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In-situ recovery of butanol during fermentation

Part 2: Fed-batch extractive fermentation*

S. R. Roffler, H. W. Blanch and **C. R. Wilke,** Berkeley

Abstract. End-product inhibition in the acetone-butanol fermentation was reduced by using extractive fermentation to continuously remove acetone and butanol from the fermentation broth. In situ removal of inhibitory products from Clostridium acetobutylicum resulted in increased reactor productivity; volumetric butanol productivity increased from 0.58 kg/(m³ h) in batch fermentation to 1.5 kg/(m³ h) in fed-batch extractive fermentation using oleyl alcohol as the extraction solvent. The use of fed-batch operation allowed glucose solutions of up to 500 kg/m³ to be fermented, resulting in a 3.5- to 5-fold decrease in waste water volume. Butanol reached a concentration of 30–35 kg/m³ in the oleyl alcohol extractant at the end of fermentation, a concentration that is 2–3 times higher than is possible in regular batch or fed-batch fermentation. Butanol productivities and glucose conversions in fed-batch extractive fermentation compare favorable with continuous fermentation and in situ product removal fermentations.

List of symbols

C_g	kg/m³	concentration of glucose in the feed
C_w	dm³/m³	concentration of water in the feed
$F(t)$	cm³/h	flowrate of feed to the fermentor at time t
$V(t)$	dm³	broth volume at time t
V_i	dm³	initial broth volume
V_{si}	dm³	volume of the i-th aqueous phase sample
α		effective fraction of water in the feed

1 Introduction

Butanol, the primary product of the fermentation of sugars or starches by Clostridium acetobutylicum, severely inhibits its further production at concentrations ranging from 10–15 kg/m³ [1–3]. This severe product inhibition leads to high water requirements, low volumetric productivity, capital intensive processes, and expensive product separation and biomass processing steps [4].

Product inhibition in the acetone-butanol fermentation can be reduced through the in situ removal of butanol by extractive fermentation [5]. In batch extractive fermentation using oleyl alcohol or a mixture of oleyl alcohol and benzyl benzoate, maximum volumetric butanol productivity was increased by over 50% compared to regular batch fermentation. In batch culture, however, the benefits of extractive fermentation are limited in that only about 100 kg/m³ of glucose can be fermented; higher concentrations of glucose inhibit the growth of the cells [6, 7]. Concentrated glucose solutions can be fermented by using fed-batch operation during extractive fermentation. In fed-batch operation, concentrated glucose is added to the fermentor at a controlled rate, so that its concentration never exceeds inhibitory levels. This article describes the application of extractive fermentation to the fed-batch culture of Clostridium acetobutylicum. Results of fed-batch extractive fermentation are compared with continuous fermentation and in situ product removal fermentations.

2 Materials and methods

2.1 Microorganism and culture conditions

All fermentations used a strain of Clostridium acetobutylicum obtained from the American Type Culture Collection (ATCC 824). Cultures were maintained as previously described [5].

2.2 Fermentations

Figure 1 shows a schematic diagram of the apparatus used in fed-batch extractive fermentations. A 3.5-dm³ New Brunswick or a 7-dm³ Chemap fermentor were used in all experiments. Fermentations were started in batch culture on a medium similar to one previously described except that all nutrient concentrations were decreased by 25% [5]. When the culture reached an optical density of 5 to 8, oleyl alcohol was transferred to the fermenter and fed-batch operation was initiated. Concentrated nutrient solution, maintained under a nitrogen atmosphere, was metered into the fermentor with a peristaltic pump. The concentrated nutrient solution used in the first two fed-batch extractive fermentations was composed of

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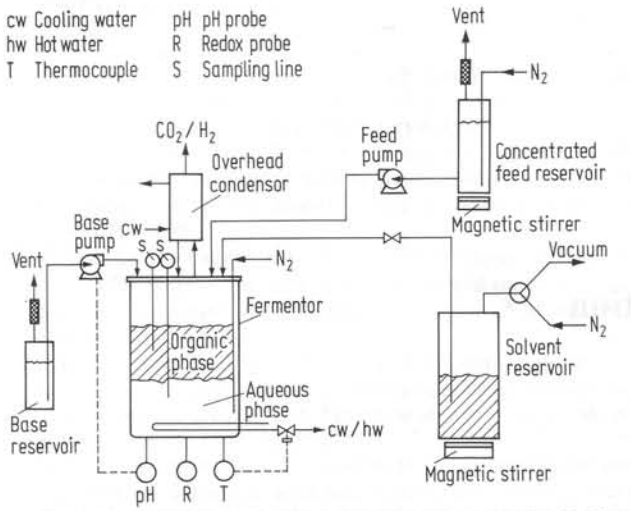

Fig. 1. Schematic of experimental apparatus used for fed-batch extractive fermentations

(in kg/m^3): 5 K_2HPO_4; 5 KH_2PO_4; 3.4 $MgSO_4(7H_2O)$; 0.041 $FeSO_4(7H_2O)$; 0.034 $MnSO_4(H_2O)$; 7.5 NaCl; 3.3 asparagine monohydrate; 1.0 cysteine; 67 yeast extract; 339 glucose; and 0.0067 resazurin. In the third extractive fermentation run, the concentration of glucose was increased to 500 kg/m^3 and the concentrations of all other nutrients were decreased by a factor of 1.34 (i.e. yeast extract concentration was 50 kg/m^3). Samples of the aqueous and organic phases were periodically collected through sampling tubes placed at appropriate heights in the fermentor. Fermentation conditions have been previously described [5].

