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Pervaporative Butanol Fermentation Using a New Bacterial Strain

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Fermentation processes for the production of butanol had an economic importance in the first part of this century. Today butanol is commercially produced from the Oxo reaction of propylene because relatively low priced propylene during the cracking of petroleum. Efforts have been made during the past decade or two to improve the productivity of butanol fermentation processes. It includes strain improvements, continuous fermentation processes, cell immobilization and simultaneous product separation. This review introduces a new butanol fermentation process using pervaporative product separation and a new bacterial strain producing less amount of organic acids. This review also compares the new process with chemical processes. This kind of new fermentation process may be able to compete with the chemical synthesis of butanol and revitalize the butanol fermentation process.

Key words: butanol, fermentation, pervaporation, chemical synthesis

INTRODUCTION

Butanol can be manufactured by chemical synthesis or by bacterial fermentation of carbohydrate-containing materials. During World War I butanol was produced by fermentation of corn starch to meet the growing demand in lacquer industry. In 1919 Weizmann developed the first industrial scale fermentation process in the United States. Even though fermentation process employing molasses or corn products with *Clostridium acetobutylicum* are still practiced in Third World countries, fermentation process was mostly replaced by chemical synthesis after early 30's because fermentation process became less cost effective. With the emergence of petrochemical industry vast quantities of relatively low priced propylene and ethylene became available during the cracking of petroleum. Today the principal commercial source of n-butanol is n-butyraldehyde, obtained from the Oxo reaction of propylene. In the Oxo process propylene reacts with carbon monoxide and hydrogen in the presence of an appropriate catalyst to give a mixture of n- and isobutyraldehydes. The aldehydes are hydrogenated to corresponding n- and isobutyl alcohols.

When crude oil price was sky-rocketed in late 70's and subsequently the price of petrochemical products raised, fuel production from renewable resources such as agricultural and forestry products regained research interests. The major research and development was focused on ethanol fermentation process. The butanol fermentation received less attention although, at this time, the cell physiology of butanol producing microorganisms was investigated extensively [1-4]. A variety of different reactor schemes were studied including the continuous stirred tank reactor (CSTR) [5, 6] and cell immobilization techniques [7, 8].

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An important difficulty with the butanol fermentation process is the strong toxicity of the major product, butanol, to the producing microorganism. Recent studies focus on genetic improvements of the butanol producing microorganisms [9, 10] and one improved strain is reported to tolerate butanol to a higher level [11]. When chemical engineering concepts were introduced into this fermentation system, many different ways of in-situ butanol removal from the fermentor have been developed to avoid inhibitory effect of butanol [12, 13]. I have developed extractive butanol fermentation processes using gas stripping [14] and pervaporation [15]. These efforts increased butanol productivity and I hope that this type of new fermentation process can compete with the chemical processes for butanol production. This article focuses on pervaporative butanol fermentation with some comparison with chemical synthesis of butanol.

Butanol Fermentation

Butanol fermentation is performed by microorganisms called *Clostridium acetobutylicum, Clostridium butylicum, Clostridium beijerinckii*, etc. In addition to butanol two other solvents are produced as co-products. *C. acetobutylicum* produces acetone and ethanol as co-products and the fermentation is called acetone-butanol-ethanol (ABE) fermentation. *C. butylicum* and *C. beijerinckii* produces isopropanol and ethanol as co-products and the process is called isopropanol-butanol-ethanol (IBE) fermentation. In addition, both ABE and IBE fermentations produce organic acids (acetic and butyric acids), carbon dioxide and hydrogen as by-products. Therefore, the fermentation broth becomes a mixture of six-components (three solvents, two organic acids and water).

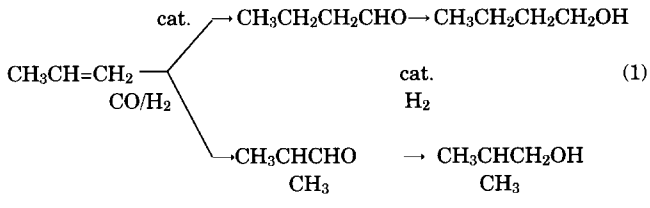
During batch fermentation, without simultaneous product removal, butanol is the primary toxic substance amongst the five products present in the fermentation broth. Butanol production is typically limited to 14-16 g/L because of its inhibitory effects. In-

hibition by other products is negligible during normal batch fermentation because strong inhibition by these products takes place at much higher levels than that obtained during batch fermentation. A complete inhibition takes place at 70 g/L of acetone, 70 g/L of ethanol, 9 g/L of butyric acid, or 11 g/L of acetic acid [16]. Based on an equal concentration level (g/L), acetic and butyric acids are actually much stronger inhibitors when compared to butanol.

