Bioprocess Engineering 2 (1987) 1–12

Bioprocess Engineering

In-situ recovery of butanol during fermentation

Part 1: Batch extractive fermentation

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

Abstract. End product inhibition can be reduced by the in situ removal of inhibitory fermentation products as they form. Extractive fermentation, in which an immiscible organic solvent is added to the fermentor in order to extract inhibitory products, was applied to the acetone-butanol fermentation. Six solvents or solvent mixtures were tested in batch extractive fermentations: kerosene, 30 wt% tetradecanol in kerosene, 50 wt% dodecanol in kerosene, oleyl alcohol, 50 wt% oleyl alcohol in a decane fraction and 50 wt% oleyl alcohol in benzyl benzoate. The best results were obtained with oleyl alcohol or a mixture of oleyl alcohol and benzyl benzoate. In normal batch fermentation of Clostridium acetobutylicum, glucose consumption is limited to about 80 kg/m^3 due to the accumulation of butanol in the broth. In extractive fermentation using oleyl alcohol or a mixture of oleyl alcohol and benzyl benzoate, over 100 kg/m^3 of glucose can be fermented. Removal of butanol from the broth as it formed also increased the rate of butanol production. Maximum volumetric butanol productivity was increased by as much as 60% in extractive fermentation compared to batch fermentation. Butanol productivities obtained in extractive fermentation compare favorably with other in situ product removal fermentations.

1 Introduction

Although many chemicals can be produced by fermentation, accumulation of products in the fermentation broth often inhibits product production, decreases fermentation rates, and limits final product concentrations. The acetone-butanol fermentation is an important case in which product inhibition affects the economics of the fermentation process. Butanol, the primary product of the fermentation of sugars or starches by Clostridium acetobutylicum, severly inhibits its further production at concentrations ranging from 10–15 kg/m^3 [1, 2, 3]. This severe product inhibition leads to high costs for the processing of biomass wastes, low volumetric productivity, capital intensive processes, high water requirements and costly product separation processes [4, 5]. The recovery of the products (acetone, butanol and ethanol) by distillation is one of the major costs of the overall operation [3].

Most of these costs can be reduced by continuously removing butanol from the fermentation broth as it is formed. Higher substrate concentrations can be used since the butanol concentration is maintained below levels toxic to the bacteria. Water requirements are lower and waste treatment costs reduced since less water is introduced into the process with a concentrated substrate. In addition, product separation costs may be lowered.

Several methods of in situ butanol removal have been investigated. Prevaporation, in which butanol and other products selectively diffuse across a membrane into a stream of gas, has been used to remove inhibitory alcohols during the butanol-isopropanol fermentation [6, 7]. Reverse osmosis membranes and solid adsorbents have been used to continuously remove butanol from fermentation broth [8, 9, 10]. Extractive fermentation can also be used for the in situ recovery of butanol. In extractive fermentation, a solvent is contacted with the broth during fermentation; inhibitory products dissolve into the solvent and product inhibition is reduced. Products dissolved in the solvent phase can be recovered by distillation or back extraction into another solvent. The solvent used in extractive fermentation can be an organic solvent or another aqueous phase. Two separate aqueous phases can be formed by adding polymers such as polyethylene glycol (PEG) and dextran to the broth. The microbes usually remain in one aqueous phase and the microbe free phase can be considered the solvent phase. Although low molecular weight products such as butanol will distribute equally between the phases, the volumes of each phase can be adjusted such that the solvent phase is much larger than the phase containing the microbes. Aqueous two-phase extraction systems have been used to produce several products by fermentation, including ethanol [11, 12], acetone and butanol [13] and acetic acid [14]. Because low molecular weight compounds are not concentrated in aqueous two-phase systems, separation costs will probably not be reduced. In addition, the polymers used in these systems are expensive. For these reasons, extractive fermentation using an organic solvent may result in lower process cost.

Dibutyl phthalate has been tested as an extraction solvent for the in situ removal of butanol during fermen-

tation [15]. In more recent studies, butanol was extracted with oleyl alcohol in batch [16] and fed batch fermentations [17]. This article describes results of batch extractive fermentations carried out on the acetone-butanol fermentation. Results of fermentation with in situ product removal are compared with conventional batch fermentation and other method of in situ product removal.

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). Freeze dried cultures obtained from ATCC were inoculated into hungate tubes containing a soluble medium composed of (in kg/m^3): 0.75 K_2HPO_4; 0.75 KH_2PO_4; 0.46 $MgSO_4$ ($7H_2O$); 0.0056 $FeSO_4$ ($7H_2O$); 0.0046 $MnSO_4$ (H_2O); 1.0 NaCl; 0.5 cysteine; 0.5 asparagine monohydrate; 5.0 yeast extract; 20.0 glucose; 1.6 succinic acid; and 0.0015 resazurin (used as a redox-potential indicator). Medium containing all components except glucose and cysteine 2 was neutralized to pH 6.5 with sodium hydroxide, placed in Hungate tubes and flushed with nitrogen to remove oxygen. The tubes were sealed with butyl rubber septems and autoclaved at 121 °C for 15 minutes. Concentrated solutions of glucose and cysteine were separately autoclaved and added to the tubes before inoculation.

Freeze dried cultures normally began growing in the Hungate tubes after 1 to 2 days. After growth was initiated, the culture was transferred to fresh medium every twelve hours for two days. After two days, cells were transferred to serum bottles containing $10^{-4}\,m^3$ of liquid medium and allowed to grow for 8 hours. Batch fermentations were then inoculated with these cells at a ratio of $10^{-4}\,m^3$ of actively growing bacteria per $10^{-3}\,m^3$ of medium.

2.2 Fermentations

Fermentations were conducted in a $3.5 \cdot 10^{-3}\,m^3$ New Brunswick fermentor or in a $7 \cdot 10^{-3}\,m^3$ Chemap fermentor. The medium used in all fermentations was composed of (in kg/m^3): 1.5 K_2HPO_4; 1.5 KH_2PO_4; 1.0 $MgSO_4$ ($7H_2O$); 0.0125 $FeSO_4$ ($7H_2O$); 0.01 $MnSO_4$ (H_2O); 2 NaCl; 1.0 asparagine monohydrate; 20 yeast extract; 100 glucose; and 0.002 resazurin. The pH of all cultures was 6.2 at the beginning of fermentation. As acids were produced, the pH of the culture decreased. The pH, however, was maintained above 5.0 by the automatic addition of 2N NH_4OH. All the fermentations were agitated at 100–150 min^{-1} and temperature was controlled at 37 °C. The redox potential of the culture was monitored with a redox probe which was calibrated using pH buffer saturated with quinhydrone. At 25 °C, quinhydrone saturated buffer has an oxidation reduction potential of 263 mV at pH 4 and 86 mV at pH 7. Nitrogen was sparged through the medium at the beginning of fermentation but was stopped when the bacteria began producing their own gas. Products contained in the gases produced during fermentation were condensed and returned to the fermentor.

Figure 1 shows a schematic diagram of the apparatus used for batch extractive fermentations. Before a solvent was added to the fermentor, it was first contacted with distilled water in order to remove water soluble impurities and then placed under vacuum to remove dissolved oxygen. Thorough removal of oxygen was important since the bacteria are strictly anaerobic and oxygen is highly soluble in the extraction solvents. Oxygen free solvent was added to $2 \cdot 10^{-3}\,m^3$ of broth after the bacteria had started producing butanol. Solvent was forced from the solvent reservoir into the fermentor through a copper tube by pressurizing the solvent reservoir with nitrogen. The impellor speed was adjusted so that two separate phases (aqueous and organic) were maintained in the fermentor. Samples of each phase were periodically removed through sampling tubes placed at appropriate heights in the fermentor.

