer higher flux should be developed. Alternately, silicalite membranes that were successfully developed for pervaporation could be attempted. The second problem was loss of ABE through the connecting tubing. It is recommended that future experiments should be conducted with a metal connecting tubing. Overall these results are superior to the batch fermentation of butanol using a commercial substrate of whey permeate.

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ACKNOWLEDGEMENTS

N. Qureshi would like to thank Michael A. Cotta (US Department of Agriculture, National Center for Agricultural Utilization Research, Fermentation Biotechnology, Peoria, IL, USA) for reading this manuscript and making valuable suggestions to improve quality of the manuscript.

The manuscript was received 3 June 2004 and accepted for publication after revision 13 December 2004.