Butanol fermentation is more complicated than ethanol fermentation. In addition to its strict anaerobic requirement, the biopathway converting glucose to butanol is a branched type [4, 17]. A detailed biopathway leading to butanol production is discussed later. Cells can produce either solvents or acids depending on their energy needs or the system conditions such as pH, nutrient level, substrate level, etc. Acid production is promoted when the cell's energy need is high, when the medium pH is near neutral, and when nutrient and substrate levels are low. In batch cultures, organic acids are produced simultaneously with cell growth. When the accumulation of organic acids causes the pH to drop below 5.0, enzymes are synthesized by the cells to shift the metabolism toward neutral solvent production.

Chemical Synthesis of Butanol

The most widely used process for the manufacture of n-butanol and isobutyl alcohol is propylene hydroformylation (Oxo reaction) followed by hydrogenation of the aldehydes formed. The hydroformylation and hydrogenation reactions are shown in Eq. (1).



(1)

In hydroformylation carbon monoxide and hydrogen are added to a carbon-carbon double bond of propylene resulting in mostly n- and isobutyraldehydes with small amounts of alcohols, formates and aldehyde condensation products.

Hydroformylation is a homogeneous catalytic reaction and there are several variations depending on the reaction conditions and catalyst. Initially cobalt was used as catalyst and a mixture of n- and isobutyraldehyde was produced at ratio of 4:1. The typical reaction temperature and pressure was 110-180°C and 200-350 atm, respectively. In the mid 70's processes using rhodium catalyst were introduced and n/iso-ratio was increased to 8:1 to 12:1. The reaction temperature and pressure were 80-120°C and 7-31 atm [18]. Using modified Rh complexes the n/iso-ratio could be increased further to 67-86 at low temperature (60-120°C) and low pressure (1-50 atm) [19].

Hydrogenation reduces n- and isobutyraldehydes obtained by hydroformylation to n- and isobutanol. Hydrogenation is heterogeneous catalytic reaction occurring in a fixed bed reactor using Ni or Co based catalysts. For example, passing a mixture of n- and isobutyraldehyde with 60:40 H_2:N_2 over a CuO-ZnO-NiO catalyst at 25-196°C and 0.7 MPa produced corresponding alcohols at 99.95% efficiency and at 98.6% conversion [18].

As a variation of hydroformylation Shell process uses a special catalyst based on cobalt and produces n-butanol directly from propylene in one step process. The n/iso-ratio was 88:12. In spite of its apparent simplicity for butanol production Shell process has some drawbacks. The catalyst is much less reactive than that for hydroformylation and 10-15% of the propylene is hydrogenated to propane [20].

Comparison of Biochemical and Chemical Synthesis

Some analogies can be made between chemical and biochemical synthesis of butanol. First, both synthesis reactions are performed by catalysts; Rh or Co based inorganic catalysts are used for chemical synthesis and organic catalysts (enzymes) are involved in biochemical synthesis. Butanol production by fermentation is essentially a series of enzyme reactions shown in Fig. 1. Initially, cells produce energy and a key metabolite (acetyl-CoA) by oxidation reactions. Subsequent enzyme reactions are reduction reactions converting acetyl-CoA to ethanol and butyryl-CoA. Butyryl-CoA is further reduced to butanol. Considering that acetyl-CoA is a common pool in cell's metabolism six key enzymes for butanol biosynthesis are thiolase (acetyl-CoA acetyltransferase), 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase and butanol dehydrogenase. Their functions are conversion of acetyl-CoA to acetoacetyl-CoA, acetoacetyl-CoA to β-hydroxybutyryl-CoA, β-hydroxybutyryl-CoA to crotonyl-CoA, crotonyl-CoA to butyryl-CoA, butyryl-CoA to butyraldehyde, butyraldehyde to butanol, respectively. These biochemical reactions are more complicated than chemical synthesis involving only two steps; one for aldehyde synthesis and the other for alcohol synthesis.