2.3 Solvent toxicity

Extraction solvents were screened for possible inhibitory effects on the growth of Clostridium acetobutylicum by injecting 1 cm^3 of solvent into Hungate tubes containing 9 cm^3 of actively growing cells. Solvents were contacted with distilled water in order to remove water soluble impurities and then autoclaved in sealed tubes before being contacted with the bacteria. Solvent toxicity was determined by visually comparing the cell density of cultures containing extraction solvents to controls without solvent.

2.4 Liquid-liquid equilibrium

Extraction capacities of organic solvents were determined by contacting 5 cm^3 of aqueous solution containing known

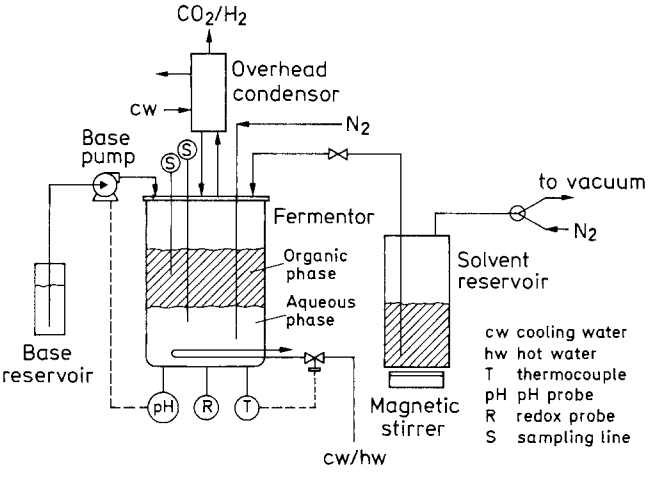

Fig. 1. Overview of the batch extractive fermentation apparatus

concentrations of acetone and butanol with 5 cm^3 of an organic solvent. The phases were contacted for 24 h in closed vials at 21 °C. After the phases were separated, the equilibrium concentrations of acetone and butanol were determined by gas chromatography. The solvents used in equilibrium experiments were of the following purities: oleyl alcohol, technical grade from Aldrich or Adol 85 from Sherex; benzyl benzoate, 99% + from Mallinckrodt; decane fraction, bp. 171 – 177 °C from Fluka.

2.5 Analytical methods

2.5.1 Aqueous phase analysis

Immediately after collection, aqueous phase samples were centrifuged at 10,000 min^{-1} for 10 minutes. The supernatants were stored in glass vials and frozen for later analysis. The centrifuged cells were resuspended in 0.154 N saline and their optical density measured at 610 nm.

Concentrations of products in the aqueous phase were determined by high performance liquid chromatography. Compounds were separated on an Aminex HPX-87X column (Biorad) using 0.01 n sulphuric acid as the eluent. The separated components were detected using a differential refractometer and peak areas of the chromatogram were automatically integrated. The components were eluted from the column in the order: acetic acid, acetoin, ethanol, acetone, butyric acid, and butanol. Residual glucose was determined enzymatically with an Instrumentation Laboratory 919 glucose analyzer.

2.5.2 Organic phase analysis

The concentrations of ethanol, acetone and butanol in organic phase samples were determined by gas chromatography. Analyses were performed on a Varian Areograph 1520 gas chromatograph equipped with a flame ionization detector. Components were separated on Porapak QS in a 152.4 cm (60 inch) long and 0.64 cm (1/4 inch) diameter stainless steel column. Helium was used as the carrier gas at a flowrate of 70 cm^3/min. The respective temperatures of the column, injector and detector were 190, 220 and 240 °C. N-propanol was used as an internal standard and peak areas were automatically integrated.

3 Results and discussion

3.1 Solvent toxicity

In order to realize the benefits of extractive fermentation, the organic solvent used must be non-toxic to the bacteria. Accordingly, several solvents were screened for their effect on Clostridium acetobutylicum growing in Hungate tubes. The quality of the phase separation between solvent and broth was also checked during toxicity tests. Phase separation was considered good if the organic and aqueous phases separated cleanly and rapidly after inverting the Hungate tubes several times. Separation was considered poor when a stable emulsion formed in the tubes. Three classes of extraction solvents were tested: alkanes, esters and alcohols. These compounds were tested because liquid-liquid equilibrium data indicated that esters and alcohols are good extractants for butanol while alkanes make good diluents for blended solvent mixtures. The results of the phase separation and solvent toxicity tests are summarized in Table 1.

In general, alkanes larger than hexane were non-toxic to the bacteria and separated cleanly and rapidly from the broth. Several of the higher molecular weight esters were also non-toxic. However, the phase separation behavior of all esters tested was poor. Dibutyl phthalate has been recommended for use in extractive fermentation of butanol [15]. The density of dibutyl phthalate, however, is very close to the density of water and it formed a stable emulsion with fermentation broth. Alcohols are promising extractants for butanol because of the high partition coefficient they exhibit for butanol. Unfortunately, most of the alcohols tested inhibited the growth of Clostridium aceto-

Table 1. Toxicity of solvent to Clostridium acetobutylicum and solvent phase separation behavior

Solvent	Bacterial growth (0 = no growth, 5 = full growth)	Phase separation
Alkanes		
hexane	0	medium
heptane	5	good
nonane	5	good
undecane	5	poor
dodecane	5	good
cyclohexane	5	medium
cyclooctane	5	medium
kerosene	5	good
Esters		
ethyl hexanoate	3	poor
amyl butyrate	1	poor
methyl octannoate	3	poor
methyl laurate	5	poor
ethyl laurate	5	poor
butyl caproate	3	poor
butyl benzoate	1	poor
benzyl proprionate	3	poor
benzyl benzoate	5	poor
diethyl phthalate	5	poor
dibutyl phthalate	5	poor
Alcohols		
pentanol	0	good
methyl cyclo hexanol	1	poor
1-octanol	3	medium
2-octanol	3	poor
decanol	3	medium
undecanol	3	good
dodecanol	5	good
oleyl alcohol	5	good

butylicum. Dodecanol and oleyl alcohol appeared to be the only alcohols tested that did not stop bacterial growth.

These results generally agree with results obtained in other studies on solvent toxicity to Clostridium acetobutylicum species. Hashimoto [15] found that corn oil, butyl octyl phthalate, butyl oleate and dibutyl phthalate were non-toxic to Clostridium acetobutylicum while hexane, *n*-octanol, and 2-octanol were toxic. In another study [18], alcohols were tested for use in the extractive fermentation of butanol. All extractants tested were found to be toxic to Clostridium acetobutylicum (172CY-02). The alcohols tested were allyl phenol, nonyl phenol, amyl alcohol, heptyl alcohol, 2-ethyl hexanol, 6-tertbutyl-2,4 xylenol, 1 undecanol and tridecanol. Another study [16] investigating the effects of extraction solvents on Clostridium acetobutylicum (IAM 19012) found that gas production, which is proportional to butanol production, was severely inhibited by 1-pentanol, 1-hexanol, 1-octanol, 1-decanol, 1-dodecanol, 1-tridecanol, 2-methyl-1-pentanol, 2-octanol, 2-ethyl-1-hexanol, 2-decanol, 2-tridecanol, 2,4,6,8-tetramethyl-1-nonanol, ethyl caproate, ethyl salicylate, 4-isopropenyl-1-methylcyclohexene, ricinoleic acid, 1,1-dihydroheptafluoro-1-butanol, 1,1-dihydrotridecafluoro-1-heptanol and mixtures of C_{12} through C_{15} alcohols. They found that C_{16}, C_{18} and C_{20} straight chain alcohols, oleyl alcohol, oleic acid, isostearic acid, freon E and octadecafluorodecalin were non-toxic to the bacteria. In general, all studies found that higher molecular weight alkanes are non-toxic, some esters are non-toxic and most alcohols are toxic to Clostridium acetobutylicum.

