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Simultaneous Fermentation and Separation in the Ethanol and Abe Fermentation

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SIMULTANEOUS FERMENTATION AND SEPARATION IN THE ETHANOL AND ABE FERMENTATION

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ABSTRACT

Simultaneous separation of solvent products during fermentation reduces product inhibition and increases reactor productivity. Separation techniques used for simultaneous extraction during ethanol fermentation and butanol-acetone fermentation are reviewed. These techniques can be classified by product removal into gas phase (vacuum fermentation, gas stripping), liquid phase (liquid-liquid extraction, aqueous two-phase system), and solid phase (adsorption). Recent developments in separation techniques use membranes. Membrane separation techniques remove products into gas phase (pervaporation) and into liquid

phase (perstraction) using either solid or liquid membranes. Liquid extractants which are nontoxic to microorganisms are required at large quantities because of their poor distribution coefficients. The required amount of solid adsorbents is also large. Gas stripping, pervaporation and perstraction share an advantage of clean product separation. Pervaporation and perstraction can overcome gas-liquid equilibrium unlike gas stripping, but have experienced flux limitation. This limitation can be resolved by developing new membranes with higher flux. Since perstraction requires alcohol recovery from extractants, pervaporation seems to be the most promising technique, but gas stripping is also attractive for large scale application.

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1. INTRODUCTION

The terms extractive bioconversion, in-situ separation and simultaneous separation describe the concept of product removal from the site of its production to increase productivity or performance of biochemical processes. Product removal increases reactor performance by reducing product inhibition or increasing product stability.[90]

Most studies on extractive bioconversion deal with extracellular products located outside the microbial cells. These products are generally small molecules such as alcohols and organic acids, which inhibit the cell membrane function. Extractive conversion of intracellular microbial products has not attracted much attention because it is difficult to release intracellular products without affecting cell viability. Intracellular products from microbial cells are separated after the cell mass is destroyed.

Extractive bioconversion is potentially more valuable in plant cell systems because plant cells grow slowly and cell mass is more valuable. Some studies on plant cell permeabilization showed that plant secondary metabolites, which are normally stored in the vacuoles, can be released outside the cell without affecting cell

viability.[6,7,108] However, it has not been shown whether product release combined with simultaneous separation will increase overall productivity of secondary metabolites.

Extractive bioconversion is more useful in continuous processes combined with retention of cell mass and cell activity using either cell immobilization or cell recycle. Open systems such as chemostat, repeated fed-batch fermentors and packed bed fermentors are preferred to closed systems such as batch fermentors for extractive bioconversion. Open systems are preferred because additional nutrients need to be added to support increased substrate conversion and because bleeding is necessary to remove products which are not easily extracted, inorganic salts, non-fermentable substrates, and aged cells.

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Many different extractive bioconversion techniques have been studied. They use product partitioning or equilibrium between gas-liquid, liquid-liquid, and liquid-solid systems with or without membrane assistance. Specific examples are shown in Table I.

The discussion of this paper is limited to extractive bioconversion applied to ethanol and butanol fermentation processes. In most of the studies the actual extraction takes place in the fermentor. Some studies extract solvents in an outside module by rapidly circulating fermentation broth in a closed loop through the fermentor and the outside module. Membrane filtration and cell recycle removing substrates along with products is not discussed.

In the following sections extractive fermentation techniques and problems associated with each technique are discussed. Extractive fermentation was reviewed previously[91] and edited as a book by Mattiasson and Holst.[90]

2. PRODUCT/BY-PRODUCT INHIBITION

Product concentrations during ethanol and butanol fermentation are low because high concentration of products have

TABLE I

Examples of extractive bioconversion techniques.

membrane	product removal into gas	liquid	solid
without (1)	vacuum gas stripping	liq-liq extraction aqueous two phase	adsorption
with (2)	pervaporation	perstraction	

(1) without membrane assistance

(2) with membrane assistance

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detrimental effects on the growth and fermentation of the microorganism by imposing severe damage to the cell membrane function. Alcohols increase membrane fluidity and decrease membrane bound enzyme activity for sugar transport.[97] Alcohols also increase membrane leakage and reduce intracellular concentration of cofactors and coenzymes essential for the activity of enzymes involved in glycolysis and alcohol production.[106] The effects of alcohols on microorganisms are discussed.[57,58]

Tolerance of microorganisms to ethanol is dependent on strain, temperature and other conditions, but microorganisms usually experience strong inhibition at approximately 5 to 8 wt% of ethanol. Butanol production is limited to 14 - 15 g/L during fermentation with a 6:3:1 product ratio (butanol:acetone:ethanol) and 1 - 3 g/L of organic acid production. Butanol is the primary toxic substance during normal fermentation without extraction because complete growth inhibition is observed at 17 g/L of butanol. Inhibition by other products took place at much higher

levels than normally obtained during fermentation (70 g/L of acetone, 70 g/L of ethanol, 9 g/L of butyric acid, or 11 g/L of acetic acid).[16] Acetic and butyric acids are much stronger potential inhibitors based on an equal concentration level (g/L).

Extractive fermentation changes the composition of the fermentation broth after operation over an extended period of time and adverse production conditions may develop. Glycerol accumulation may cause problems in extractive ethanol fermentation.[71] For extractive fermentation processes removing volatile components preferentialy, formic acid and acetic acid have been shown to be the most inhibitory by-products during ethanol fermentation, with 80% cell mass reduction in continuous cultures at concentrations of 2.7 and 7.5%, respectively.[82] As formic acid is removed preferentially because of its higher volatility, acetic acid is likely to become the most toxic by-product. Acetic and butyric acids were the major inhibitory compounds in extractive butanol fermentation.[107] Bleeding may decrease the build-up of non-extractable or non-volatile by-products to a limited extent but the system eventually confronts new limitations by the accumulation of unwanted products.

A new strain *Clostridium acetobutylicum* B18 is potentially useful for extractive butanol fermentation because it completely recycles butyric acid for butanol production under certain conditions, and acetic acid production is low.[109,124]

3. NON-MEMBRANE BASED SEPARATION TECHNIQUES

3.1 Product Removal into Gas Phase

Vacuum fermentation and gas stripping remove products into the gas phase using product volatility. The limit of these processes is given by vapor-liquid equilibrium of aqueous product solution. Recent researches have been focused on gas stripping because alcohol (ethanol or butanol) fermentation produces gases

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which impose problems in vacuum fermentation but serve as free extractants in gas stripping processes.

3.1.1 Vacuum Fermentation

Vacuum fermentation systems preferentially remove fermentation products that exert a greater vapor pressure than water (Figure 1a). A pressure is chosen such that the fermentation broth boils at the fermentation temperature, for example 32 mm Hg at 30°C. In vacuum fermentation the fermentation gas must also be removed, which increases the processing cost. Vacuum fermentation requires a very large compressor and is plagued by severe ethanol condensation problems due to the large amount of a non-condensable gas, CO_2.

As ethanol and water vapor are removed during vacuum fermentation, nonvolatile feed components, such as salts, and nonvolatile by products, such as organic acids and longer chain alcohols, accumulate in the fermentor. Most of these species are ionic or polar, and depress the activity and the vapor pressure of water, resulting in an increase in the relative ethanol volatility. Roychoudhury et al.[125] reported that the maximum relative volatility of ethanol (13.92) obtained by using ethanol-cellulase-treated rice straw-nutrients-water mix was twice that of the ethanol-water system. This volatility effect diminished as the liquid ethanol concentration increased, but remained significant: 47% at 22 wt % ethanol concentration.

Ramalingham and Finn[116] showed that oxygen deficiencies occurred under vacuum conditions, and therefore ethanol fermentation was not as vigorous as expected even after saturating the feed stream with oxygen.

Cysewski and Wilke[18] studied cell recycle fermentations with atmospheric and vacuum conditions. Ethanol productivity increased from 29 g/L-h to 82 g/L-h under vacuum conditions. In order to maintain a viable yeast culture in the vacuum fermentor, a bleed and pure oxygen sparging was required.