2.3 Analytical method

2.3.1 Aqueous phase analysis

Concentrations of products in aqueous phase samples were determined by high performance liquid chromatography as previously described [5].

2.3.2 Organic phase analysis

The concentrations of acetone and butanol in organic phase samples were determined by gas chromatography as previously described [5].

2.4 Calculations

Overall volumetric butanol productivities were calculated by dividing the total mass of butanol produced during fermentation by the fermentation time and average aqueous phase volume. Overall solvent productivities were similarly calculated with the sum of the total mass of butanol, ethanol, and acetone used in the numerator. Instantaneous butanol productivities were also estimated. First, a least squares routine was used to fit a polynomial to the curve of total butanol production vs. time. The instantaneous butanol productivity at a particular time was then calculated by differentiating the polynomial with respect to time and divided by the volume of broth at that time.

The volume of broth varied throughout fermentation as samples were withdrawn and concentrated feed was added to the fermentor. In cases where the volume of broth could not be visually followed, it was estimated by measuring the volume of feed added and samples removed during fermentation. Not all of the feed added, however, contributed to an increase in the broth volume. Part of the glucose added to the fermentor is converted to gases, and some of the liquid products are extracted into the organic phase. Thus, only the water in the feed solution and the water produced from the fermentation of glucose were assumed to contribute to the volume of broth. The volume of broth at any time, $V(t)$, can then be estimated from

$$V(t) = V_i + \alpha \int_0^t F(t)\, dt - \sum_{i=0}^{n} V_{si}\,, \tag{1}$$

with V_i being the initial broth volume, $F(t)$ the volumetric flowrate of feed into the fermentor, α the effective fraction of water in the feed, V_{si} the volume of the i-th aqueous phase sample, and n being the number of samples taken up to time t. The effective fraction of water in the feed is the sum of the water actually in the feed and the amount of water produced from the fermentation of the glucose contained in the feed. The concentration of water in a glucose solution is tabulated [8] and can be calculated from

$$C_w = 1000 - 0.634\, C_g\,, \tag{2}$$

with C_w and C_g being the respective concentrations of water and glucose in the feed in kg/m^3.

According to stoichiometric equations derived for butyric acid bacteria [9] 1.86 moles of water are produced per mole of glucose fermented. The effective fraction of water in the feed, α, is thus given by

$$\alpha = (C_w + 0.186\, C_g)/1000\,. \tag{3}$$

Using the above equations for a feed containing 500 kg/m^3 glucose, one obtains $C_w = 683$ ml/L and $\alpha = 0.78$. Thus, only about three-fourths of the feed volume contributes to increasing the broth volume, when a 500 kg/m^3 glucose feed is used. The broth volume given by Eq. (1) was found to predict actual broth volume in fermentations in which the volume could be visually followed.

3 Discussion of results

Oleyl alcohol has been shown to be a good extractant for use in extractive fermentation [5]. In batch extractive fermentation using oleyl alcohol, overall volumetric butanol productivity was increased by 25% and glucose consump-

tion increased from 81 kg/m^3 to over 98 kg/m^3 compared to regular batch fermentation. Attempts to ferment more than 100 kg/m^3 of glucose in batch extractive fermentation, however, were unsuccessful due to catabolite repression of the cells by the high concentrations of glucose in the medium. In order to relieve catabolite repression of the cells, fed-batch operation was used during extractive fermentation. By slowly adding glucose to the fermentor, the concentration of glucose is maintained below inhibitory levels.

3.1 Fed-batch extractive fermentations

Oleyl alcohol (Adol 85, Sherex Chemical Co.) was used as the extraction solvent in the three fed-batch extractive fermentations carried out in this study. Table 1 summarizes the conditions used in each run. The primary variable tested, the ratio of extraction solvent to broth was increased in each run. The concentration of glucose in the feed solution was also increased in run 3. All fermentations were started in batch operation with an initial glucose concentration of 75 kg/m^3. Fed-batch operation was initiated after oleyl alcohol was added to the fermentor.

Figure 2 shows results of the first fed-batch extractive fermentation. the feed solution contained 339 kg/m^3 of glucose and the broth volume was 2 dm^3 at the beginning of fermentation. After 9 hours of fermentation, 2 dm^3 of oleyl alcohol were added to the fermentor and fed-batch operation was initiated. The feed flow rate was not monitored during this run. A majority of butanol produced during this run was extracted into the oleyl alcohol phase and butanol inhibition was reduced throughout the fermentation. At the end of fermentation, the organic phase contained over 30 kg/m^3 butanol and 5 kg/m^3 acetone. This is over twice the concentration of butanol that can be obtained in conventional batch fermentation. The concentrations of products in the aqueous phase at the end of fermentation were (in kg/m^3): 8 butanol; 8 acetone; 2.5 ethanol; 3.1 acetic acid; and 1.8 butyric acid. The cell optical density during this run increased in a stepwise manner: periods of rapid cell growth were followed by periods of slow growth. This stepwise increase in cell density may have been the result of two factors: changing cell morphology and dilution of the broth by feed additions. The cells formed large clumps ranging from about 2 to 5 cm in diameter during extractive fermentation. During clump formation, the bacteria appeared to be excreting a polysaccharide or other macromolecule as the broth became viscous and slimy. Dilution of the medium by the feed may also have contributed to the stepwise increase in cell concentration, because the feed was periodically turned off and on. The presence of cell clumps made accurate cell density measurements difficult in the latter stages of fermentation.