3.2 *Batch fermentation*

A batch fermentation without any extraction solvent was carried out as a control for comparison with extractive fermentations. Figure 2 shows the product distribution and substrate uptake over the course of batch fermentation. During the early stages of the fermentation, butyric acid accumulated to a concentration of about 6 kg/m^3. At this time, its concentration rapidly dropped and acetone, butanol and ethanol accumulated in the medium. The concentrations of products at the end of fermentation, which was completed after about 24 hours, were (in kg/m^3): 14.6 butanol; 8.3 acetone; 3.4 ethanol; 0.1 butyric acid; 4.3 acetic acid; and 0.9 glycerol. A small amount of acetoin was also detected in the broth. 83 kg/m^3 of glucose was consumed during fermentation. Product accumulation in the broth prevented complete glucose conversion; 18 kg/m^3 of glucose remained at the end of fermentation.

3.3 *Batch extractive fermentations*

In order to determine if the possible benefits of extractive fermentation can be realized, several batch extractive fermentations were carried out. In all cases, the initial glucose concentration was about 100 kg/m^3 the same as used in regular batch fermentation. In batch extractive fermentations, between 1 to $2 \cdot 10^{-3}$ m^3 of solvent was added to $2 \cdot 10^{-3}$ m^3 of fermentation broth after butanol production had began. Six solvents or solvent mixtures were tested: kerosene, 50 wt% dodecanol in kerosene, 30 wt% tetradecanol in kerosene, oleyl alcohol, 50 wt% oleyl alcohol in a decane fraction, and 50 wt% oleyl alcohol in benzyl benzoate. Table 2 summarizes the experimental conditions used in batch extractive fermentations.

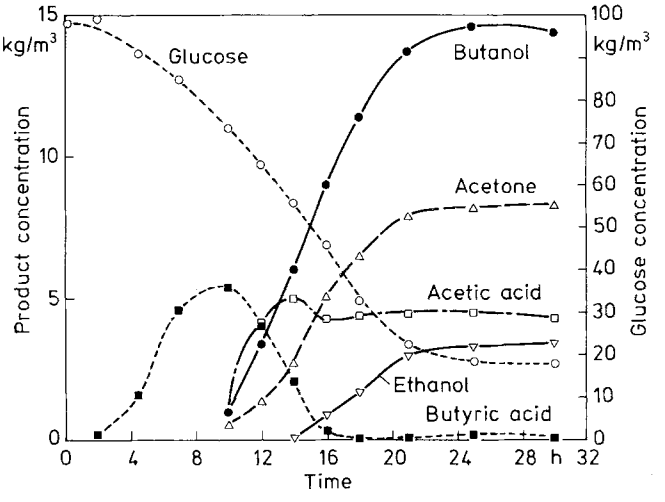

Fig. 2. Batch fermentation of C. acetobutylicum. Initial glucose concentration was 99 kg/m^3

Table 2. Summary of extractive fermentation experimental conditions

Solvent	Diluent	Solvent in diluent [%]	Volume solvent mixture added [m^3]	Ratio of solvent to broth volume
none	kerosene	–	$2 \cdot 10^{-3}$	1
dodecanol	kerosene	50	$1.2 \cdot 10^{-3}$	0.6
tetradecanol	kerosene	30	10^{-3}	0.5
oleyl alcohol	none	–	$1.1 \cdot 10^{-3}$	0.55
oleyl alcohol	decane fraction	50	$2 \cdot 10^{-3}$	1
oleyl alcohol	benzyl benzoate	50	$1.2 \cdot 10^{-3}$	0.6

3.3.1 Kerosene based extractive fermentations

Many extraction solvents must be diluted in another organic solvent before they can be used in an extraction process; the solvent may be a solid at process temperature or be too viscous to be an effective extractant. Kerosene was tested in extractive fermentation because it is often used to dilute solid or viscous extractants. Figure 3 shows results of an extractive fermentation in which $2 \cdot 10^{-3}\,m^3$ of kerosene was added to $2 \cdot 10^{-3}\,m^3$ of actively growing cells. In contrast to regular batch fermentation in which only $82\,kg/m^3$ of glucose was converted to products, $96\,kg/m^3$ of glucose was converted to products in extractive fermentation with kerosene. The increased glucose conversion can be attributed to the removal of some inhibitory butanol and acetone from the broth into the kerosene phase. The final concentrations of products in the broth were (in kg/m^3): 13.7 butanol; 7.9 acetone; 3.0 ethanol; 5.5 acetic acid; and 0.2 butyric acid. The kerosene phase contained about $2.7\,kg/m^3$ butanol and $0.8\,kg/m^3$ acetone at the end of fermentation. Kerosene is a poor extractant for both butanol and acetone as can be seen by the small amounts of these products extracted into the kerosene phase in Fig. 3. The distribution coefficients for butanol and acetone distributing between broth and kerosene are only 0.25 and 0.13, respectively. Although extractive fermentation using kerosene can reduce end product inhibition, an excessive amount of solvent would be required on an industrial scale. In addition, the fermentation products are diluted in the kerosene phase and their recovery would probably be more expensive than in regular batch fermentation. This experiment, however, did show that kerosene is compatible with Clostridium acetobutylicum and can be used as a diluent for viscous or solid extractants.

The extraction of butanol can be improved by adding an organic extractant to the kerosene phase. Accordingly, two long-chain alcohols were used as extractants in mixed solvent systems with kerosene: 50 wt% dodecanol in kerosene and 30 wt% tetradecanol in kerosene. In both cases, extraction of butanol was greatly increased by the addition of the alcohol extractants to the kerosene. For example, the butanol distribution coefficient was about 2.4 with the tetradecanol mixture and about 3.8 with the dodecanol mixture compared to 0.25 for kerosene alone. In extractive fermentations using these solvent mixtures, however, glucose conversion was reduced, indicating that these alcohols are partially toxic to Clostridium acetobutylicum. Glucose conversion was 78% with the dodecanol-kerosene mixture and 89% with the tetradecanol-kerosene mixture. Figure 4 shows the results of the extractive fermentation in which $1 \cdot 10^{-3}\,m^3$ of a tetradecanol-kero-

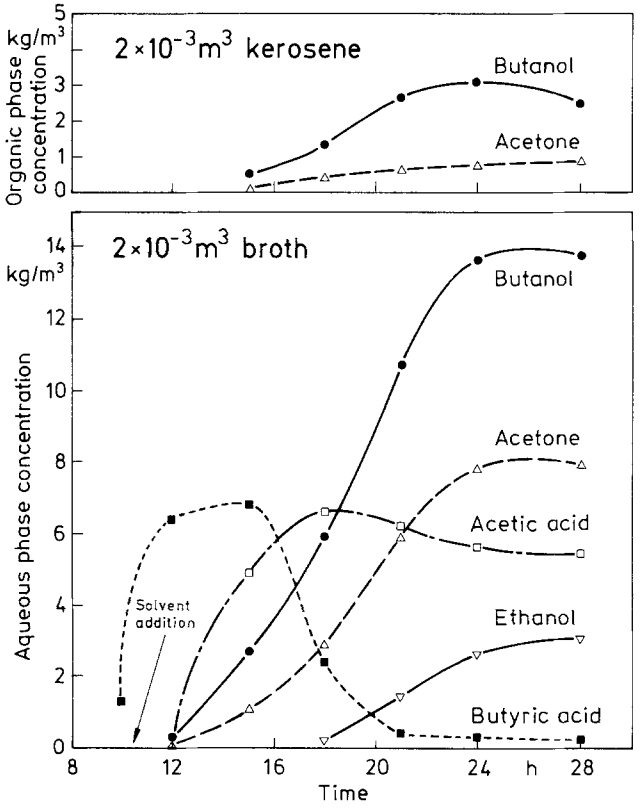

Fig. 3. Batch extractive fermentation of C. acetobutylicum using kerosene as the extraction solvent. $2 \cdot 10^{-3}\,m^3$ of kerosene were added to $2 \cdot 10^{-3}\,m^3$ of broth. Initial glucose concentration was $99\,kg/m^3$