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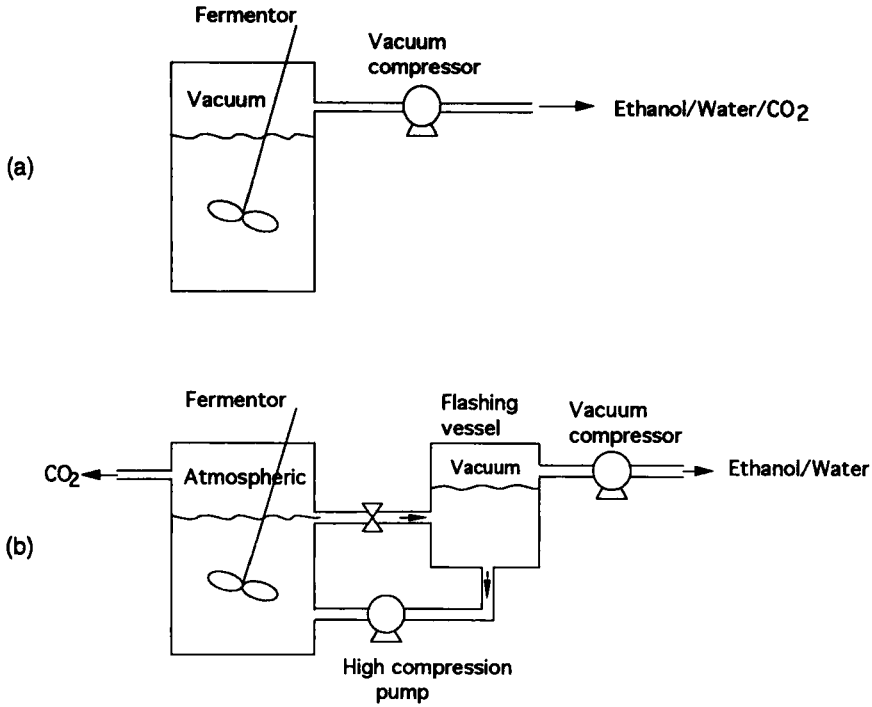

Figure 1. Vacuum fermentation and "Flashferm" vacuum fermentation

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To overcome drawbacks related to the gases (carbon dioxide and unconsumed oxygen) present in the vapor product stream, "Flashferm" was proposed.[139] In this process fermentation was conducted at atmospheric pressure, and ethanol-rich vapor was removed from a separate reduced-pressure flash vessel through which the broth was continually cycled (Figure 1b). Flashferm with *Zymomonas mobilis* was studied with cell recycling.[75] Productivity of 85 g/L-h was obtained with a condensate ethanol concentration of 200 g/L. In an economic analysis neither "Vacuferm" nor "Flashferm" offered any cost advantage over recycling CSTR (continuous stirred tank reactor).[83]

Ghose et al.[41] studied simultaneous saccharification and fermentation (SSF) of lignocellulosics to ethanol under vacuum cycling and feeding. Vacuum cycling was done in a flash chamber at 80 mm Hg as follows: as soon as the ethanol concentration in the fermentor reached 22-23 g/L, the broth was circulated between the flash vessel and the fermentor for 1 hr. Without removal of ethanol, only 23 g/L of ethanol could be produced because of ethanol inhibition of saccharification. Intermittent substrate feeding in conjunction with vacuum cycling increased ethanol productivities more than three times as compared with SSF without vacuum cycling. Also, SSF with vacuum cycling itself increased the ethanol productivity 1.4-fold compared to SSF without vacuum cycling. Vacuum-cycling operation increased cellulose utilization by 40% compared to SSF without vacuum cycling.

Vacuferm has an attractive feature of clean product separation, and ethanol productivity of up to 82 g/L-h has been reported.[18,19] However, unresolved difficulties with Vacuferm process are the necessity of pure oxygen sparging to meet oxygen demand[19], bleeding to reduce the accumulation of toxic by-products[18], and a very large compressor because of large amount of CO_2 production.[40]

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3.1.2 Gas Stripping

Gas stripping is a process driven by vapor-liquid equilibrium. Even though Walsh et al.[137] used CO_2 gas stripping in a pulsed fed, suspension culture of *S. cerevisiae*, the purpose was to produce a clean ethanol feed stream for a subsequent adsorption process. Dale et al.[21] first reported improved ethanol productivity using gas stripping in an immobilized cell reactor-separator (ICRS). The ICRS consists of two glass columns: In the enricher the trickling liquid is in cocurrent contact with fermentation gas, and in the stripper the liquid is in countercurrent contact with gas upflow (Figure 2). Ethanol productivity increased from 66 to 73.5 g/L-h in the enricher and from 4.3 to 16.4 g/L-h in the stripper

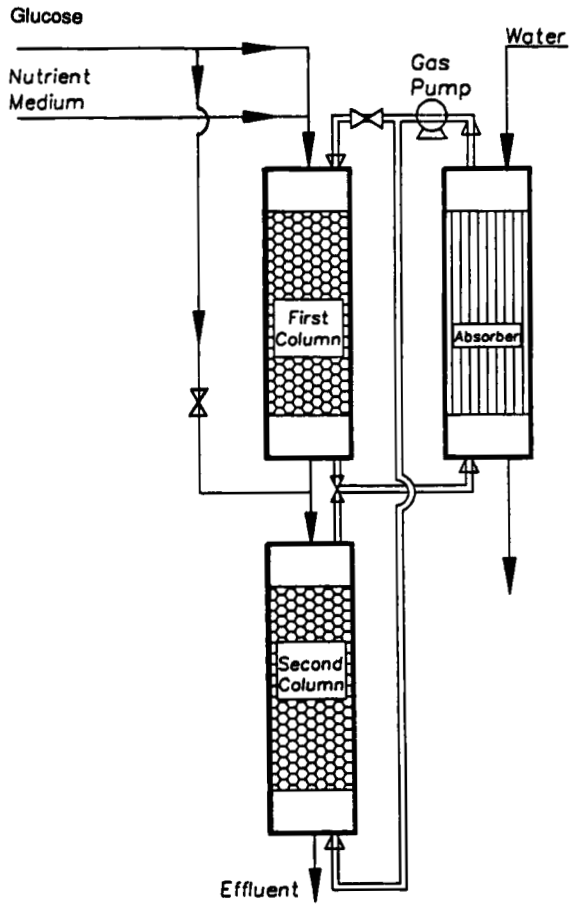

Figure 2. Extractive fermentation with gas stripping in an immobilized cell trickle bed reactor; single line (———) is liquid, double line (═══) is gas.

with gas stripping. This productivity improvement agreed with the theoretical analysis.[20] The advantages of using gas stripping during ethanol fermentation in a CSTR were discussed theoretically[79,80], but the study was not supported by experimental study.

Gas stripping was applied to acetone-butanol fermentation in a batch culture with *C. acetobutylicum* P262 using whey permeate[29].

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A stripping-gas (nitrogen) flow rate of 2.7 L/L-min was required to achieve an average butanol removal rate of 0.30 g/L-hr over a 12-hr period. The selectivity of butanol removal by gas stripping/condensation was found to be 19.3 using the equation proposed by Groot et al.[42] Overall fermentation productivity increased from 0.22 g solvent/L-hr for the control to 0.31 g solvent/L-h with gas stripping.

Ennis et al.[30] compared gas-stripping (using nitrogen), an adsorbent resin (XAD-16) and a molecular sieve (silicalite) for use in a two-stage continuous reactor. Cells of *C. acetobutylicum* P262 were immobilized by adsorption onto bonechar, and solvents were removed in between stages. Gas stripping was the most successful method, possibly because the other techniques removed essential nutrients in addition to solvents. Gas stripping removed significant quantities of acetone, butanol and ethanol but not acetic and butyric acids.

Extractive isopropanol-butanol fermentation using gas stripping was studied in batch and continuous fermentors with free cells.[46] Butanol in the fermentation broth was recovered in an external stripper, and the broth was recycled to the fermentor. In comparison with a control fermentor without product removal, gas stripping increased substrate consumption from 37 g/L to 126 g/L in batch fermentation, and productivity from 0.36 g/L-h to 1.0 g/L-h in continuous fermentation.