Secondly, in both synthesis butanol is not produced as a single product. Two products are formed from chemical synthesis, which is less than five products for biochemical synthesis. Selectivity is also better for chemical process and up to 95% of n-butanol production was reported. For typical biochemical reaction, solvents at ratio of butanol:acetone:ethanol is 6:3:1 and 1 to 3 g/L of organic acids are produced. This results in a typical butanol selectivity of 50%. The butanol selectivity increased to 70.6% for a new strain *C. acetobutylicum* B18 [21]. This selectivity is less than 76-81% for classical hydroformylation and 88% for Shell process [20].

For product separation, chemical and biochemical processes need different techniques. Distillation is used to separate aldehyde products and catalyst in hydroformylation. Distillation is also used in hydrogenation to separate alcohol products. For fermentation, the products are diluted in water, and distillation is not a preferred separation technique since butanol and water makes a binary azeotrope at 42.4 butanol wt% [18]. Pervaporation is a better choice for fermentation butanol because it can break azeotrope. The operation of chemical processes is continuous whereas fermentation processes are usually performed in a batch mode.

A unique feature of biochemical reaction is organic acid recycle. Organic acids are produced in the cells for energy (ATP) generation, and then recycled to alcohols by enzymatic reactions. Phosphotransbutyrylase con-