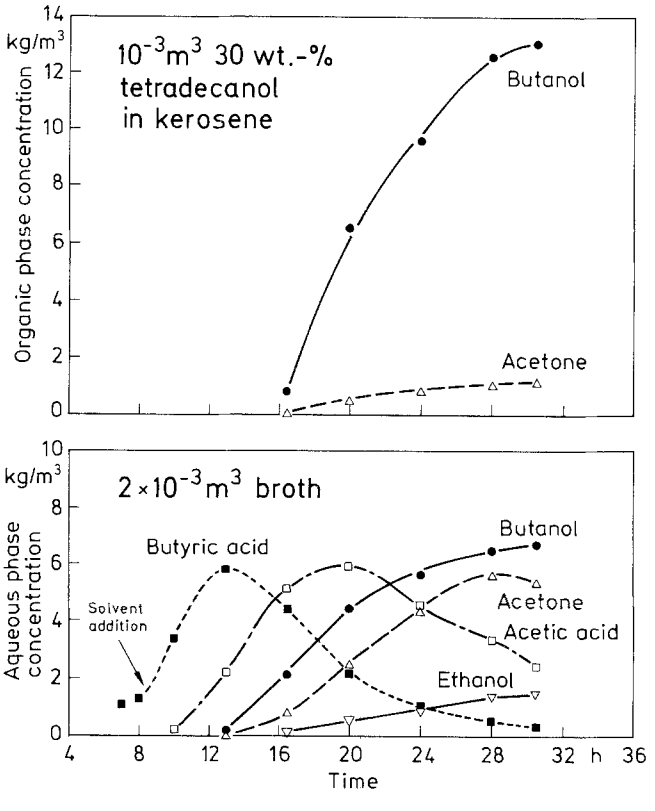

Fig. 4. Batch extractive fermentation of C. acetobutylicum using 30 wt% tetradecanol in kerosene as the extraction solvent. $1 \cdot 10^{-3}\,m^3$ of solvent was added to $2 \cdot 10^{-3}\,m^3$ of broth after the eighth hour of fermentation. Initial glucose concentration was $92\,kg/m^3$

sene mixture was added to $2 \cdot 10^{-3}$ m^3 of fermentation broth. At the end of fermentation, the broth phase contained 6.4 kg/m^3 butanol and 5.6 kg/m^3 acetone while the organic phase contained 12.4 kg/m^3 butanol and 1.0 kg/m^3 acetone. Fermentation stopped after about 40 h even though the concentration of butanol in the broth was below inhibitory levels again indicating that tetradecanol is partially toxic to Clostridium acetobutylicum. Similar results were obtained when a mixture of dodecanol and kerosene was used. At the end of fermentation, the aqueous phase contained 5 kg/m^3 butanol and 6.8 kg/m^3 acetone while the organic phase contained 15.7 kg/m^3 butanol and 1.8 kg/m^3 acetone. Although these solvent mixtures extract butanol well, they are not suitable for a large scale process because they inhibit the growth of the bacteria; extractive fermentation with these solvents shows no improvement over conventional batch-fermentation.

3.3.2 Oleyl alcohol based extractive fermentation

Oleyl alcohol was also tested for use in extractive fermentation. Oleyl alcohol has several properties that make it a good candidate for use in extractive fermentation. In contrast to dodecanol and tetradecanol, oleyl alcohol is a liquid at room temperature and can be used directly in extractive fermentations. Oleyl alcohol also appeared to be non-toxic to Clostridium acetobutylicum in initial toxicity tests. In addition, oleyl alcohol extracts butanol well. Figure 5 shows equilibrium curves for butanol distributing between water and several organic extraction solvents: oleyl alcohol, benzyl benzoate, 50 wt% oleyl alcohol in a decane fraction, and 50 wt% oleyl alcohol in benzyl benzoate. Of these solvents, oleyl alcohol was the best extractant for butanol with an average distribution coefficient (ratio of butanol in the solvent to butanol in the aqueous phase on a weight basis) of 4.4. Average distribution coefficients for the other solvents were: 2.8 oleyl alcohol in benzyl benzoate; 2.6 oleyl alcohol in decane fraction; and 1.4 benzyl benzoate. Oleyl alcohol not only extracts butanol well, but it also separated cleanly from the aqueous phase after extraction. For these reasons, oleyl alcohol was tested in extractive batch fermentation.

Figure 6 shows results of an extractive fermentation in which $1.1 \cdot 10^{-3}$ m^3 of oleyl alcohol were added to $2 \cdot 10^{-3}$ m^3 of broth. Fermentation was rapid and all glucose was consumed after 20 h. The concentration of butanol in the organic phase continued to increase for several more hours, indicating that the rate of mass transfer from the aqueous phase to the organic phase was slower than the rate of butanol production for much of the fermentation. At the end of fermentation, the aqueous phase contained 6.6 kg/m^3 butanol and 6.6 kg/m^3 acetone while the oleyl alcohol phase contained 24 kg/m^3 butanol and 2.6 kg/m^3 acetone. Butanol was maintained below inhibiting levels throughout the fermentation allowing rapid and complete conversion of glucose. In addition, because of the favor-

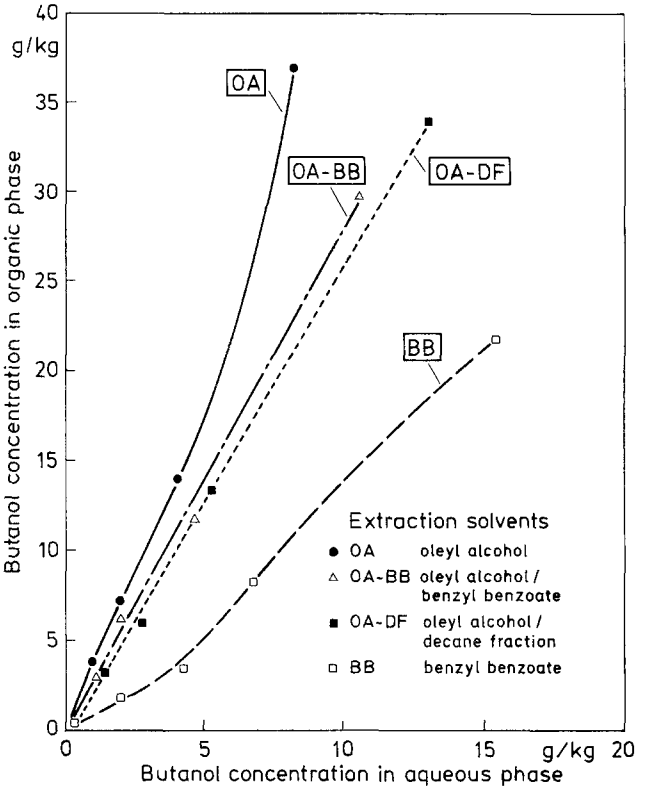

Fig. 5. Liquid-liquid equilibrium curves for butanol distributing between water and several organic solvents. Solvent mixtures contained 50 wt% of each component. Equilibration temperature was 21 °C