Qureshi and Maddox[115] studied extractive acetone-butanol fermentation by gas stripping using nitrogen gas. Cells were immobilized onto bonechar in a fluidized bed reactor. Gas stripping took place in an external sparger made of glass reactor vessel controlled at 65-67°C. At a dilution rate of 1.37 hr^{-1} a reactor productivity of 5.1 g/L-h was achieved. The solvents in the stripping gas were condensed to give a solution of 53.7 g/L. They explained that the high solvent yield was due to the fact that acetic and butyric acid were not removed by gas stripping.

Extractive acetone-butanol fermentation was applied to a trickle bed reactor.[107] The mode of gas-liquid contact was

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essentially the same as Dale et al.[21] for ethanol fermentation. Cells were immobilized on polyester sponge strips which were fixed by fabricated iron wire screens. Solvents were stripped preferentially from the fermentation broth. Butanol removal was as efficient as acetone removal in spite of butanol's high boiling point (117°C) because of butanol's high volatility at fermentation concentrations. Since most of the butanol was removed by gas stripping, organic acids played major inhibiting roles. Experiments showed that up to 87.4% of butanol and up to 37.3% and 18.3% of butyric and acetic acids, respectively were recovered by using a water absorber. With this removal of toxic products from the fermentor, glucose conversion improved by 33.6 and 54.7% at feed glucose concentrations of 60 and 80 g/L, respectively. Numerical calculations predicted that glucose concentrations higher than 60 g/L could be converted, but this could not be shown experimentally because of increased cell degeneration.

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Gas stripping is a relatively new technology in extractive fermentation, and its potential application to large scale extractive fermentation is high because of its relative simplicity. Volatile products are separated in a clean form because non-volatile products (glycerol or organic acids) as well as nutrients and cells are not removed by gas stripping. Stripping gas does not have to be purchased because fermentation produces gas as much as 40-50 wt% of the consumed sugar on a carbon basis. Gas stripping is not so selective to alcohols as pervaporation using solvent selective membranes because the selectivity of gas stripping is determined by gas-liquid equilibrium of products. However, unlike pervaporation mass transfer for gas stripping is not limited by the diffusion rate through the membrane. Mass transfer can be increased by improving gas-liquid contact mode. Countercurrent contact of trickling liquid with gas stream over a structured packing material is more efficient compared with bubbling fermentation gas through a liquid continuous fermentor, and this mode of gas-liquid contact is being used in an ethanol fermentation plant with 7,500 liters of structured packing.[22]

3.2 Product Removal into Liquid Phase

3.2.1 Liquid-Liquid Extraction

Product extraction into organic solvents is determined by distribution coefficient and selectivity which are defined as follows.

$$\text{Distribution Coefficient } (D_p) = \frac{C_{p,s}}{C_{p,w}}$$

$$= \frac{\text{concentration of the product in the solvent}}{\text{concentration of the product in aqueous phase}}$$

$$\text{Selectivity } (S) = \frac{D_p}{D_w} = \frac{\text{distribution coefficient of product}}{\text{distribution coefficient of water}}$$

The requirements desired for the extractant (solvent) are a high distribution coefficient for products, and a high selectivity for products compared with water. These values strongly affect the extractor size, and the solvent flow requirement. A lack of toxicity to the microorganism is also important. A typical fermentation system coupled with liquid-liquid extraction is shown in Figure 3.

Since organic solvents with high distribution coefficient are often toxic to the cells[88,101,119], a compromise must be made between solvent biocompatibility and extraction capacity. Solvent biocompatibility varies depending on the particular strain of microorganism. Therefore, potential extraction solvents must be tested with the process microorganism before biocompatibility can

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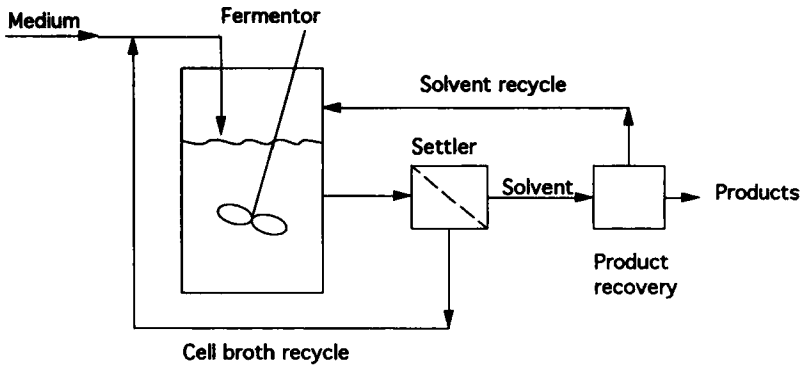

Figure 3. Extractive fermentation with liquid-liquid extraction

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be assured.[123] Many extraction solvents that have high distribution coefficients have poor selectivities[101] and a compromise must usually be reached between solvent capacity and selectivity. It is desirable to extract as little water and by-products (for example, organic acids in butanol fermentation) as possible. Other solvent properties to consider are viscosity, interfacial tension, volatility, water solubility, cost, toxicity to people, corrosiveness, and stability. The solvent can be regenerated by distillation or back-extraction.

The order of extraction capacity of ethanol from water mixtures was hydrocarbon < ether < ketone< amine< ester< alcohol.[118] Hashimoto[51] reported that corn oil, butyloctyl phthalate, butyl oleate, and dibutylphthalate were nontoxic but hexane, n-octanol, and 2-octanol were toxic to *Clostridium acetobutylicum*.

Matsumura and Märkl[88] found excellent solvents mainly among the alcohols and esters. N-octanol, 2-octanol, 2-ethyl-1-hexanol, 3-phenyl-1-propanol, tributylphosphate, iso-eugenol, and 2-ethyl-1-butanol inhibited the growth of several ethanol-producing microorganisms. Methyl crotonate, 2-ethyl-1,3-hexanediol, and polypropylene glycol P-1200 had little effect on cell growth. However, the price of methyl crotonate is currently too high for

TABLE II

Industrial solvent examples for ethanol extraction (for details see reference 88)

solvents	distribution coefficient	selectivity	cell growth	reference
2-ethyl-1-butanol	0.83	103.8	poor	88
sec-octanol	0.60	75.0	none	88
tri-n-butyl phosphate	0.79	29.3	poor	88
dodecanol	0.35	n.a.	good	96

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industrial scale application. 2-ethyl-1,3-hexanediol and polypropylene glycol P-1200 were viscous and had a tendency to form an emulsion with aqueous ethanol solutions. Examples of solvents for ethanol extraction are shown in Table II.

Extraction of ethanol which is not coupled with fermentation was studied using solvents composed of phenol derivatives[4] and solvents of Lewis acid have much more favorable combination of capacity and selectivity than solvents of Lewis bases.[100] A scheme of extraction using white light paraffin oil at 115°C was proposed[94], but it is not suitable to extractive fermentation. A conceptual processing scheme was proposed for separating and recovering ethanol from aqueous solution by solvent extraction followed by gas stripping.[28]

3.2.1.1 Ethanol Fermentation

Improvement in ethanol productivity during extractive fermentation has been reported by using dibutylphthalate[51,117],

dodecanol[96], and oleic acid[1] as extractants. Large volumes of solvent were required because of low solvent distribution coefficients. Minier and Goma[96] found that *S. cerevisiae* (UG5) was not inhibited by alcohols higher than dodecanol (C_{12}).

By co-immobilizing cells and a low-density adsorbent with a strong affinity for the solvent in gel beads, Matsumura and Märkl[88] succeeded in protecting the ethanol producing strains against solvent toxicity. Porapack Q (100-120 mesh) trapped the toxic solvent molecules coming into the gel beads, and the function was maintained for a relatively long period. However, this barrier function was expected to diminish when adsorbents became saturated with solvent molecules.