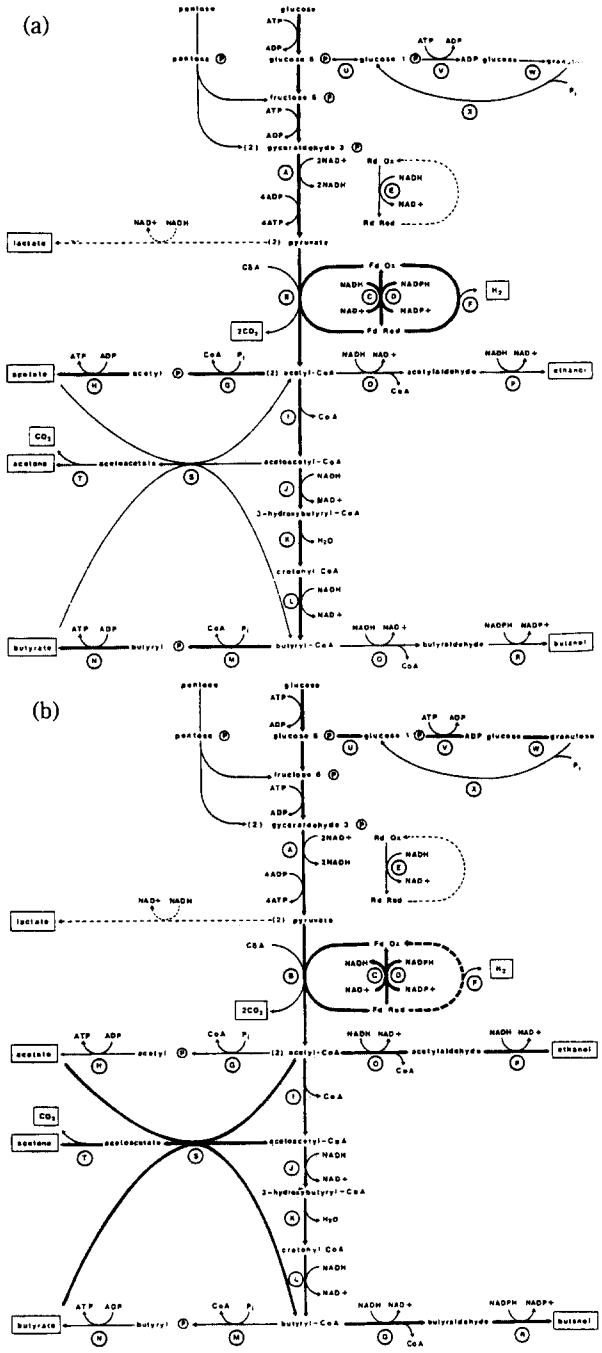


Fig. 1. Biochemical pathways in *C. acetobutylicum*. Reactions which predominate during the acidogenic phase (a) and the solventogenic phase (b) of the fermentation are shown by thick arrows. Enzymes are indicated by letters as follows: (A) glyceraldehyde 3-phosphate dehydrogenase; (B) pyruvate-ferredoxin oxidoreductase: (C) NADH-ferredoxin oxidoreductase; (D) NADPH-ferredoxin oxidoreductase; (E) NADH rubredoxin oxidoreductase; (F) hydrogenase; (G) phosphate acetyltransferase (phosphotransacetylase); (H) acetate kinase; (I) thiolase (acetyl-CoA acetyltransferase); (J) 3-hydroxybutyryl-CoA dehydrogenase; (K) crotonase; (L) butyryl-CoA dehydrogenase; (M) phosphate butyltransferase (phosphotransbutyrylase); (N) butyrate kinase; (O) acetaldehyde dehydrogenase; (P) ethanol dehydrogenase; (Q) butyraldehyde dehydrogenase; (R) butanol dehydrogenase; (S) acetoacetyl-CoA:acetate/butyrate:CoA transferase; (T) acetoacetate decarboxylase; (U) phosphoglucomutase; (V) ADP-glucose pyrophosphorylase; (W) granulose (glycogen) synthase; (X) granulose phosphrylase. (Reprinted with permission from [4] by courtesy of American Society for Microbiology).

verts butyryl CoA to butyryl~PO_4 and butyrate kinase converts butyryl~PO_4 to butyrate. During solvent production butyric acid is recycled back through butyryl-CoA to butanol via both by an induced CoA-transferase enzyme and by reversal of butyrate-producing enzymes (see Fig. 1, reactions S and M-N, respectively).

Simultaneous Fermentation and Separation of Butanol

Fermentation is a process in which a microorganism grows and produces certain metabolites from carbohydrates and other nutrient sources. Even with enough carbon source and other nutrients present in the medium cell growth is often limited because of product toxicity. As stated above, in the case of butanol fermentation, a concentration at 15 g/L is toxic enough to stop the cell growth. Cells can possibly produce more butanol if butanol is removed from the fermentation broth. The technique of removing toxic products during fermentation is called simultaneous fermentation and separation. Various simultaneous fermentation and separation techniques have been reported in the literature [12, 13] and most of these have been applied to butanol removal from the fermentors.

Pervaporation is a new actively growing membrane process [22] and applied to simultaneous fermentation and separation. Pervaporation is a combined process of permeation and evaporation. Components of interest permeate through the membrane and subsequently evaporate at the other surface of the membrane because their partial pressure on the permeate side is lower than saturation vapor pressure. The driving force for pervaporation is generated by applying a vacuum or by using an inert carrier gas. Pervaporation uses either solvent-selective or water-selective membranes which modify the vapor-liquid equilibrium (V.L.E.) of the system. V.L.E. of ethanol-water system and its modification by different membranes are shown in Fig. 2.

Pervaporation applied to butanol fermentation need to use solvent-selective membranes (composite or silicone rubber membranes in Fig. 2) first to extract bu-

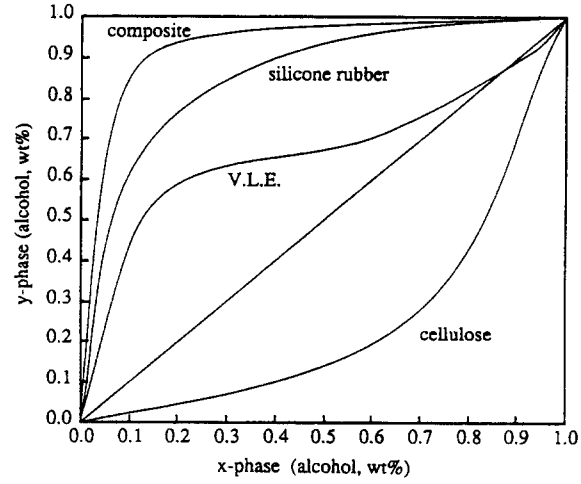


Fig. 2. Vapor-liquid equilibrium curve (V.L.E.) of ethanol-water system and its modification by different membranes. Composite and silicone rubber membranes are alcohol-selective and the cellulose acetate membrane is water-selective. (Reprinted with permission from [13] by courtesy of Marcel Dekker).