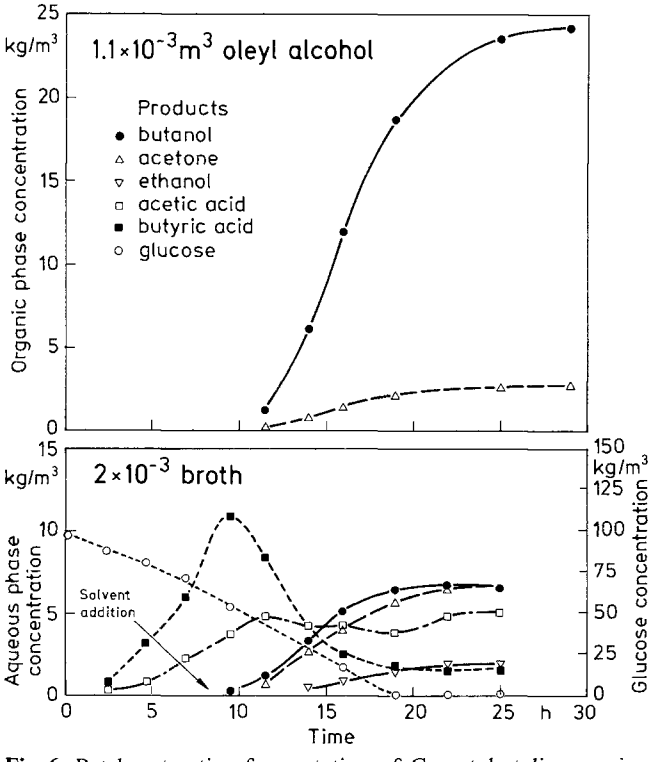

Fig. 6. Batch extractive fermentation of C. acetobutylicum using oleyl alcohol as the extraction solvent. $1.1 \cdot 10^{-3}$ m^3 of oleyl alcohol were added to $2 \cdot 10^{-3}$ m^3 of broth after the eighth hour of fermentation. Initial glucose concentration was 98 kg/m^3

able partition of butanol between oleyl alcohol and the aqueous phase, the concentration of butanol in the oleyl alcohol was higher than can be obtained in batch fermentation. This would help reduce down-stream separation costs.

Figure 7 compares butanol production in batch and extractive fermentations using oleyl alcohol, kerosene or tetradecanol in kerosene. The rate of butanol production in extractive fermentation using a mixture of tetradecanol and kerosene was slower than in batch fermentation, perhaps due to tetradecanol toxicity to the bacteria. Extractive fermentation using kerosene or oleyl alcohol, on the other hand, resulted in both greater and faster butanol production compared to conventional batch fermentation, indicating that end product inhibition can be reduced by the in situ removal of toxic metabolites.

Although extractive fermentation with oleyl alcohol was successful, there are several problems with scaling up a process using oleyl alcohol. Oleyl alcohol is fairly viscous, resulting in slow rates of mass transfer and phase separation. Extraction equipment would be large and expensive. Viscous solvents also tend to entrain water droplets which would have to be removed in subsequent separation steps. In addition, oleyl alcohol has a high boiling point range (282–349 °C) so that the distillation of butanol from oleyl alcohol would require the use of expensive high pressure steam in the reboiler. These problems can be reduced by diluting oleyl alcohol in a less viscous, lower boiling diluent. The solvent mixture will be less viscous, mass transfer and phase separation will be improved and less water will be entrained in the solvent. Also, a volatile diluent will lower the reboiler temperature of the butanol-solvent distillation so that less expensive, low pressure steam can be used. We used an inexpensive decane fraction with a boiling point range of 171 to 177 °C as a diluent for oleyl alcohol. Dilution of oleyl alcohol with this diluent effectively decreased the mixture viscosity. Figure 8 compares the viscosity of oleyl alcohol and a mixture of oleyl alcohol and decane fraction as a function of temperature. At 37 °C, oleyl alcohol has a viscosity of $1.7 \cdot 10^{-2}$ Ns/m^2 while the mixture of oleyl alcohol and decane fraction has a viscosity of only $3.1 \cdot 10^{-3}$ Ns/m^2.

A batch extractive fermentation was carried out in which $2 \cdot 10^{-3}$ m^3 of a 50 wt% oleyl alcohol in decane fraction mixture was added to 2 liters of broth. Fermentation was rapid and all glucose was consumed after 21 h. At the end of fermentation, the aqueous phase obtained 5 kg/m^3 butanol and 5.5 kg/m^3 acetone while the organic phase contained 10.7 kg/m^3 butanol and 1.6 kg/m^3 acetone. The absolute amounts of acetone, butanol, and ethanol at the end of fermentation, however, were less than those obtained when oleyl alcohol was used alone. The decrease in the production of these products appeared to be due to incomplete conversion of butyric acid. Butyric acid concentration at the end of fermentation was 4.8 kg/m^3 compared to 0.1 kg/m^3 in batch fermentation. The high concentration of butyric acid may be the result of toxicity of the decane fraction to the bacteria, or a shift in the bacterial metabolism due to the presence of the solvent. The decane fraction used in a complex mixture of many compounds having similar boiling points. Its effect on the bacteria could thus be reduced by using a higher boiling point mixture or using a more pure alkane diluent. In any case, the effect of the decane fraction on the bacteria was not severe as all glucose was consumed and butanol accumulation was rapid.

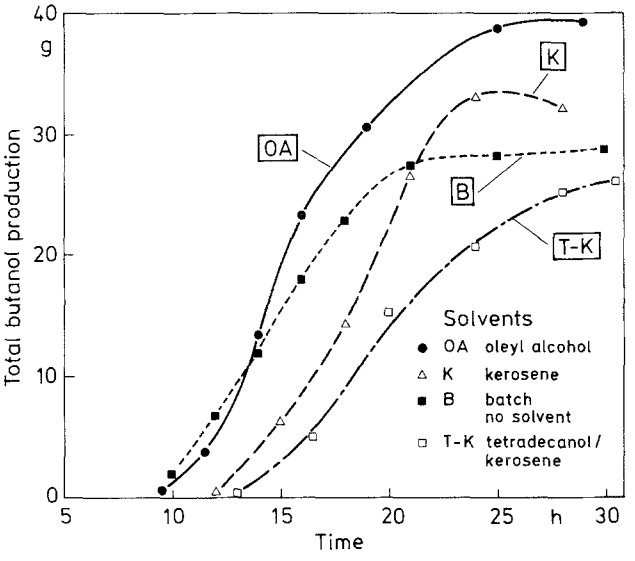

Fig. 7. Comparison butanol production in batch and batch extractive fermentations using oleyl alcohol, kerosene, or 30 wt% tetradecanol in kerosene as the extraction solvents. Broth volumes were $2 \cdot 10^{-3}$ m^3 and initial glucose concentrations ca. 100 kg/m^3

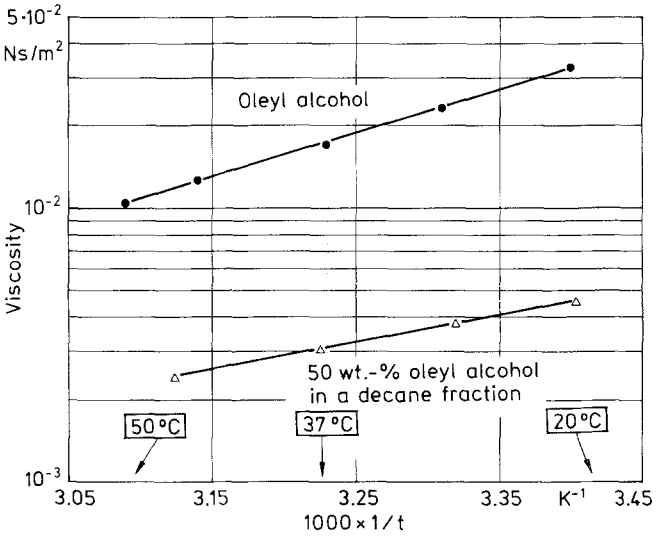

Fig. 8. Reduction of oleyl alcohol viscosity by dilution in a decane fraction. The decane fraction has a boiling point range of 171 to 177 °C