Several advantages over batch fermentation were demonstrated in the first fed-batch extractive fermentation. Taking into account butanol removed in aqueous and organic phase samples, a total of 75 g of butanol was produced. This is equivalent to over 32 g of butanol produced per dm^3 of broth based on final aqueous phase volume, or over twice the amount of butanol that can be

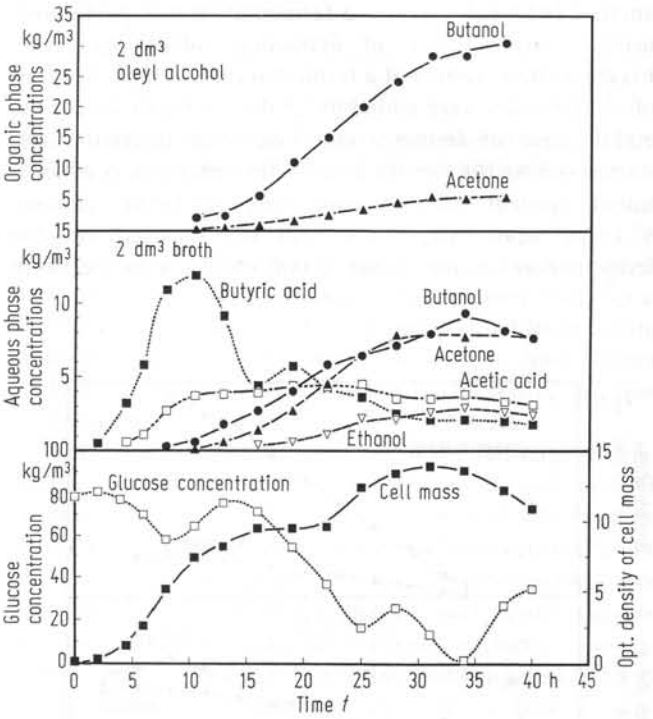

Fig. 2. Fed-batch extractive fermentation of Clostridium acetobutylicum using 2 dm^3 of oleyl alcohol. Initial broth volume was 2 dm^3. Glucose concentration in the feed was 339 kg/m^3

Table 1. Experimental conditions used in fed-batch extractive fermentations

Run	Initial broth volume [dm^3]	Average broth volume [dm^3]	Final broth volume [dm^3]	Ratio of solvent volume to initial broth volume	Glucose concentration in feed [kg/m^3]	Ratio of yeast extract to glucose in feed
1	2	2.1	2.2	1	339	0.2
2	1.5	1.7	2.1	1.5	339	0.2
3	1.5	1.65	2.0	2.3	500	0.1

produced per dm^3 of broth in batch fermentation. Approximately 310 g of glucose were consumed, equivalent to 140 g of glucose consumed per dm^3 of broth. In batch fermentation only 81 kg/m^3 of glucose can be fermented due to the accumulation of butanol in the broth, while in batch extractive fermentation glucose consumption was limited to around 100 kg/m^3 due to catabolite repression. Butyric acid production was also minimized in fed batch extraction fermentation. Only 1.8 kg/m^3 of butyric acid remained in the broth at the end of fermentation, corresponding to a yield of 0.006 g of butyric acid per g glucose. This yield is almost an order of magnitude smaller than the yield of butyric acid in batch extractive fermentation. It appears that the longer fermentation times allowed in fed-batch culture results in greater conversion of butyric acid to products.

If more inhibitory products are removed from the broth during fermentation, it should be possible to ferment more glucose. In order to test this hypothesis another fed-batch extractive fermentation was performed, using a higher ratio of extraction solvent to broth. Figure 3 shows results of a fermentation in which 2.25 dm^3 of oleyl alcohol were added to 1.5 dm^3 of broth during the eighth hour of fermentation. Fed-batch operation was started during the twelfth hour of fermentation. A concentrated nutrient solution containing 339 kg/m^3 glucose, 67 kg/m^3 yeast extract, and salts was metered into the fermentor as needed. Glucose concentration in the broth

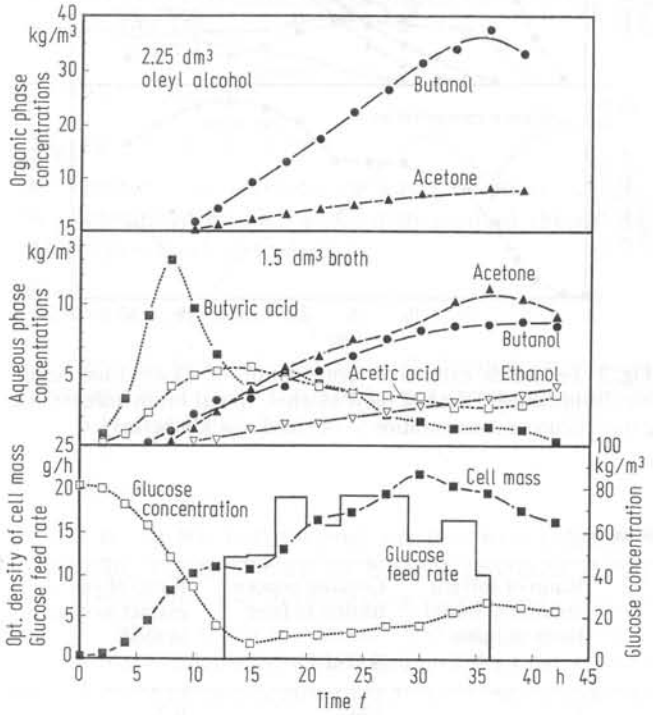

Fig. 3. Fed-batch extractive fermentation of Clostridium acetobutylicum using 2.25 dm^3 of oleyl alcohol. Initial broth volume was 1.5 dm^3. Glucose concentration in the feed was 339 kg/m^3