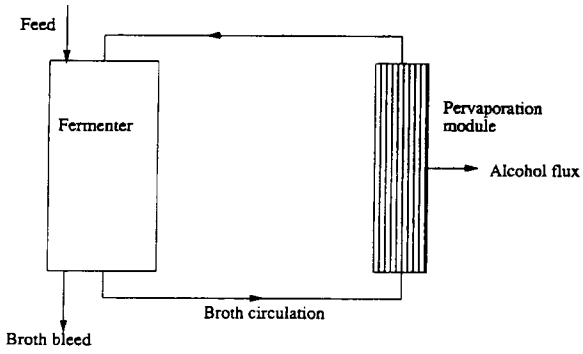


Fig. 3. Extractive fermentation with a pervaporation membrane module.

tanol from a dilute aqueous solution. After we obtain a more concentrated butanol permeate from solvent-selective separation, we need to process the permeate further by using water-selective membranes (cellulose membrane in Fig. 2). The latter is called dehydration process (water removal from liquid organics) and accounts for the majority of plants built to date. However, membranes and processes for selective removal of organics from aqueous streams became also commercially available around 1989. The best known of the commercial processes is the GFT process for dealcoholization of beers, wines and liquors.

Pervaporation seems to be a suitable method to remove butanol from water-butanol mixture because of its high selectivity and because it can separate the azeotrope mixtures. A concept diagram of the pervaporative butanol fermentation system using an external pervaporation module is shown in Fig. 3. Fermentation broth is pumped to the pervaporation module for butanol separation and the retentate is recycled back to the fermentor. In a small scale application the pervaporation module can also be placed inside the fermentor as shown in Fig. 4.

The goal of simultaneous fermentation and separation is to raise the product formation by increasing the sugar consumption. In doing so, the composition of the fermentation broth changes after operation over a period of time and adverse production conditions may develop. In extractive ethanol fermentation problems caused by the accumulation of glycerol [23], formic acid and acetic acid [24] were reported. In the case of pervaporative butanol fermentation, acetic acid and butyric acid will accumulate in the fermentor and their concentrations can be increased to the toxic levels during fed-batch fermentation. In other words, in the absence of butanol toxicity acetic acid and butyric acid will be the major toxic substances limiting further progress of the fed-batch fermentation. Fed-batch fermentation is a modification of batch fermentation by repeated addition of nutrient sources and/or by removing a portion of the culture broth. This implies that we need to avoid organic acid accumulation in the medium to achieve our goal of increasing butanol production. However, this cannot be accomplished easily because pervaporation membranes for butanol removal are not efficient in removing organic acids. Moreover, organic acid formation is an indispensable biochemical reaction for energy generation in the cell. And organic acid removal is not beneficial for solvent production because butanol production initiates only when butyric acid concentration reaches a certain level. We found a way of avoiding acid accumulation by using a new strain of *C. acetobutylicum* B18.

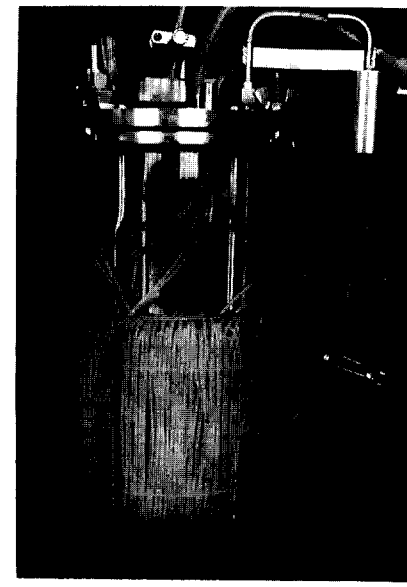


Fig. 4. A fermentor with a pervaporation module made of silicone tubings. (Reprinted from [15]).

Clostridium acetobutylicum B18

We could avoid the acid inhibition problem, which occurs during pervaporative fermentation, by using a strain which produces a lower amount of organic acids. The strain *C. acetobutylicum* B18 was first isolated by Rogers and Palosaari [25]. They showed that this strain produced mostly solvents in pH-uncontrolled batch experiments using serum bottles of working volume 100 mL and 50-55 g/L of glucose. This strain produced butanol with less butyric acid because of higher activity of butanol-producing enzymes and butyrate-recycling enzymes rather than lower activity of butyrate-producing enzymes.