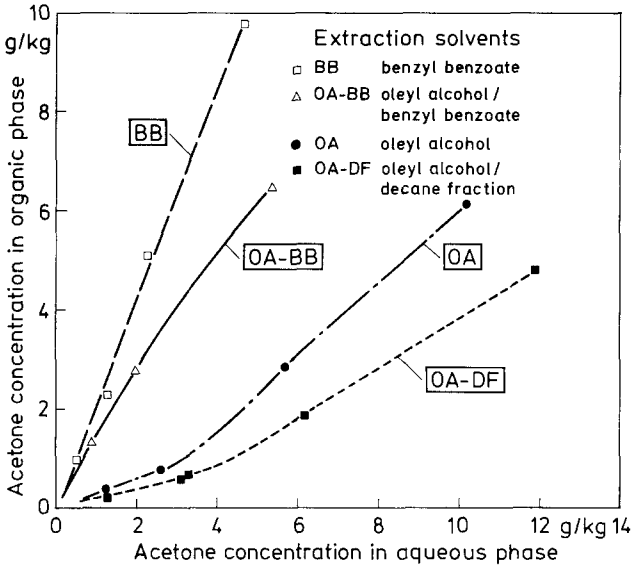

Fig. 9. Liquid-liquid equilibrium curves for acetone distributing between water and several organic solvents. Solvent mixtures contained 50 wt% of each component. Equilibrium temperature was 21 °C

Although oleyl alcohol extracts butanol well, it is a poor extractant for acetone. In cases where it is desirable to extract both acetone and butanol, another solvent might be more suitable. Benzyl benzoate was found to extract acetone well in liquid-liquid equilibrium studies. Figure 9 shows equilibrium curves for acetone distributing between four organic solvents and water. Of these solvents, benzyl benzoate is the best extractant for acetone with an average distribution coefficient (on a weight basis) of 2.1. Oleyl alcohol, on the other hand, extracts acetone poorly, having an average distribution coefficient of only 0.4. As expected, oleyl alcohol diluted in a decane fraction also extracted acetone poorly with a distribution coefficient of 0.3. Although benzyl benzoate is a good extractant for acetone, it extracts butanol poorly. Both acetone and butanol can be extracted, however, by using a mixture of benzyl benzoate and oleyl alcohol as the extraction solvent. A mixture of 50 wt% benzyl benzoate and oleyl alcohol gave an average distribution coefficient of 1.2 for acetone and 2.8 for butanol. This solvent mixture was thus tested in an extractive fermentation in which $1.2 \cdot 10^{-3}\,m^3$ of solvent mixture was added to $2 \cdot 10^{-3}\,m^3$ of broth. Figure 10 shows results of that fermentation. Again, glucose was rapidly depleted, indicating a reduction of end product inhibition by extractive fermentation. In addition, more acetone was produced than normal. At the end of fermentation, the aqueous phase contained 7 kg/m^3 butanol and 7.6 kg/m^3 acetone while the organic phase contained 20.5 kg/m^3 butanol and 7.4 kg/m^3 acetone. The yields of butanol and acetone were 0.18 and 0.115 respectively in extractive fermentation using the oleyl alcohol-benzyl benzoate mixture compared with 0.19 and 0.08 for extractive using oleyl alcohol alone. It thus appears that the product distribution is shifted toward overproduction of acetone when more acetone is extracted from the fermentation broth.

Table 3 lists the concentrations of products (based on the volume of broth) obtained in batch and extractive batch fermentation while Table 4 lists the yields of the products based on glucose conversion. The best results were obtained in extractive batch fermentation using oleyl alcohol or a mixture or oleyl alcohol and benzyl benzoate as the extraction solvent. For example, using oleyl alcohol as the solvent, 19.7 kg/m^3 butanol was produced compared to 14.6 kg/m^3 in regular batch fermentation. In all extractive fermentations, however, the yield of ethanol was lower than in batch fermentation. The yield of acetone was also lower in all runs except the one in which benzyl benzoate and oleyl alcohol were used together. The presence of the extraction solvents may have acted to shift the product distribution to other by-products. Acetoin was detected in all fermentations but its final concentration was always less than 1 kg/m^3. The yield of butanol was not affected when oleyl alcohol or a mixture of oleyl alcohol and benzyl benzoate were used. Thus, these solvents appear to have the most promise for use in the extractive fermentation of butanol.

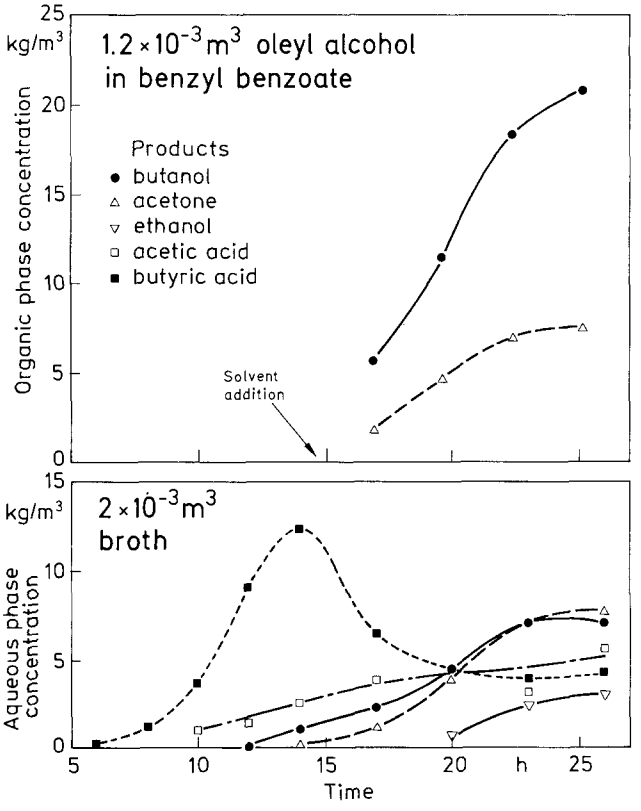

Fig. 10. Batch extractive fermentation of C. acetobutylicum using 50 wt% oleyl alcohol in benzyl benzoate as the extraction solvent. $1.2 \cdot 10^{-3}\,m^3$ of solvent was added to $2 \cdot 10^{-3}\,m^3$ of broth after the fourteenth hour of fermentation. Initial glucose concentration was 103 kg/m^3

Table 3. Product accumulation during batch and extractive batch fermentation

	Batch	Kerosene	Dodecanol in kerosene [50 wt%]	Tetradecanol in kerosene [30 wt%]	Oleyl alcohol	Oleyl alcohol in decane fraction [50 wt%]	Oleyl alcohol in benzyl benzoate [50 wt%]
Solvent/broth volume	–	0.95	0.6	0.5	0.55	1	0.6
Butanol [kg/m^3]	14.6	16.4	14.4	13.9	19.7	15.4	19.3
Acetone [kg/m^3]	8.3	8.7	7.9	6.9	8.0	7.0	12.0
Ethanol [kg/m^3]	3.4	3.0	1.8	1.7	2.1	1.5	3.0
Acetic acid [kg/m^3]	4.3	5.5	3.4	5.8	4.8	4.9	4.3
Butyric acid [kg/m^3]	0.1	0.3	2.5	0.3	0.16	4.8	4.0
Glucose conversion	82%	97%	78%	89%	100%	100%	100%