was regularly monitored. The flow of concentrated nutrients into the fermentors was varied such that a relatively constant glucose concentration was maintained in the broth. After 39 hours of fermentation, the broth volume had increased to 2.1 dm^3 and contained (in kg/m^3): 8.8 butanol; 10.4 acetone; 3.6 ethanol; 2.9 acetic acid; and 1.1 butyric acid. The organic phase contained about 35 kg/m^3 butanol and 8.2 kg/m^3 acetone at the end of fermentation. As more solvent was used in this experiment, more butanol and acetone were removed from the broth, and, consequently, more glucose was consumed.

A total of 435 g of glucose was consumed, and 95 g of butanol were produced in this fed-batch extractive fermentation. Based on final broth volume, 45 g of butanol were produced and 207 g of glucose were consumed per dm^3 of broth. This represents a 300% increase over regular batch fermentation and translates into reduced waste water treatment costs. In other words, only a third of the water used in batch fermentation would have to be processed in fed-batch extractive fermentation to produce the same amount of butanol. As in the first extractive fermentation, very little butyric acid remained at the end of the second run. Although about 20 kg/m^3 of glucose remained in the broth at the end of the second extractive fermentation, residual glucose could be reduced by using on-line glucose analysis, so that the flowrate of glucose into the fermentor could be properly adjusted.

Cell concentration reached a maximum of 22 optical density units corresponding to a dry weight of 14 kg/m^3. The broth again appeared slimy during the second half of fermentation and large clumps of cells formed in the broth. Cell concentration followed a stepwise increase similar to that observed in the first fed-batch run. The stepwise increase in cell density appeared to be due to the combined effect of cell clumping and dilution of the broth by the feed.

Another fed-batch extractive fermentation was carried out by using a higher ratio of solvent to broth than in the previous two runs. In addition, the concentration of glucose in the feed was increased to 500 kg/m^3, while the concentrations of salts and yeast extract were decreased by a factor of 1.34. Midway through this run very large clumps of bacteria formed at the aqueous-organic interface and at the bottom of the aqueous phase. The bacteria appeared to outgrow the aqueous phase and begin growing in the organic phase. The agitation rate was increased at this time, creating a relatively homogeneous emulsion of broth in the solvent phase. Due to the presence of large cell clumps and the formation of an emulsion, it was not possible to measure cell optical density during this run.

Figure 4 shows results of the third fed-batch extractive fermentation run, in which 3.5 dm^3 of oleyl alcohol were added to 1.5 dm^3 of broth. Butanol was produced only slowly during the first 25 hours of fermentation, but was rapidly produced during the second half of fermentation.

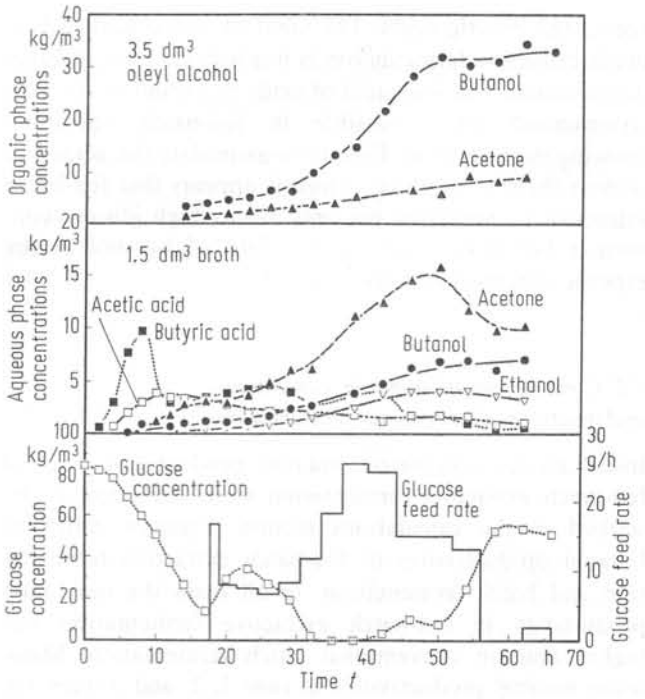

Fig. 4. Fed-batch extractive fermentation of Clostridium acetobutylicum using 3.5 dm^3 of oleyl alcohol. Initial broth volume was 1.5 dm^3. Glucose concentration in the feed was 500 kg/m^3