We determined the fermentation characteristics of this strain in batch cultures of 1-L working volume [21]. The strain produced solvents at high yields and recycled butyric acid completely at glucose concentrations of 40 g/L or above. The acetic acid concentration was low (0.32-0.41 g/L) (Table 1). When we increased glucose concentration, solvent yield increased, and acid yield decreased (Table 1). The solvent products (butanol/acetone/ethanol) ratio (wt/wt) for this strain was significantly different from those of other *C. acetobutylicum* strains because of its low ethanol production (Table 1). Butanol selectivity out of five products (three solvents and two organic acids) was 70.6%.

We determined the level of undissociated butyric acid (UBA) required at the onset of solvent production for strain B18 in pH-uncontrolled batch experiments. The level of UBA for strain B18 was much smaller than other strains of *C. acetobutylicum* (Table 2). For 1 mM (0.07 g/L) of butanol production, strain B18 required approximately 0.5 g/L of UBA as compared to 1.14-1.80 g/L of UBA for American Type Culture Collection (ATCC) strains, and 1.27-1.72 g/L of UBA for Deutsche Sammlung von Mikroorganismen (DSM) strains (Table 2) [21].

Rogers and Palosaari [25] explained how the strain

Table 1. Fermentation characteristics of *Clostridium acetobutylicum* B18. (Reprinted with permission from [21] by courtesy of Springer-Verlag)

	Uncontrolled-pH							Controlled-pH
nominal initial glucose conc.(g/L)	4(1)	10	20	30	40	60(2)	80(2)	60(2)
glucose consumption	4.3	10.3	23.2	27.7	41.0	56.2	61.2	64.2
solvent yield(%)	8.4	25.1	30.3	31.4	32.0	35.8	34.7	29.2
butanol yield(%)	7.3	20.4	22.0	23.3	25.0	25.7	24.6	21.4
acid yield(%)	56.1	16.3	5.86	1.67	1.60	0.59	0.59	4.50
total liquid product yield(%)	64.5	41.4	36.2	33.1	33.6	36.4	35.2	33.7
lowest pH	4.67	4.50	4.40	4.66	4.54	4.75	4.39	6.0
butanol/acetone/ethanol	12:1:1	28:7:1	26:9:1	29:9:1	35:10:1	25:9:1	27:11:1	20:6:1
butanol selectivity(%)	88	78	72	74	76	71	71	73
max. acetic acid conc.(g/L)	1.02	1.11	1.16	0.93	0.67	0.96	1.18	4.74
final acetic acid conc.(g/L)	0.85	0.99	0.97	0.37	0.34	0.32	0.41	2.48
max. butyric acid conc.(g/L)	1.90	0.95	1.05	0.92	1.01	1.01	1.52	4.32
	1.90	0.69	0.39	0.13	0.0	0.0	0.0	0.35
undissociated butyric acid at the onset of butanol production (g/L) and corresponding [butanol concentration]	0.80 [0.03]	0.62 [0.14]	0.76 [0.29]	0.47 [0.06]	0.59 [0.07]	0.46 [0.03]	1.11 [0.21]	0.24 [0.31]
specific growth rate (hr^{-1})	0.54	0.35	0.32	0.28	0.27	0.21	0.20	0.37
cell mass(g/L)	0.9	1.2	2.1	2.7	4.0	5.2	4.4	5.3

The superscripts in nominal initial glucose concentration row are explained as follows.
(1) Carbohydrate(0.6 g/L) from yeast extract is considered in yield calculations.
(2) Extra nutrients were added after the start of fermentation.

Table 2. Comparison of strain B18 with other *C. acetobutylicum* strains (Reprinted with permission from [21] by courtesy of Springer-Verlag)