Modeling of extractive ethanol fermentation in CSTR predicted that the most pronounced increase in ethanol productivity is achieved by the fermentation of concentrated feeds. When glucose feed concentration was increased from 500 g/L to 750 g/L, model predicted ethanol productivity increase from 48 g/L-h to 83 g/L-h.[68] Based on theoretical screening of 1500 solvents for biocompatibility, 62 were chosen and tested with yeast culture. Fifteen including dibutylphthalate were completely biocompatible as predicted by the theory.[69] In 1.3 L CSTR extraction using commercially available solvent made of oleyl alcohol improved ethanol productivity (g/L-h) from 4.2 to 8.4 with 147 g/L glucose. Ethanol productivity increased to 18 g/L-h at 535 g/L glucose.[70] The process was scaled up to 7 L with more sophisticated ancillary equipment including a thermal recovery unit to separate the product from the extracting solvent.[23] The technical feasibility of extractive fermentation of concentrated glucose feed (up to 53 % w/v) in CSTR has been established. The economics of ethanol production by extractive fermentation using liquid-liquid extraction is discussed by Daugulis et al.[24]

3.2.1.2 Butanol Fermentation

Many studies have reported the effects of organic solvents on *Clostridium acetobutylicum* used for butanol production. Most

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researchers found that alcohols smaller than decanol or tetradecanol inhibit the growth of these cells. Traxler et al.[135] found that hexanol, octanol, decanol, cyclohexanol, and 4-methyl cyclohexanol inhibited the growth of *C. acetobutylicum*, but hexadecanol and ethylcaproate were biocompatible and increased butanol yield. Roffler et al.[120,121] examined the toxicity of several alkanes, esters, and alcohols to *C. acetobutylicum*. Biocompatible solvents included kerosene, cyclooctane, cyclohexane, dodecane, undecanone, nonane, benzyl benzoate, diethylphthalate, dibutylphthalate, dodecanol, and oleyl alcohol. Alkanes smaller than heptane, alcohols smaller than dodecanol, and most esters inhibited the growth of the cells to some degree. Ishii et al.[61] found that oxocol (branched-chained C_{14}-C_{15} alcohols), C_{16} guerbet alcohol, oleyl alcohol, fine oxocol (branched-chained C_{18} alcohol), C_{20} guerbet alcohol, oleic acid, isosteric acid, Freon E, and octadecafluorodecalin were biocompatible with *C. acetobutylicium* (IAM 19012). However, oleyl alcohol and C_{20} guerbet alcohol were chosen for extractive butanol fermentation considering their negligible emulsibility and the high partition coefficients of butanol. In general, alkanes larger than hexane or heptane, alkyl phthalates, and high-molecular-weight esters were found to be biocompatible with *C. acetobutylicum*.

Jeon and Lee[62] reported that n-dodecanol, dibutyl phthalate and tributyl phosphate are excellent extractants, but are toxic to *C. acetobutylicum* ATCC 824. Shukla et al.[127] found that 1-octanol and 2-ethyl-1-hexanol are toxic to *C. acetobutylicum* NRRL B-592.

Oleyl alcohol[122] or a mixture of oleyl alcohol and benzyl benzoate[120,121] increased butanol productivity by 70% and 60%, respectively. Productivity was improved using oleyl alcohol or guerbet alcohol.[61] The amount of butanol production increased four times, but fermentation slowed down because other by-products accumulated.[134] A mixed extractant that contained 20% decanol in oleyl alcohol enhanced butanol formation by 72%. Decanol itself was a good extractant but toxic to the cells.[32]

Liquid-liquid extractions have the potential for energy savings in the recovery of fermentation products as compared to

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distillation. However, this potential has not been fully realized in extractive fermentation because good extractants are usually toxic to the cells. Techniques have been reported to circumvent extractant toxicity. Matsumura and Märkl[88] made a barrier to solvent molecules beneath the surface of the gel beads by immobilizing the cells. A Ca-alginate immobilized cell system, with entrapped vegetable oil, has been reported to provide protection from the toxic solvents 2-octanol, benzene, toluene, and phenol. For 0.1% 2-octanol, one batch was not finished even after 35 hrs without vegetable oil, but four repeated batch fermentation were completed in 35 hrs with the new immobilized cell system using vegetable oils.[132] Liquid-liquid extraction using solvents of poor distribution coefficients seems to be not practical because solvent requirement is large.

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3.2.3. Aqueous Two-Phase Systems

Aqueous two-phase systems use aqueous solutions of two different polymers, one of them acting as an extractant. An ideal extractant is required to have a high distribution coefficient K for the product which is defined as the ratio of the concentration of the product in the top phase to that in the bottom phase. Application of this technique to extractive solvent fermentation has been marginal.

Kühn[71] studied extractive ethanol fermentation. The concept is explained as follows. When poly(ethylene glycol) (PEG) 6000, Dextran 500, and water are mixed in suitable proportions, two phases occur, the upper phase containing most of the PEG and the lower containing most of the dextran. If yeast cells are added, they will separate with the lower, dextran-rich phase. By choosing appropriate polymer concentrations, various volume ratios between the two phases can be chosen. If an alcoholic fermentation is made in a polymer mixture giving a volume ratio of 9:1, upper:lower phase, the upper phase will contain 90% of the produced alcohol, while the lower phase will contain most of the yeast cells and only

10% of the alcohol. The alcohol in the upper phase can be removed by distillation, and the upper phase can be returned to the concentrated yeast cells. The fermentation can begin anew with no product inhibition. Experimental results showed that the fermentative capacity went down after 10 cycles because glycerol and other nonvolatile by-products accumulated. The system was regenerated by dialyzing the broth and adding fresh yeast cells.

The fermentation of glucose to acetone-butanol by *Clostridium acetobutylicum* was studied in a 25% (w/w) PEG and 6% (w/w) dextran T-40 system.[92] The onset of solvent production was seen to be faster in the extractive fermentation system. However, the mean productivity in the aqueous two-phase system (0.24 g/L-hr) was no better than that of an ordinary batch process (0.26 g/L-hr), with 13 g/L butanol produced after 50 hr.

Hahn-Hägerdal et al.[50] showed that during cellulose bioconversion in an aqueous two phase system of 6% (w/w) Dextran T-40 and 7.5% (w/w) Carbowax PEG 8000, the amount of ethanol produced was almost the same in both systems, indicating that the polymers of the aqueous system do not impair the fermentation rate of the yeast cells.

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3.3 Product Removal into Solid Phase

3.3.1 Adsorption

Adsorbents with high alcohol adsorbing capacity, easy regeneration, no toxicity and low costs are desirable. Direct addition of adsorbents into the fermentor does not appear to be desirable. Because of the numerous components and the yeast suspension present in fermentor liquids, substrates may be adsorbed[2] and cells may form a biofilm on the surface of adsorption particle. Reasonable adsorption processes are either off-line whole broth treatment, or off-line adsorption from a cell-free broth obtained by membrane filtration or centrifugal systems.

Adsorbents used for ethanol removal are activated carbon[2,76,138], silicalite[15,78,95], and polymeric adsorbents such as

divinylbenzene crosslinked polystyrene resins and experimental proprietary molecular sieves with hydrophobic properties.[78,110]

Several studies were made to adsorb alcohols from aqueous solutions. Milestone and Bibby[95] investigated the possibility of using silicalate for the adsorption of various alcohols. Butanol was concentrated from 0.5 to 98% (w/v) by adsorption and subsequent thermal desorption. Zeolites have also been used.[73] Capacities of 82 mg butanol/g adsorbent and 99 mg butyric acid/g adsorbent were reported for a nitrated divinylbenzene-styrene copolymer when adsorbing from an aqueous solution.[74]

3.3.1.1 Ethanol Fermentation

Fermentation rate as well as cell growth was enhanced by extraction using silicalite, which resulted in a 30% reduction in fermentation time.[15] Ethanol concentration in the broth could be maintained below 5 g/L. Considering the large amount of adsorbent required, an efficient use of the adsorbent in an on-line extraction system would require that it be used in a packed bed arrangement in an external loop. A typical extractive fermentation system using adsorption is shown in Figure 4.