Table 4. Product yields during batch and batch extractive fermentation

	Batch	Kerosene	Dodecanol in kerosene [50 wt%]	Tetradecanol in kerosene [30 wt%]	Oleyl alcohol	Oleyl alcohol in decane fraction [50 wt%]	Oleyl alcohol in benzyl benzoate [50 wt%]
Butanol	0.18	0.17	0.17	0.16	0.19	0.17	0.18
Acetone	0.10	0.09	0.09	0.08	0.08	0.08	0.115
Ethanol	0.04	0.03	0.02	0.02	0.02	0.02	0.03
Acetic acid	0.05	0.06	0.04	0.03	0.05	0.06	0.05
Butyric acid	0.001	0.002	0.03	0.002	0.02	0.05	0.04

Yields expressed as kg product/kg glucose

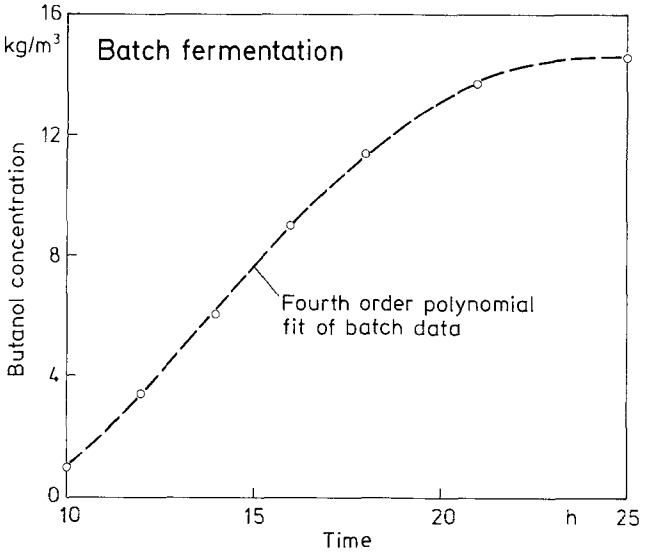

Fig. 11. Example of polynomial fit of butanol concentration data used in the calculation of instantaneous volumetric butanol productivities. A fourth order polynomial was fit through this data using a least squares routine

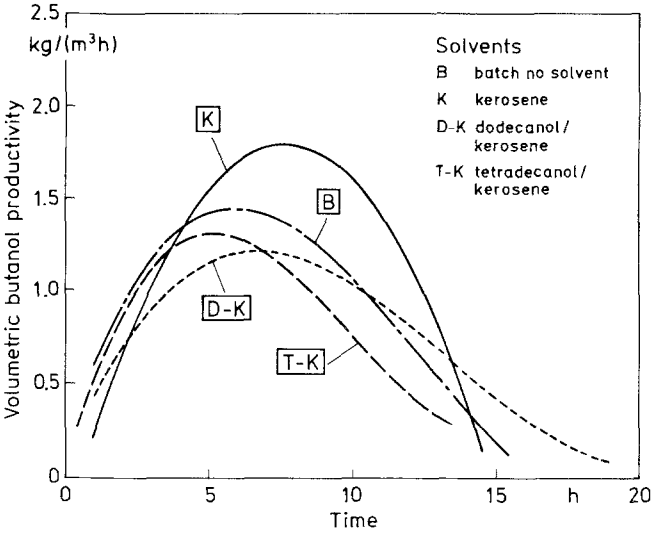

Fig. 12. Volumetric butanol productivities during batch extractive fermentations of C. acetobutylicum using kerosene based extraction solvents. Solvent mixtures contained 50 wt% of each component. Productivities were based on the broth volume ($2 \cdot 10^{-3}$ m^3)

3.4 Comparison of in situ product removal fermentations

In order to compare results of batch extractive fermentation with regular batch fermentation and other methods of in situ product removal, volumetric butanol productivities were calculated for the fermenations carried out in this work. Volumetric butanol productivities were based on the volume of the aqueous phase. Overall butanol productivities were calculated as total grams of butanol divided by the fermentation time and volume of broth. Instantaneous butanol productivities were also calculated. First a least squares routine was used to fit polynomials to the

total butanol concentration data, again based on the volume of the aqueous phase. Figure 11 shows the total butanol concentration data for a regular batch fermentation along with the polynomial fit through the data. Instantaneous volumetric productivities were then calculated by differentiating the polynomials with respect to time. Figures 12 and 13 show the instantaneous butanol volumetric productivities over the course of fermentation for batch and extractive batch fermentations. Figure 12 compares productivities in batch and kerosene based extractive fermentations. Extractive fermentation using kerosene resulted in a higher productivity than could be obtained in batch culture indicating that end product inhibition was reduced by extractive fermentation. Productivities were lowered, however, when tetradecanol or dodecanol were used with kerosene due to their toxicity to the microbes. Figure 13 compares productivities in batch and oleyl alcohol based extractive fermentations. Extractive fermentations using oleyl alcohol based extractants has maximum butanol productivities 40 to 60% greater than regular batch fermentation. The highest productivity was obtained in extractive batch fermentation using a mixture of oleyl alcohol and benzyl benzoate. This is probably because this solvent mixture can extract both butanol and acetone so that end product inhibition due to both compounds is reduced.

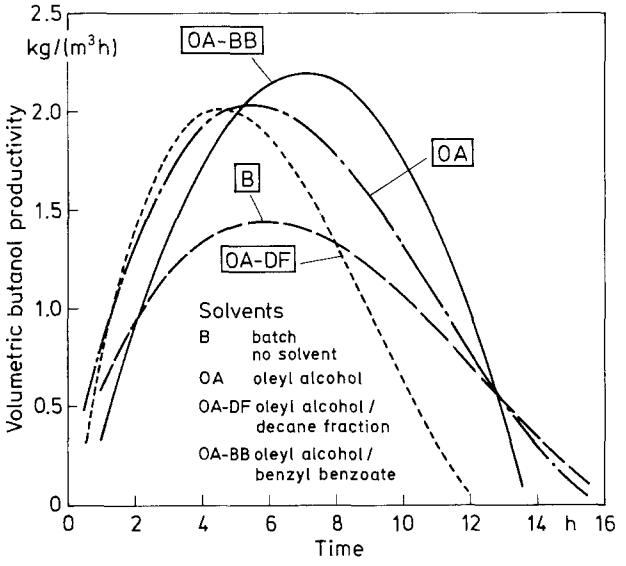

Fig. 13. Volumetric butanol productivities during batch extractive fermentations of C. acetobutylicum using oleyl alcohol based extraction solvents. Solvent mixtures contained 50 wt% of each component. Productivities were based on the broth volume ($2 \cdot 10^{-3}$ m^3)

Table 5 summarizes the results from this work and compares them with results obtained in other studies of in situ product removal. Regular batch fermentation in this work had a maximum butanol productivity of 1.4 kg/(m^3 h) and an overall productivity of 0.58 kg/(m^3 h). This compares favorably with results obtained in other studies. The maximum productivity obtained with extractive fermentation in this study was 2.2 kg/(m^3 h) with an overall productivity of 0.7 kg/(m^3 h). The productivities are sev-

Table 5. Comparison of batch fermentation with and without in situ product removal

	Maximum butanol volumetric productivity [kg/(m^3 h)]	Overall butanol volumetric productivity [kg/(m^3 h)]	Glucose consumed [kg/m^3]
Traditional			
batch fermentation	1.4	0.58	81
batch [4]	1.3	0.51	65
batch [16]	0.62	0.23	29
batch [13]	–	0.26	40
batch [20]	–	0.22	40
Extractive batch fermentation			
kerosene	1.8	0.69	96
dodecanol in kerosene	1.2	0.53	85
tetradecanol in kerosene	1.3	0.43	82
oleyl alcohol	2.0	0.72	98
oleyl alcohol in decane fraction	2.0	0.71	90
oleyl alcohol in benzyl benzoate	2.2	0.74	103
oleyl alcohol [16]	0.24	0.13	73
Other fermentations			
solid adsorbent [9]	–	0.17	400*
prevaporation [6]	0.32	0.13	71
aqueous two phase [13]	–	0.24	40

* Used fed-batch operation

eral times greater than those obtained using oleyl alcohol in another study [16], possibly due to differences in the strain of Clostridium acetobutylicum used.