Strain	Fermentor type	Feed glucose (g/L)	pH control	UBA(g/L) and [pH] for onset of solvent production	final product conc. Butanol (g/L)	Butyric acid (g/L)	Reference and comments
ATCC 824	batch	60	no control	1.5[4.5]	11.5	1.0	[26]
		55	4.2	1.6	n.a.	2.1	
		55	4.5	1.7	n.a.	2.5	
		55	5.0	1.8	n.a.	4.8	
		55	5.5	1.6	n.a.	9.5	
		55	6.0	0.88	<1.0	14.2	
ATCC 824	batch	55	4.5	1.69	12.0	1.5	[27]
		55	5.0	1.80	12.5	3.5	
		55	5.5	1.64	7.5	5.5	
		55	6.0	0.88	<1.0	14.5	
NCIB 8052	batch	20	no control	0.26[5.4]	4.1	<0.4	
(ATCC 824)		40	7.0	n.a.	1.3	4.9	[28]
		20	7.0	0.06	3.8	n.a.	8.8 g/L of butyric acid
ATCC 4259	batch	60	no control	1.14[4.7]	10.9	2.4	[29]
DSM 792	batch	76	no control	1.69[4.0]	3.7	0.4	
		76	no control→4.3	1.54[4.3]	5.9	0.7	[1]
		76	6.0→no control	0.99[5.5]	2.4	5.7	
		76	4.3	1.29	7.3	<0.4	
							[2]
DSM 1731	chemostat	3.72	6.0	1.36[4.3]	0.07		1.76 g/L butyric acid added
				2.64[4.8]	0.07		5.28 g/L butyric acid added
DSM 1731	batch	60	no control	1.27[5.1]	6.8	1.76	[30]
DSM 1732	chemostat	60	6.0	>0.72	0	12.0	[31]
			4.3	>0.62	12.6	0.8	
	batch	60	no control	1.72	8.3	0.4	
B18	batch	60	no control	0.5[4.75]	14.0	0	[21]

(1) UBA---undissociated butyric acid
(2) n.a.---the data are not available
(3) The date for UBA, [pH], and final product concentrations were estimated from figures and tables in the references.
(4) UBA at the onset of solvent production was estimated using butyric acid concentration and pH corresponding to 1 mM (0.07 g/L) of butanol or closest estimation to that.

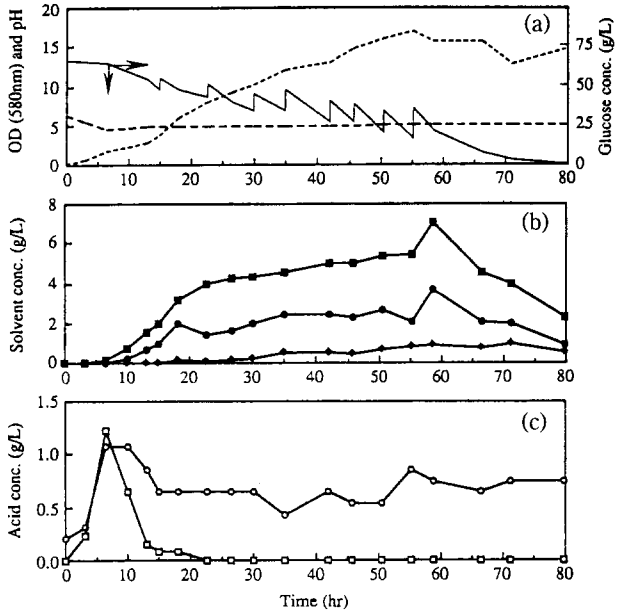


Fig. 5. Profiles of pH, cell growth, glucose and product concentrations during simultaneous fermentation and pervaporation in fed-batch culture. (a) glucose—; pH – –; OD ---. (b) acetone —●—; butanol —■—; ethanol —◆—; (c) acetate —○—; butyrate —□—. The vertical increases in glucose concentration indicate addition of supplemental medium. (Reprinted from [15]).

B18 produced solvents with less butyric acid by determining enzyme activities involved in butanol fermentation. They observed a twofold higher activity levels of butanol-producing enzymes (butanol dehydrogenase and butyraldehyde dehydrogenase). On the other hand, there was no significant activity decrease in the butyrate-producing enzyme (phosphobutyryl transferase and butyrate kinase) even though little butyrate was present. In addition, they found an approximately 30% decrease in acetate producing enzyme (phosphoacetyl transferase and acetate kinase) activities in strain B18 compared to the parent strain. The recycling of butyrate and acetate is believed to occur through acetoacetyl-CoA:acetate/butyrate:CoA transferase, by which butyryl-CoA and acetyl-CoA are produced and funneled into alcohol production [32]. The fact that acetate was not completely recycled by strain B18 is consistent with the finding that CoA transferase prefers conversion of butyrate to butyryl-CoA over acetate to acetyl-CoA [33].