The addition of the molecular sieve, silicalite, to fermentation broths greatly reduced the concentration of ethanol present, but did not increase the glucose utilization rate to the extent predicted by product-inhibition kinetic models.[78] Addition of two polymeric adsorbents (XAD-4 and XAD-7) greatly inhibited cell growth because of nutrient adsorption by the resin.

3.3.1.2 Butanol Fermentation

Maddox[81] used silicalite, a zeolite analogue, to adsorb n-butanol from fermentation liquors. 85 mg butanol/g silicalite could be adsorbed. Groot and Luyben[44] used activated carbon and polymeric resins (XAD series). Adsorbent fouling by cells and medium components was severe, but this had no measured effect on the adsorption capacity of butanol in at least three successive

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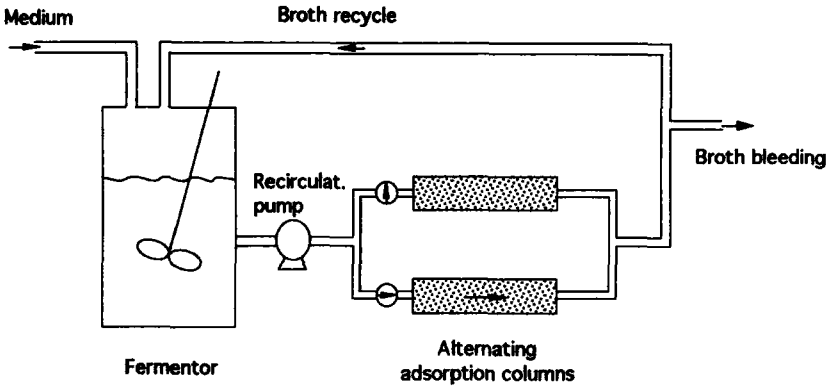

Figure 4. Extractive fermentation by adsorption

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fermentations. The fermentation was drawn towards the production of butyric and acetic acids. This may be due to the adsorption of the acids (intermediary products in the fermentation), or the adsorption of medium components leading to a more acidic fermentation course. Larrson et al.[74] reported that adsorption capacity of a polymeric resin for butyric acid was larger than butanol.

The capacity of Amberlite XAD-4 and Bonopore (a copolymer of divinylbenzene and styrene) for adsorption of butanol from a water solution were 83 and 74 mg butanol/g adsorbent.[104] These capacities decreased to 27 and 23 mg butanol/g adsorbent when the adsorbent was used in cell-free spent broths because of the presence of sugar and nutrients likely to be adsorbed. Nutrient adsorption was found to be a serious problem when using XAD-4, and no growth or butanol formation was found in media treated with XAD-4. However, the media could be restored by adding yeast extract. Bonopore did not affect the fermentability of the medium. A pH change to 8.0 was used to avoid adsorption of dissociated form of acetic and butyric acids.

Adsorption is disadvantageous for butanol fermentation because it removes intermediate products (organic acids) along with products (alcohols). Adsorption also can remove nutrients and

sugar. In butanol fermentation adsorption removes butyric acid, and fermentation is changed toward the production of organic acids. Requirement of a large amount of adsorbent is also a disadvantage. One area of further improvement in adsorption is to modify the hydrophobicity and the pore size of zeolites so that selectivity can be increased. Adsorption may work for extractive alcohol fermentation if adsorbents become more alcohol specific.

4. MEMBRANE BASED SEPARATION TECHNIQUES

4.1 Membrane Based Product Removal into Gas Phase

4.1.1 Pervaporation

Pervaporation is a membrane separation process that combines evaporation and permeation through a semipermeable membrane. The separation is not based on relative volatilities like distillation or evaporation, but is based on the relative permeation rates through the membrane.[54] Vapor-liquid equilibrium of ethanol-water system and its modification by different membranes are shown in Figure 5. The prevailing model for pervaporation is a solution-diffusion mechanism.[5]

The vapor-liquid equilibrium is modified when a polymeric membrane is placed between the two phases of a binary mixture. Depending on the changes in vapor-liquid equilibrium, pervaporation is either solvent-selective or water selective. Examples of water-selective pervaporation are solvent dehydration and dehydration of aqueous solutions at their azeotrope using a hydrophillic membrane. This type of application has been commercially developed using polyvinyl alcohol (PVA) membranes for dehydrating aqueous mixtures (ethanol, isopropanol, acetone, etc.). These applications are typically most effective when the concentration of the water to be removed is less than 10 wt%.[54]

For an extractive solvent fermentation process, solvent-selective pervaporation is required because fermentation broth is a

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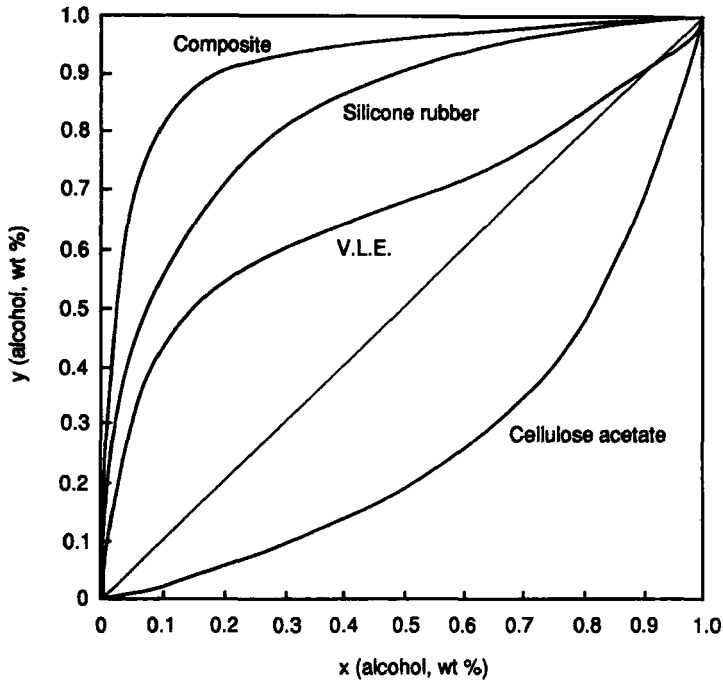

Figure 5. Vapor-liquid equilibrium curve (V.L.E.) of ethanol-water system and its modification by different membranes: composite, composite membrane composed of styrene-fluoroalkyl acrylate graft copolymer and cross-linked PDMS membrane.[59] Composite membrane and silicone rubber membrane are alcohol-selective, and cellulose acetate membrane is water selective.

dilute solution of products. Membranes and processes are commercially available for selective permeation of organics from aqueous streams.[33] For the preparation of solvent-selective membranes, poly(dimethylsiloxane), poly(methoxysilane), poly-tetrafluoroethylene (PTFE)[8], silicone rubber[56,66,126] or similar rubbery polymers were used as the actual selective barrier and poly(sulfone) or poly(acrylonitrile) are used as the microporous support. Most applied development has focused on the well known

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silicone rubber. Extensive lists of pervaporation data are available for ethanol , acetone and butanol selective membranes made of silicone rubber, miscellaneous silicone-based material and fluoride-containing material.[77]

The mass transport in pervaporation can be broken down into three consecutive steps.[131]

1. Sorption of components from a liquid phase at the membrane surface facing the feed solution.
2. Diffusion of the sorbed components through the polymer matrix.
3. Desorption and evaporation from the polymer matrix into the vapor phase on the permeate side of the membrane.

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Membrane selectivity ($S_{i,j}$) is a ratio of the mass fractions of components i and j for the permeate and the retentate. For the selective permeation of component i, the definition of separation factor is as follows.

$$S_{i,j} = \frac{X_i''/X_j''}{X_i'/X_j'} \qquad \begin{matrix} '' \text{ permeate} \\ ' \text{ retentate} \end{matrix}$$

Selectivity in pervaporation (or separation factor $a_{i,j}$) is determined by membrane selectivity and selectivity due to evaporation as follows.

$$a_{i,j} = S_{i,j} \times \frac{f_i P_i^o}{f_j P_j^o}$$

,where f_i, f_j are activity coefficients of component i and j, and P_i^o, P_j^o are their saturation pressure. A value of $a_{i,j}$ greater than unity indicates the selective permeation of i over j.