In a study that used pervaporation to remove butanol and isopropanol from a fermentation using Clostridium beyerinckii, maximum alcohol productivity was only 0.32 kg/(m^3 h) [6]. In another study, butanol was removed from a fed-batch fermentation using a solid adsorbent [9]. The overall butanol productivity, however, was only 0.17 kg/(m^3 h). In a study that attempted to reduce butanol toxicity by using an aqueous two-phase extraction system, the volumetric productivity was not improved over that obtained in regular batch fermentation. In fact, the methods of in situ product removal previously tested in batch operation appear to actually decrease the volumetric productivity of the acetone-butanol fermentation as compared to regular batch fermentation. The results of this study, however, show that an increase in volumetric productivity is possible through the use of extractive fermentation.

4 Conclusion

Several improvements can be made in the extractive fermentation system used in this study. Economically, it is an advantage to use concentrated feed stocks in order to reduce equipment sizes and waste treatment costs. Although 100 kg/m^3 of glucose could be fermented in the extractive fermentation system, attempts to ferment 150 kg/m^3 glucose were unsuccessful, perhaps due to catabolite repression. Others have also observed that glucose concentrations above 100 kg/m^3 retarded fermentation by Clostridium acetobutylicum [17, 19]. More concentrated feeds could be used if a fed-batch fermentation was used. Concentrated substrate could be slowly added to the fermentor so that its concentration never exceeds inhibitory levels. Another advantage of fed-batch operation is that the fermentation could be carried out for extended periods of time so that less time would be wasted on turn arounds. Also, longer fermentation times might allow more butyric acid to be converted to products.

An improved extractive fermentation system requires that the solvent used be not only a good extractant for butanol, but that the recovery of butanol from the solvent be economical. An economic analysis of the entire process is necessary in order to determine the optimum solvent mixture resulting in minimum process costs. Other changes such as increasing the rate of mass transfer during extraction and continuously feeding fresh solvent to the fermenter or extraction vessel will also improve the extractive fermentation process.

Extractive fermentation has been shown to be beneficial to the acetone butanol fermentation. The best results were obtained with oleyl alcohol or a mixture of oleyl alcohol and benzyl benzoate. Glucose conversion was increased from 81 kg/m^3 in batch fermentation to over 100 kg/m^3 in extractive fermentation using these solvents. Similarly, the maximum butanol volumetric productivity was increased from 1.4 kg/(m^3 h) in batch fermentation to over 2 kg/(m^3 h) in extractive fermentation using these solvents. Extractive fermentation has promise for use on an industrial scale and should be relatively easy to scale up using standard chemical engineering techniques.

Acknowledgement

The authors wish to acknowledge the help of Joe Cho, Bernie Edrada, Andy Lin and Randy Lee. This research was supported by a grant from the Center for Biotechnology Research, San Francisco, California.

References

1. Hastings, J. J. H.: Acetone-butyl alcohol fermentation. In: Rose, A. H. (Ed.): Economic Microbiology, vol. 2, pp. 31–44. New York: Academic Press 1978
2. Walton, M. T.; Martin, J. L.: Production of butanol-acetone by fermentation. In: Peppler, H. J.; Perlman, D. (Eds.): Microbial Technology, vol. 1, pp. 187–209. New York: Academic Press 1979
3. Leung, J. C. Y.; Wang, D. I. C.: Production of acetone and butanol by Clostridium acetobutylicum in continuous culture using free cells and immobilized cells. In: Robinson, C. W. (Ed.): Proceedings of the 2nd World Congress of Chemical Engineering and World Chemical Exposition, pp. 348–352. Montreal, Canada, October 4–9, 1981
4. Murphy, T. K.; Blanch, H. W.; Wilke, C. R.: Water recycle in extractive fermentation. Process Biochem. 17 (1982) 6–9
5. Linden, J. C.; Moreira, A. R.; Lenz, T. G.: Acetone and butanol. In: Moo-Young, M. (Ed.): Comprehensive Biotechnology, vol. 3, pp. 915–931. New York: Pergamon Press 1985
6. Groot, W. J.; Van den Oever, C. E.; Kossen, N. W. F.: Pervaporation for simultaneous product recovery in the butanol/isopropanol batch fermentation. Biotechnol. Lett. 6 (1984) 709–714
7. Groot, W. J.; Schoutens, G. H.; Van Beelen, P. N.; Van den Oever, C. E.; Kossen, N. W. F.: Increase of substrate conversion by pervaporation in the continuous butanol fermentation. Biotechnol. Lett. 6 (1984) 789–792
8. Garcia, A.; Iannotti, E. L.; Fischer, J. R.: Reverse osmosis application for butanol-acetone fermentation. Biotechnol. Bioeng. Symp. No. 14 (1984) 543–552
9. Larsson, M.; Holst, O.; Mattiasson, B.: Butanol fermentation using a selective adsorbent for product recovery. In: Third European Congress on Biotechnology, vol. 2, pp. 313–316. München, Fed. Rep. of Germany, September 10–14, 1984
10. Heady, R. E.; Frankiewicz, J. R.: U.S. Patent 4,520,104 (1985)
11. Kuhn, I.: Alcoholic fermentation in an aqueous two-phase system. Biotechnol. Bioeng. 22 (1980) 2393–2398
12. Hahn-Hagerdal, B.; Mattiasson, B.; Albertsson, P.-A.: Extraction bioconversion in aqueous two phase systems. A model study on the conversion of cellulose to ethanol. Biotechnol. Lett. 3 (1981) 53–58

13. Mattiasson, B.; Suominen, M.; Andersson, E.; Haggstrom, L.; Albertsson, P.-A.; Hahn-Hagerdal, B.: Solvent production by Clostridium acetobutylicum in aqueous two-phase systems. In: Chibata, I.; Fukui, S.; Wingard Jr., L. B. (Eds.): Enzyme Engineering, vol. 6, pp. 153–155. New York: Plenum Press 1982
14. Mattiasson, B.; Hahn-Hagerdal, B.: Utilization of aqueous two-phase systems for generating soluble immobilized preparations of biocatalysts. In: Mattiasson, B. (Ed.): Immobilized cells and organelles, vol. 1, pp. 121–134. Boca Raton, Florida: CRC Press 1983
15. Hashimoto, Y.: Optimization of an extractive fermentation process for the production of butanol. M.Sc. Thesis, Univ. of Pennsylvannia, Philadelphia 1979
16. Ishii, S.; Taya, M.; Kobayashi, T.: Production of butanol by Clostridium acetobutylicum in extractive fermentation system. J. Chem. Engr. Japan. 18 (1985) 125–130
17. Taya, M.; Ishii, S.; Kobayashi, T.: Monitoring and control for extractive fermentation of Clostridium acetobutylicum. J. Ferment. Technol. 63 (1985) 181–187
18. Annual Technical Report of the Research Assoc. for Petroleum Alternatives Develop., Chiyoda-ku, Tokyo 101, Japan, September, 1985
19. Linden, J. C.; Paige, M. R.; Doremus, M. D.: Fed-Batch Fermentations of Clostridium acetobutylicum. Presented at the AIChE National Meeting, San Francisco, CA, November 27, 1984
20. Ennis, B. M.; Maddox, I. S.: Use of Clostridium acetobutylicum P262 for production of solvents from whey permeate. Biotechnol. Lett. 7 (1985) 601–606

Received May 27, 1986

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