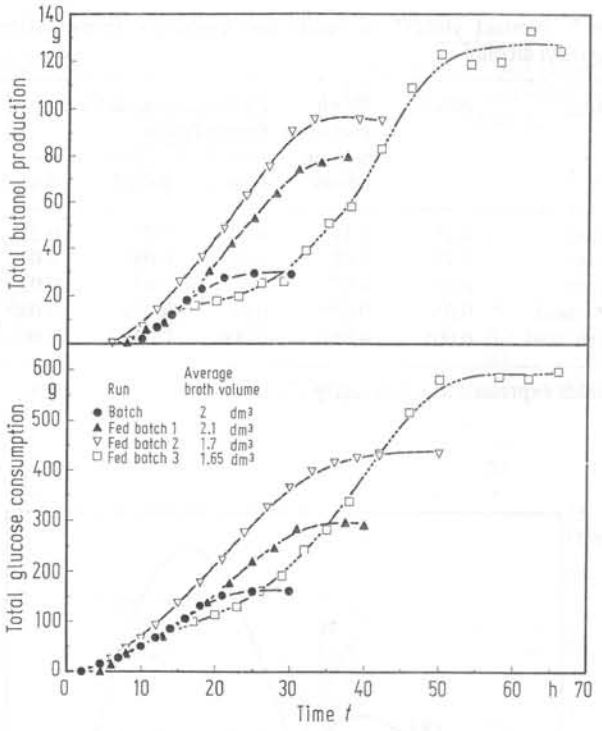

Fig. 5. Comparison of total butanol production and glucose consumption in batch and fed-batch extractive fermentation

Table 2. Results of fed-batch extractive fermentations using oleyl alcohol

	Run 1	Run 2	Run 3
Ratio of solvent volume to initial broth volume	1	1.5	2.3
Average broth volume [dm^3]	2.1	1.7	1.65
Total glucose consumed [g]	310	435	605
Total butanol produced [g]	75	95	125
Total acetone produced [g]	28	42	56
Total ethanol produced [g]	8	11	12
Final butanol concentration in solvent [kg/m^3]	30	35	33

The concentration of acetone in the aqueous phase reached a maximum of 15.8 kg/m^3 after 50 hours of fermentation. By the end of fermentation the aqueous phase acetone concentration had decreased to 10 kg/m^3 due to the combined effect of acetone transferring to the organic phase and the dilution of the aqueous phase by the feed. After 50 hours of fermentation, the concentrations of products in the aqueous phase were (in kg/m^3): 7 butanol; 15.8 acetone; 4.0 ethanol; 1.8 acetic acid; and 1.7 butyric acid. The organic phase contained 32 kg/m^3 butanol and 8 kg/m^3 acetone at this time. A total of 605 g of glucose were consumed and 125 g of butanol were produced, equivalent to a glucose consumption of 302 g and butanol production of 63 g per dm^3 of broth. This represents a 400% increase in glucose consumption and butanol production compared to batch fermentation. At the end of fermentation, there was 50 kg/m^3 of glucose remaining in the broth. This high residual glucose resulted from adding nutrient solution too rapidly at the end of fermentation and could be eliminated by properly controlling the flow of nutrients into the fermentor.

Table 2 summarizes the results of the fed-batch extractive fermentations. As the ratio of oleyl alcohol to broth increased, product inhibition was relieved for progressively longer times. More glucose had been consumed and more products had been produced before fermentation stopped. Figure 5 compares total butanol production and glucose consumption during fed-batch extractive fermentation and regular batch fermentation. Compared to batch fermentation total butanol production and glucose consumption were greatly increased in fed-batch extractive fermentation. In all cases fermentation stopped when butanol concentration in the oleyl alcohol reached 30–35 kg/m^3. Higher butanol concentrations may be possible, if the fermentation is optimized. The concentrations of salt and yeast extract in the feed solution were not optimized in this study. Bacteria can tolerate only a narrow range of water activities [10]. More importantly, many ions specifically inhibit enzyme activity and interfere with cellular transport processes [11]. The yeast extract used in this study (Tastone 154, Universal Foods Corp.) contains 6–8% salts. Thus, the accumulation of salts in the broth may have inhibited the growth of the bacteria or increased their sensitivity to butanol inhibition.

Table 3. Product yields* in batch and extractive fermentation using oleyl alcohol

Product	Batch	Batch extractive fermentation	Fed-batch extractive fermentation		
			Run 1	Run 2	Run 3
Butanol	0.18	0.19	0.24	0.22	0.21
Acetone	0.10	0.08	0.09	0.095	0.095
Ethanol	0.04	0.02	0.03	0.03	0.02
Acetic acid	0.05	0.06	0.01	0.009	0.002
Butyric acid	0.001	0.05	0.006	0.0007	0.001

* Yields expressed as g product/g glucose

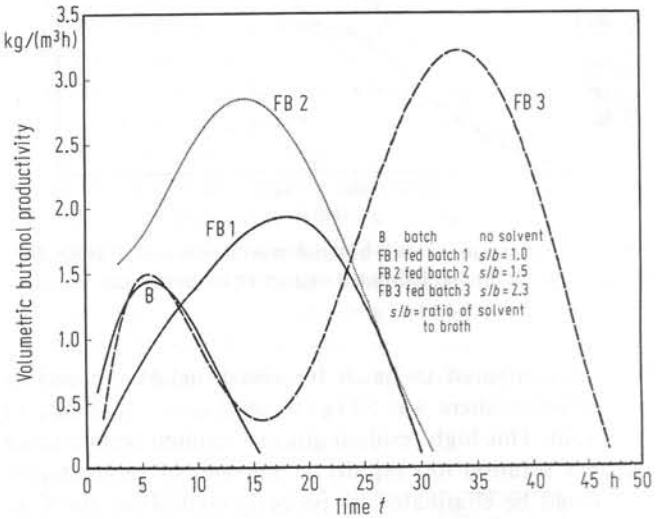

Fig. 6. Comparison of volumetric butanol productivities in batch and fed-batch extractive fermentation. Volumetric productivities are based on broth volume