The enrichment factor is defined as follows.

$$b_i = \frac{X_i''}{X_i'} = \frac{\text{molar fractions of component i in the permeate}}{\text{molar fractions of component i in the retentate}}$$

The separation factor $a_{i,j}$ of pervaporation may either be larger than the separation factor obtained by distillation, when $S_{i,j} > 1$, or smaller, when $S_{i,j} < 1$. It should be noted that the separation factor $a_{i,j}$ and the enrichment factor b_i are defined as always >1.

The selectivity of a composite membrane is determined by

1. Selectivity of the selective barrier polymer
2. Porosity of the support structure
3. Selectivity of the support structure polymer

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The transmembrane flux of the various components is determined by their partial pressure gradient across the membrane (determined by the distribution coefficient of component i between the feed solution and the permeate) and their permeability in the membrane (determined by the diffusion coefficient in the membrane) and porosity of the support structure. Since the flux rate is roughly inversely proportional to the membrane thickness, the composite film consists of 0.1 - 5 mm thick actual selective barrier (polymer film) deposited on a microporous support structure. Fluxes in pervaporation are generally low (< 12 kg/m^2-h) compared to conventional membrane processes such as ultrafiltration or reverse osmosis. Selectivities can be extremely high often exceeding 1,000.[34]

The driving force of pervaporation is induced by lowering the partial pressure of the permeands on the downstream side of the membrane. Thus, every permeand must undergo a phase change. The required latent heat of evaporation is drawn from the feed solution.[131] The process is perpetually driven by condensation of permeate creating a significant vacuum. In contrast to reverse osmosis, the osmotic pressure is not limiting because the permeate is kept under saturation pressure.

There are three ways of lowering the permeate side concentration of the permeands; vacuum pervaporation[105], sweep gas

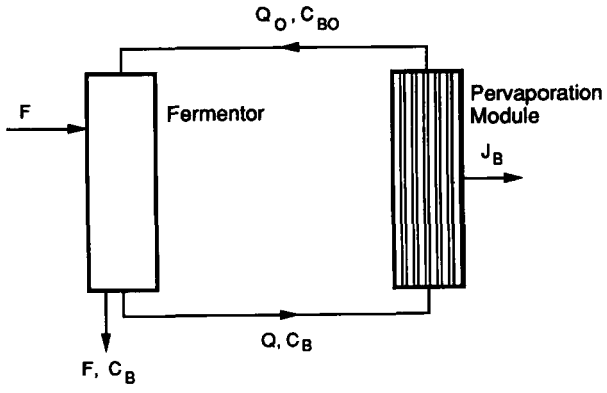

Figure 6. Extractive fermentation with a pervaporation membrane module

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pervaporation[56] and thermopervaporation.[3] Vacuum pervaporation was superior to sweep gas pervaporation and thermopervaporation in both flux and selectivity, even at an elevated permeate pressure of 30 mbar. The selectivity of thermopervaporation was lower than the selectivity of vacuum pervaporation when feed temperature was low. When feed and condensation temperatures were 50°C and -20°C, the selectivity became equivalent to vacuum pervaporation. Flux in thermopervaporation was approximately 60% of that of vacuum pervaporation.[3]

Pervaporation is the solvent-selective removal process, and has the biggest potential for simultaneous pre-concentration of the product. Pervaporation can keep the fermentation broth in the separator under conditions identical to those in the fermentor.[99] A typical combination of a fermentor and a pervaporation module is shown in Figure6. The permeabilty of the solvent-selective membranes towards CO_2 and O_2 are high, and initial O_2 supply for cell growth and removal of fermentation gas (CO_2) can be accomplished. Pervaporation includes both vaporization and condensation, so energy efficiency of alcohol recovery is less than that in reverse osmosis.

4.1.1.1 Pervaporation Without Fermentation

Different membranes have been studied for ethanol pervaporation without fermentation. A classic example is a silicone membrane which was used to separate ethanol from water with ethanol separation factor of about 9.[56] Selectivity of ethanol increased from 7 to 39 and total flux increased from 175 to 390 g/m^2-h when up to 70% of zeolites was added to silicone rubber membrane.[52,53] The overall mass transfer coefficient of poly-tetrafluoroethylene (PTFE) membrane was higher than that of polypropylene or silicone membranes[8]. PTFE membrane may provide viable alternative to silicone rubber membranes. High transmembrane fluxs of more than 2,000 - 3,000 g/m^2-h and selectivities in excess of 50 was obtained using membranes made of substituted polyacetylene/polydimethylsiloxane graft copolymer.[102] Higher transmembrane ethanol flux of 4,000 g/m^2 with corresponding separation factor 12 at 30°C was obtained using poly [1-(trimethylsilyl)-1-propyne] (PTMSP) membrane.[60,84] PTMSP membrane has performance equivalent or superior to that of silicone rubber membrane. However, the performance of the PTMSP membrane was dramatically reduced by contact with fermentation broth compared to its performance with pure ethanol and water solutions.[98] Other membranes studied are polydimethyl silicone-type membranes[31], poly(dimethyl siloxane) (PDMS) (500 mm) and composite of PDMS with poly vinyl fluoride (PVF) membranes (40 mm)[10], and N-vinylpyrolidone (NVP)-isobutylmethacrylate (IBMA) copolymer membrane.[140] Many of these new membranes with higher flux and better selectivity have not be used in fermentation systems.

4.1.1.2 Pervaporation With Fermentation

4.1.1.2.1 Pervaporative Ethanol Fermentation

Extractive ethanol fermentation by pervaporation increased the specific rate of ethanol production.[103] Silicone rubber (120 mm

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thickness), hydrophobic polypropylene (25 mm thickness), and polytetrafluoroethylene (PTFE) (80 mm thickness) were tested using an ethanol-water mixture, and PTFE was found to have the best separation characteristics of flux and selectivity. The fermented ethanol was continuously extracted from the membrane bioreactor, and simultaneously concentrated by pervaporation. The extracted ethanol concentration was 6 to 8 times higher than in the broth. Permeate flux was constant during fermentation at 3,960 g/m^2-h. However, in order to achieve a high ethanol productivity, part of the fermentation broth had to be removed from the membrane bioreactor.

Silicone membrane was more effective than a polypropylene membrane; ethanol permeate concentrations were 33.5 and 25.6 wt % for silicone and polypropylene, respectively.[64] Calibo et al.[9] performed extractive ethanol fermentation using PTFE membrane (400 mm thickness). The PTFE module removed a high concentration of ethanol from the fermentation broth and thus maintained a low ethanol concentration in the broth. Ethanol flux was 50 - 100 g/m^2-h, and the specific ethanol production rate was higher with ethanol stripping.

A continuous extraction of ethanol by thermopervaporation using a PTFE membrane (120 mm thickness) resulted in an 87% increase in ethanol productivity from 0.99 to 1.85 g/L-h.[136] Permeate flux was 6 L/m^2-h initially with a feed temperature of 37°C and a cold temperature of 18°C, but decreased to 3 L/m^2-h after 90 hrs of operation because of membrane fouling by the culture medium or yeast cells.

4.1.1.2.2 Pervaporative Butanol Fermentation

Extractive butanol fermentation by pervaporation was first reported using silicone tubing as membrane (400 mm) in a batch suspension isopropanol-butanol-ethanol (IBE) fermentation.[42] In a continuous immobilized fermentation, both the glucose conversion and the reactor productivity were 65 - 70% higher than in a

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continuous fermentation without product removal.[43] In their preliminary experiments involving a n-butanol/water binary separation using sweep gas, butanol flux and selectivity were 2.2 - 4.4 g butanol/m^2-h and 47 - 57, respectively for 4.3 - 9.4 g/L of butanol concentration. Experiments using actual fermentations produced selectivities between 20 and 30.