Pervaporative Butanol Fermentation Using Strain B18

We showed that pervaporation using a silicone tubing module was efficient for the removal of butanol and acetone from dilute aqueous solutions [15]. The module was made of silicone tubing (1.95 mm outside diameter and 240 μm thick walls) that was woven through the holes on two flat autoclavable plastic holders horizontally mounted in a fermentor (Fig. 4). The module was immersed in one liter make-up solutions or fermentation broth at 32°C. Air was supplied at a controlled rate through the lumen side of the tubings using an air pump. The flux of each solvent component was linearly dependent on its solution concentration. At the concentration ranges studied in our work, solvent diffusivities were independent each other and increased at higher sweep air flow rates. Organic acid removal by pervaporation was inefficient for butyric acid (20% removal) and negligible for acetic acid. However, the acids did not accumulate up to toxic levels because *C. acetobutylicum* B18 produced little organic acids and recycled existing organic acids efficiently.

Our results showed that strain B18 is a very promising strain for extractive butanol fermentation using pervaporation. With a limited separation capacity (surface area 0.17 m^2) we could maintain butanol concentration in the fermentation broth below 4.5 g/L. The glucose consumption rate with pervaporation was 2.0 g/L-h, which was faster compared to that without pervaporation. The glucose consumption was nearly three fold compared with batch fermentation without product removal, and butanol was produced steadily during fed-batch operation for 80 h. Profiles of pH, cell growth, glucose and product concentrations during simultaneous fermentation and pervaporation in fed-batch culture are shown in Fig. 5.

Competitiveness of Pervaporative Fermentation Process

Ethyl alcohol produced by microbial fermentation from agricultural resources such as corn is currently blended with gasoline. Butyl alcohol can also be produced by fermentation and it has some better physical and chemical characteristics as a motor fuel blender compared with ethanol. Notable characteristics of butanol are its low vapor pressure, low miscibility with water and higher heat of combustion [34]. Total heat value of fermentation products by *C. acetobutylicum* B 18 is approximately the same as that of ethanol fermentation products based on the same sugar consumption (unpublished data). This makes butanol as a potential substitute of ethanol for fuel blender.

Engine performance test using butanol fuel blends showed that butanol can be used as a gasoline or diesel fuel supplement in percentages ranging from 0 to 20 percent and 0 to 40 percent, respectively, without significantly affecting unmodified engine performance [35]. A more interesting finding is that solvent mixture of ABE fermentation was shown as efficient spark ignition engine fuel. Solvent mixtures of butanol (51 wt%), acetone (25 wt%) and ethanol (6 wt%) with some water (18 wt%) produced power and thermal efficiency roughly equivalent to gasoline, provided the engine is operated in performance regions where mixture maldistribution is not severe [36]. This means that the mixture of ABE fermentation product can possibly be used as an engine fuel after simple concentration by pervaporation. This will improve the economics of fermentation butanol production because we can eliminate the steps necessary to separate butanol from acetone and ethanol. It is also reported that oxides of nitrogen (NO_x) emissions were substantially lower for mixture of butanol, acetone and ethanol [36].

Our study showed that pervaporative butanol fermentation using *C. acetobutylicum* B18 is very efficient for butanol production [15]. One limitation of our study was low butanol flux (4 g/m^2-h at 6 g/L of butanol concentration), which can be increased by employing thinner membranes. Development and application of membranes with higher butanol flux will further improve the efficiency of our pervaporation system. Some membranes with transmembrane flux of

more than 2,000-3,000 g/m^2-h and selectivity in excess of 50 was reported [37]. A spin coating technique is also available for the preparation of ultrathin composite membranes [38]. Using these kinds of membranes, a smaller module can accomplish a large separation job.

The economic viability of fermentation process producing low value added material such as butanol is particularly dependent upon the feed stock cost, capital investment and separation cost. The process described in this article markedly improved downstream processing and it opened a possibility of continuous operation in a fed-batch mode. With other efforts on using lower value feed stock I expect that pervaporative butanol fermentation will be commercially viable in the future.

The viability of the fermentation process is also affected by the oil price. Oil demand is increasing recently in the Third World because of growing industry and increasing vehicle demand. However, oil producers are in no rush to expand their capacity at today's low oil prices. This imbalance will lead to rising oil prices and eventually to higher cost for chemically processed butanol. Under this circumstances the newly developed fermentation processes such as described in this article will be a more attractive alternative.

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