Fed-batch extractive fermentation has been shown to allow highly concentrated glucose feeds to be fermented and large amounts of butanol to be produced. It is important that the yields of products are not adversely affected by the presence of the extraction solvent. Ideally, the yields of butanol, acetone, and ethanol should be maximized, while the yields of acetic and butyric acids are minimized. Table 3 compares the yields of products in batch and extractive fermentation using oleyl alcohol. In regular batch fermentation or batch extractive fermentation the yield of butanol was about 0.18 g per g of glucose consumed. In fed-batch extractive fermentation, however, the yield of butanol increased to an average value of 0.22. The yields of acetone and ethanol in fed-batch extractive fermentation, on the other hand, were the same or lower than in batch fermentation. It appears that the metabolism of the bacteria is shifted to overproduction of butanol, when butanol is extracted from the broth. The increased butanol yield is matched by decreased yields of acetic and butyric acids. The yield of acetic acid in fed-batch extractive fermentation is much lower than in batch fermentation. The low yield of acids is a result of the long fermentation times possible in fed-batch operation, allowing the cells more time to re-assimilate the acids and convert them to products. Thus, it appears that fed-batch extractive fermentation not only allows high glucose conversion but also improves the yield of butanol at the expense of acetic and butyric acids.

3.2 Comparison of fed-batch, continuous, and in-situ product removal fermentations

Instantaneous volumetric butanol productivities during fed-batch extraction fermentation were estimated as described in the calculations section. Figure 6 compares butanol productivities in fed-batch extractive fermentation and batch fermentation. In all cases the maximum productivity in fed-batch extractive fermentation was higher than in conventional batch fermentation. Maximum butanol productivities in runs 1, 2, and 3 were 1.9, 2.7, and 3.2 kg/(m³ h), respectively, compared to 1.4 kg/(m³ h) in batch fermentation. Maximum butanol productivity in fed-batch extractive fermentation increased as the ratio of solvent-to-broth increased. The larger the solvent reservoir, the longer the concentration of butanol in the aqueous phase was maintained below inhibitory levels. Cell densities reached higher concentrations and volumetric butanol productivity was correspondingly increased. Overall butanol productivities were also higher in fed-batch extractive fermentation. Overall productivities were 0.9, 1.5, and 1.3 kg/(m³ h) in runs 1, 2, and 3, respectively. Even though run 3 had the highest maximum butanol productivity, overall productivity was greatest in run 2. This is the result of the long lag phase in run 3, which decreased its overall productivity. Figure 6 shows that during run 3 butanol productivity passed through a minimum during the twenty-first hour of fermentation. The decreasing productivity coincided with the addition of oleyl alcohol to the fermentor and the formation of large clumps of bacteria. It is likely that bacteria inside these clumps were starved or were inhibited by the accumulation of toxic metabolites inside the clumps. Effective cell concentration was thus reduced, resulting in decreased volumetric productivity. The impeller speed was later increased to break up these clumps and prevent their further formation, accounting for the increased productivity later in the run. Neglecting the lag phase, overall butanol productivity was 1.6–1.7 kg/(m³ h) in run 3.

Table 4 compares productivities obtained in batch, fed-batch, continuous, and in situ product removal fermentations. The concentration and amount of glucose consumed are also compared. Glucose concentration and total glucose conversion are variables important in determining waste treatment costs.

Table 4. Comparison of acetone-butanol fermentations

Fermentation type	Bacterial strain [1]	Volumetric butanol productivity			Concentration of glucose fermented	Total glucose [3] consumed per liter broth
		Maximum	Overall	Overall solvent [2] productivity		
		[kg/(m³ h)]	[kg/(m³ h)]	[kg/(m³ h)]	[kg/m³]	
Batch						
[27]	f	–	0.16	0.22	40	40
[28]	e	0.62	0.23	0.40	29	29
[29]	a	–	0.26	0.42	40	40
[3]	a	1.3	0.51	0.87	65	65
[5]	a	1.4	0.58	1.0	81	81
Fed-batch						
[7]	a	–	0.12	0.2	80	50
[12]	b	–	0.14	0.23	200	58
Continuous						
[17]	a	0.19	0.19	0.31	25.5	
[19]	a	0.25	0.25	0.40	45	
[15]	c	0.24	0.24	0.35	54	
		0.54	0.54	0.79	24	
[16]	c	0.29	0.29	0.43	13.5	
		0.40	0.40	0.58	17.6	
[13]	a	0.32	0.32	0.53	20	
[18]	a	0.51	0.51	0.75	40	
[3]	a	1.5	1.5	2.5	50	
Continuous with cell recycle						
[14]	a	1.1	1.1	1.4	19	
[20]	a	1.8	1.8	3.0	< 60	
		2.7	2.7	4.5	25	
Continuous with immobilized cells						
[21]	a	0.6	0.6	0.72	13	
[22]	a	0.92	0.92	1.5	18	
[23]	d	1.0	1.0	1.6	24	
In-situ product removal						
Fed-batch and solid adsorbant [24]	a	–	0.17	0.29		267
Fed-batch extractive fermentation [6]	e	0.22	0.12	0.20	500	120
Continuous and pervaporation [25]	d	1.0	1.0	1.7	35	
Continuous and activated carbon [26]	b	0.9	0.9	1.5	135	
This work						
Fed-batch extractive fermentation 1	a	1.9	0.9	1.4	339	148
Fed-batch extractive fermentation 2	a	2.7	1.5	2.3	339	207
Fed-batch extractive fermentation 3	a	3.2	1.3	2.0	500	303