Larrayoz and Puigjaner[72] used silicone tubing (1000 mm) and sweep gas in batch suspension culture of *C. acetobutylicum* ATCC 824. The selectivity decreased from 32.2 to 25.7 and the n-butanol flux increased from 4.42 to 11.05 g/m^2-h for n-butanol feed concentrations ranging from 1.38 to 1.72 wt %. For an initial glucose concentration of 100 g/L, glucose consumption increased from 73 to 95 g/L with pervaporation. The authors attributed incomplete glucose conversion to the production of autolysines inducing cell lysis. However, the authors did not report the level of organic acids with pervaporation.

Groot and Luyben[45] used silicon tubing (400 mm) which had a selectivity of 11 and a flux of 2.6 mL/h at 30°C in butanol fermentation. Sodeck et al.[129] used PDMS membrane in acetone-butanol fermentation. No membrane fouling was observed. The selectivities for a feed temperature of 41°C were 78, 66 and 9.6 for n-butanol, acetone, and ethanol, respectively. The component permeation rates were 3.44, 1.66 and 0.065 g/m^2-h, respectively. PDMS membrane was used in acetone-butanol-ethanol fermentation.[49] The membrane's flux and selectivity were not found to degrade over a 30 day study. With a downstream pressure of 8 mbar, a total flux of 600 g/m^2-h was observed for a 5 wt % feed. Enrichment factors between 5.5 and 6 were also observed at these conditions.

Groot et al.[47] studied IBE fermentation in an immobilized CSTR and fluidized bed reactor. Using a silicone tubing (thickness 250 mm) module and sweep gas, substrate consumption was increased by a factor of four compared with continuous fermentations without in-situ separation. Mathematical modeling and simulation showed that high productivity and high substrate consumption should be possible.[48] It was concluded that because of the accumulation of

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components and byproducts in the medium, the concentration of these components in the broth following the removal of water is of great importance.

Friedl et al.[38] studied ABE fermentation by *C. acetobutylicum* P262 in a immobilized cell, packed bed reactor. Using polypropylene hollow fiber membrane (thickness 400 mm) and sweep gas, solvent flux was 3.0 - 7.1 g/m^2-h and selectivity was 3 - 5. The acid flux was about 0.45 - 0.55 g/m^2-h for acetic acid and 0.1 - 0.2 g/m^2-h for butyric acid. Productivity was not improved with pervaporation.

4.1.1.3 Liquid Membrane

Liquid membranes were used recently in pervaporation and perstraction process. The fluidity of liquid organic films leads to high diffusion coefficients of solutes and thus high fluxes compared with permeation through dense polymeric membranes. The liquid membrane process requires a small amount of solvent just enough to cover the support membrane. A drawback is possible fragility of the film. Stability is an essential parameter in liquid membrane utilization. Pervaporation and perstraction processes utilizing liquid membranes are illustrated in Figure 7.

In an effort to reduce the barrier to mass transfer and to increase selectivity of butanol, Matsumura and Kataoka[85] studied pervaporation through a liquid membrane supported with a hydrophobic microporous polypropylene, flat sheet, Celgard 2500 membrane (thickness 25 mm). The results were compared with that of silicone rubber membrane (thickness 180 mm). The stability of a liquid membrane under vacuum (down stream pressure of 0.133 kPa) was checked using several liquid membranes prepared with higher alcohols and esters with high boiling points. Liquid membranes remained stable as long as the surface tension of the feed solution was less than the critical surface tension (35 mN/m) of the support membrane. The liquid membrane prepared with oleyl alcohol, di-n-butylphthalate, and tricresyl phosphate proved to be stable (Table III). Oleyl alcohol was selected based on the separation factor rather than on the permeation flux because the permeation rate can

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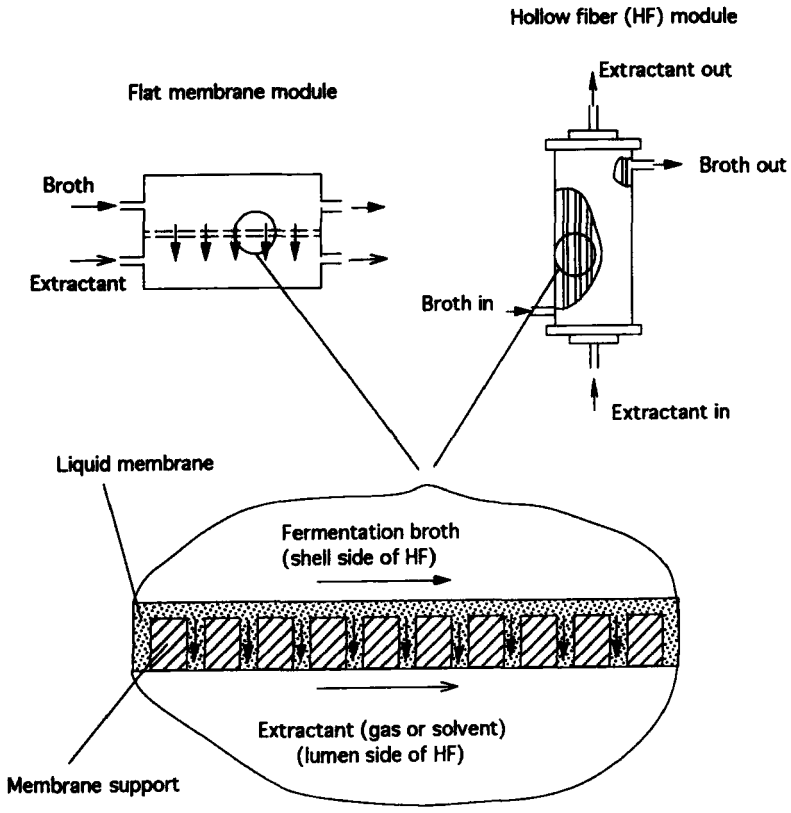

Figure 7. Liquid membranes for pervaporation/perstraction using flat membrane and hollow fiber membrane supports

be increased rather easily by using a hollow-fiber module with high contact area. The liquid membrane prepared with oleyl alcohol was found to be superior by far in both selectivity and permeability of butanol to the better known silicon rubber membrane (separation factor 70 for membrane thickness 180 mm), and the liquid membrane could be used for 100 hrs. Dilute aqueous butanol solutions of around 4 g/L obtained in acetone-butanol fermentation could be concentrated up to 100 times. Although oleyl alcohol was selected mainly for the separation of butanol, the liquid membrane also showed rather high selectivity for acetone (160) and isopropanol.[86]

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TABLE III

Separation performance of liquid membrane prepared from organic solvents at 30°C. (from reference 85)

solvent	boiling point (°C)	distribution coefficient	separation factor	permeation flux (kg/m^2-h)
oleyl alcohol	207/1.7 kPa	3.8	180	0.080
di-n-butyl phthalate	339	1.8	90	0.112
tricresyl phosphate	250/0.5 kPa	2.3	105	0.055

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Christen et al.[14] compared perstraction and pervaporation using a porous polytetrafluoroethylene (PTFE) sheet support soaked with isotridecanol. For perstraction using pure water, the membrane (thickness 60 mm) was stable up to 170 h. With perstraction the viability of the cells was improved and ethanol productivity increased from 0.5 to 1.2 g/L-h. For pervaporation using air, the membrane (thickness 65 mm) was stable for 14 days. Ethanol flux for pervaporation was inferior to that obtained with perstraction at similar broth concentrations. The ethanol concentration was about 4 times higher in the permeate. The selectivity of the isotridecanol membrane for ethanol and water separation remained between 5.5 and 11 throughout the 330 h extractive fermentation. For a similar level of ethanol, the ethanol flux at 30°C, estimated from the permeability of silicone rubber tubes[56], is between 1.6 - 5.9 g/m^2-h with a transmembrane pressure drop of about 1 atm. With isotridecanol membrane pervaporation, the flux of ethanol reached 16.5 g/m^2-h without a transmembrane pressure gradient.