[1] Bacterial strain: a: C. acetobutylicum; ATCC 824, NCIB 8052
b: C. acetobutylicum; ATCC 4259; NCIB 619
c: C. acetobutylicum; DSM 1731
d: C. butylicum or C. beyerinckii; LMD 27.6
e: C. acetobutylicum; IAM 19012
f: C. acetobutylicum; P262

[2] Solvents: ethanol, acetone and butanol

[3] Total glucose converted to products divided by final broth volume in batch or fed-batch culture

In batch culture overall butanol productivities range from 0.16 to 0.58 kg/(m³ h) and glucose conversions range from 29 to 81 kg/m³. The highest productivities were obtained with cultures of Clostridium acetobutylicum (ATCC 824) grown on a complex medium [3, 5]. In all batch cultures glucose conversion was limited below about 80 kg/m³ due to accumulation of butanol in the broth. More concentrated feeds can be fermented in fed-batch culture. In a study applying fed-batch culture of the acetone and butanol fermentation [12], a glucose feed of 200 kg/m³ was used. Butanol inhibition, however, limited glucose consumption to 50–60 kg/m³. Fed-batch fermentations had overall volumetric butanol productivities of only 0.12 and 0.14 kg/(m³ h) [7, 12], which is lower than the productivities possible in normal batch fermentation. Fed-batch culture offers little improvement over conventional batch fermentation, because butanol inhibition of the cells is not reduced.

Many researchers have attempted to increase the productivity of the acetone-butanol fermentation by using continuous culture. Butanol production, however, can degrade over time when Clostridium acetobutylicum is grown in continuous culture [13]. Studies have therefore been carried out, in which the concentrations of glucose [13, 14], phosphate [13, 15, 16], nitrogen [13, 17], sulphate [16], or other nutrients [13, 14] have been purposely limited to prevent decreasing butanol yields in continuous culture. By limiting phosphate in continuous culture, Bahl et al. [15] obtained a butanol productivity of 0.24 kg/(m³ h), while fermenting 54 kg/m³ of glucose and producing 9.6 kg/m³ of butanol. Butanol productivity was increased to 0.54 kg/(m³ h) at higher dilution rates, but only 20 kg/m³ of glucose were consumed and 4.2 kg/m³ of butanol were produced. In a two-stage continuous system butanol concentration and glucose consumption increased to 12.6 and 60 kg/m³, respectively. Butanol productivity was 0.3 kg/(m³ h) in the two-stage continuous fermentation. In another study of the continuous culture of Clostridium acetobutylicum [16] butanol productivities reached 0.4 and 0.29 kg/(m³ h) under phosphate and sulphate limitation. Glucose conversion, however, never exceeded 18 kg/m³. Monot and Engasser [17] used nitrogen limitation to maintain butanol production in continuous culture. At a butanol productivity of 0.19 kg/(m³ h), 5 kg/m³ of butanol was produced and 25 kg/m³ glucose was consumed.

Not all studies of acetone-butanol production in continuous culture have purposely limited nutrient concentrations in order to maintain butanol productivity. Leung and Wang [3] obtained high butanol productivities with cells grown on a complex medium. 50 kg/m³ of glucose were fermented, producing 9.5 kg/m³ of butanol at a butanol productivity of 2.5 kg/(m³ h). Frick et al. [18] maintained stable operation of a continuous acetone-butanol culture for 2 months using a complex medium. Butanol productivity was 0.51 kg/(m³ h) at a butanol concentration of 9 kg/m³. In a study using a synthetic medium [19], butanol productivity was 0.25 kg/(m³ h), 49 kg/m³ of glucose were consumed and 7.6 kg/m³ of butanol were produced. These studies highlight some of the problems with using continuous culture to produce acetone and butanol. Although butanol productivity increases as the dilution rate to the fermentor is increased, glucose consumption and butanol concentration both decrease. High concentrations of butanol are desirable, because product recovery costs are strongly dependent on butanol concentration in the product stream [4]. High concentrations of butanol can be produced in continuous culture, but the productivity of the fermentation is greatly reduced. In batch culture butanol inhibition affects productivity primarily at the end of fermentation, when butanol reaches its maximum concentration. In continuous culture, however, a high butanol concentration continuously limits productivity.

Several investigations have attempted to improve continuous culture by using cell recycle to maintain high cell concentrations in the fermentor. Meyer and Papoutsakis [14] combined cell recycle with continuous culture under iron or glucose limitation. By recycling 50% of the cells back to the fermentor they obtained butanol productivities of 1.1 kg/(m³ h) under iron limitation, and 0.16 kg/(m³ h) under glucose limitation. Butanol concentration, however, was less than 4 kg/m³. They also performed experiments in which they sparged the medium with carbon monoxide to increase butanol yield. With no cell recycle butanol productivity was 0.9 kg/(m³ h), and butanol concentration 10.4 kg/m³. Using 75% cell recycle, butanol productivity was increased to 1.17 kg/(m³ h), but butanol concentration dropped to 5.9 kg/m³. Afschar et al. [20] also used cell recycle to improve the continuous fermentation of Clostridium acetobutylicum. Using a glucose feed concentration of 60 kg/m³, they were able to demonstrate a butanol productivity of 2.7 kg/(m³ h) by maintaining a high cell density in the fermentor. Total solvent concentration, however, was only 7 kg/m³, and 35 kg/m³ residual glucose remained in the product stream. In a two-stage fermentor glucose conversion was increased, and 7.2 kg/m³ of butanol were produced at a productivity of 3 kg/(m³ h). By slowing the feed rate to the reactors, butanol concentration reached 9 kg/m³, although butanol productivity dropped to 2.3 kg/(m³ h). Immobilized cells have also been used for the continuous production of acetone and butanol [21–23]. Butanol productivities as high as 1 kg/(m³ h) were obtained. Glucose conversion, however, never exceeded 25 kg/m³.

In all continuous fermentations, including those using cell recycle or immobilized cells, the accumulation of butanol in the broth limited the concentration of glucose in the feed to less than 60 kg/m³. More concentrated glucose feeds can be fermented, if butanol is removed from the broth during fermentation. In a study that used a polymeric resin to absorb butanol and other inhibitory

products during a fed-batch fermentation [24], a total of 400 g of glucose were fermented, corresponding to 267 g of glucose consumed per dm^3 of broth. Overall butanol productivity, however, was only 0.17 kg/(m^3 h). Butanol productivity was also low in a fed-batch extractive fermentation carried out by Taya et al. [6]. However, they were able to ferment a glucose feed solution of 500 kg/m^3 in their system. This represents a large decrease in the amount of water that must be processed after fermentation. In-situ product removal has also been used with continuous culture. In one study [25] isopropanol and butanol were removed from broth by allowing them to diffuse across a membrane into a gas stream. Butanol productivity was 1 kg/(m^3 h), but only 35 kg/m^3 of glucose were fermented. Better results were obtained in a study that used activated carbon to absorb butanol from broth during the continuous culture of Clostridium acetobutylicum on hydrolyzed starch [26]. 135 kg/m^3 of starch was fermented at a butanol productivity of 0.9 kg/(m^3 h).

In the fed-batch extractive fermentations carried out in this work, highly concentrated glucose feeds were fermented at high butanol productivities. A feed solution of 339 kg/m^3 glucose was fermented at an overall butanol productivity of 1.5 kg/(m^3 h). A glucose solution of 500 kg/m^3 was also fermented at a maximum butanol productivity of 3.2 kg/(m^3 h) and an overall butanol productivity of 1.3 kg/(m^3 h). Based on final broth volume, 302 kg/m^3 of glucose were fermented, representing a 3.5- to 5-fold decrease in water required compared to batch and continuous fermentations. Volumetric butanol productivities in fed-batch extractive fermentation were higher than those obtained in all other methods of fermentation, except for continuous fermentation with cell recycle. Further improvement of extractive fermentation is thus possible by combining extractive fermentation with continuous culture using cell recycle.

4 Conclusions

Fed-batch extractive fermentation using oleyl alcohol has been shown to help relieve end-product inhibition in the acetone-butanol fermentation. Volumetric butanol productivity was increased from 0.58 kg/(m^3 h) in batch fermentation to 1.5 kg/(m^3 h) in fed-batch extractive fermentation. Glucose concentrations of up to 500 kg/m^3 could be fermented with little production of butyric and acetic acids. Butanol yields in fed-batch extractive fermentation were better than those obtained in batch or batch extractive fermentation.

When separate aqueous and organic phases are maintained in the fermentor during extractive fermentation, the rate of butanol transfer from the aqueous to organic phase may be much slower than the rate of butanol production. In large-scale extractive fermentation butanol would accumulate in the aqueous phase faster than it could be extracted into the organic phase due to the limited interfacial area between the organic and aqueous phases. The interfacial area could be increased by rapidly stirring the phases, so that the organic phase was dispersed in the aqueous phase. Although this would improve mass transfer, stable emulsions tend to form with high agitation, making phase separation difficult. In large-scale extractive fermentation it may be preferable to contact the solvent and broth in a separate extraction vessel. In many types of extractors, such as reciprocating plate or pulsed columns, shear rate is relatively uniform over the cross-sectional area of the column and emulsion formation is minimized. Interfacial area can thus be increased and mass transfer improved without forming stable emulsions, that are difficult to break. Fed-batch extractive fermentation of acetone and butanol may be improved by optimizing the composition of the concentrated feed solution. Salt and yeast extract in the feed should be minimized to prevent inhibition of the cells by high salt concentrations. In addition, very productive systems may result, if extractive fermentation is coupled with a continuous acetone-butanol fermentation in which cell recycle is used to maintain high cell concentrations.

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S. R. Roffler
Prof. H. W. Blanch
C. R. Wilke
Department of Chemical Engineering
University of California
Berkeley, California, 94720
(USA)