TABLE IV

Performance of pervaporation with supported liquid membrane. (from reference 87)

	Weight fraction in permeate		Permeation flux (g/m^2-h)		selectivity	
	butanol	iso-propanol	butanol	iso-propanol	butanol	iso-propanol
Experimental value	0.23	0.07	3.3	1.1	66	24
Theoretical value	0.27	0.06	12.1	2.1	160	35

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Matsumura et al.[87] applied pervaporation using oleyl alcohol liquid membrane to a continuous butanol/isopropanol fermentation with immobilized *Clostridium isopropylicum* (IAM 19239) in a down-flow column reactor packed with Na-alginate beads. Pervaporation took place in an external module. The support material for the liquid membrane was a 25 mm thick, microporous polypropylene flat sheet membrane, Celgard 2500. In comparison with the continuous fermentation without product removal, the specific butanol production rate was 2 times higher. The butanol concentration in the permeate was 230 kg/m^3, which was about 50 times higher than that in the culture broth. However, experimental values for butanol permeation flux were much lower than the theoretical value (Table IV). The membrane surface after the continuous fermentation was completely fouled with some viscous materials. The circulated broth did not distribute equally into each permeation cell of the module.

Pervaporation seems to be the most promising process in extractive alcohol fermentation. Pervaporation shares the advantage of clean product separation with gas stripping. However, pervaporation can overcome vapor-liquid equilibrium limitation and can produce more concentrated permeate. Two stage pervaporation process using combination of solvent selective membranes followed by water selective membranes can be used to concentrate dilute fermentation broth to highly concentrated product without experiencing the azeotropic limitation. Current limitation in pervaporation is low transmembrane flux. Use of membranes with higher transmembrane flux will solve this problem. For example, pervaporation membranes with transmembrane flux of more than 2,000-3,000 g/m^2-h and selectivity in excess of 50 have been developed on a laboratory scale[102] and should soon be commercially available.

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4.2 Membrane Based Product Removal into Liquid Phase

4.2.1. Perstraction

Perstraction is a solvent extraction process combined with membrane permeation. Hydrophobic membranes in a flat or a hollow fiber shape are used in extractive alcohol fermentation. Hollow fiber membranes are most advantageous due to the high surface area per volume.[112,113] Perstraction may possibly reduce the problems associated with solvent toxicity, emulsification, and cell aggregation at the liquid-liquid interface during liquid-liquid extraction process.[17] Perstraction allows independent variation of process stream flow rates. Dialysis is a kind of perstraction using water as the extractant.

4.2.1.1 Perstraction Without Fermentation

Solvent extraction without dispersion of the solvent into the aqueous phase has been studied for acetic acid extraction using methyl isobutyl ketone and xylene and a microporous hydrophobic

membrane (Celgard 2400)[67] and several membranes (Celgard 2400, 2500, Goretex 1, 2).[111] In this technique, the interface of the immiscible aqueous and organic phases is immobilized at the pore mouths of a microporous hydrophobic membrane. The membrane is wetted by the organic solvent by maintaining the aqueous phase at a pressure higher than that of the organic phase.

Dispersion-free solvent extraction has been carried out with microporous hydrophillic and microporous composite hydrophobic-hydrophillic membranes in flat shape; Celgard 2400 (hydrophobic, polypropylene), cellulose acetate, regenerated cellulose, and Goretex 1 (hydrophobic, Teflon).[112] For hydrophillic membranes, the overall mass transfer coefficient based on the organic phase was varied from 1-5 $x10^{-3}$ cm/sec depending on the flow rate of the organic phase. For composite membranes, the value decreased to 0.7-1.8 $x10^{-3}$ cm/sec. Hydrophillic films are particularly attractive for a system with a low distribution coefficient since the mass transfer resistance of such a system is lower than that for a hydrophobic film. Earlier attempts to utilize hydrophillic membranes for solvent extraction had problems of phase intermixing because proper pressure conditions were not maintained (higher pressure on the organic phase along the whole length of the hollow fiber).[65]

Since composite films with asymmetric wetting characteristics (the hydrophobic section of the composite membrane is wetted by the organic phase, while the hydrophillic section is preferentially wetted by the aqueous phase) can operate dispersion-free with an excess pressure in either phase, they are ideal for handling accidental process pressure fluctuations. However, the overall solute extraction flux will always be lower than that obtained with either a hydrophillic or a hydrophobic membrane.[112]

Microporous hollow fibers were used for a system of n-butanol-water-succinic acid[114], for extraction of p-nitrophenol into amylacetate and acetic acid into methyl amyl ketone[25], and applied to extractive ethanol fermentation.[36,37,89]

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4.2.1.2 Perstraction With Fermentation

4.2.1.2.1 Perstractive Ethanol Fermentation

In-situ recovery of ethanol by perstraction was studied using a tubular bioreactor-separator containing a large number of axially located microporous hydrophobic hollow fiber membranes evenly distributed among 24.7 g/L of dry cell immobilized on wood chips.[36] Increased flow of dibutyl phthalate through the hollow fiber lumens decreased ethanol concentration in the fermentor. In a subsequent study[37] cell density was reduced to 6.8 g/L to decrease volumetric productivity to observe the effects of solvent extraction more readily. Additionally, the hollow fibers were used to supply oxygen throughout the reactor, while removing CO_2. The increase in ethanol productivity was only marginal.

Extractive ethanol fermentation by *S. cerevisiae* was studied using a multi-membrane bioreactor of flat membranes (hydrophobic membrane for broth extractant interface) with tributyl phosphate as an extracting solvent.[11] Some increase in ethanol production was observed in the extractive fermentation system when concentrated nutrient solutions were added periodically. Glucose consumption increased by 60% on day 10. However, the reactor system was complex, and the available membrane surface area per unit bioreactor volume was low compared to a hollow fiber extractor-bioreactor.

The reactor performance was improved with a pressure swing operation, termed pressure cycling, in which the substrate- and product-laden suspension medium is convectively forced into and out of the cell layer between hydrophobic and hydrophillic membranes.[27] Long term (3000 h) operation of this reactor was performed using *S. cerevisiae* and *Zymomonas mobilis*. *Z. mobilis* appeared to be less attractive than *S. cerevisiae* for such a reactor because it formed filaments that reduced the effectiveness of the pressure cycle.[130]

Ethanol inhibition to *S. cerevisiae* was completely removed by perstraction using countercurrent contact of aqueous ethanol

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solution with tri-n-butylphosphate as extractant through hollow fibers made of cuproammonium cellulose.[89] A thicker membrane (TH10) gave a lower overall volumetric mass transfer coefficient of 5.7 L/h compared with 10.4 L/h of thinner membrane (TE10 module), but the leakage of solvent was reduced. The solvent requirement per consumed glucose (6.0 L solvent per kg glucose) was smaller compared with that of non-membrane assisted liquid-liquid extraction by Minier and Goma[96] (43 L solvent per kg glucose) who used non-toxic n-dodecanol with a poor ethanol distribution coefficient as the extractant. The protection effect of the silanized silica gel adsorbent in the gel beads was lost rather quickly, and exchange of the packed column for a new one before it attains the break point for tri-n-butylphosphate is suggested.

Mathematical modeling and analysis showed that a microporous hollow-fiber membrane extractive fermentor (fermentation in the shell side and extraction in the lumen side) has a volumetric productivity significantly higher than that possible using conventional fermentors such as a plug flow fermentor and CSTR.[35] The model predicted the existence of an optimum volume fraction of hollow fibers in the fermentor that maximizes the total volumetric productivity.

Using hydrophobic hollow fibers and cell immobilization on chopped hydrophillic hollow fibers, productivity increased significantly as the solvent/substrate flow ratio increased.[63] At a ratio of 3 productivity increased by 39%. The glucose consumption increased from 177 to 259 g/L as the oleyl alcohol/substrate flow ratio was increased from 0 to 3 at a fixed substrate flow rate of 9 mL/hr. Oleyl alcohol was more efficient than dibutyl phthalate because of its higher distribution coefficient.

4.2.1.2.2 Extractive Butanol Fermentation

Perstraction was studied using a semipermeable silicone membrane tubing with oleyl alcohol and polypropylene glycol as extractants.[62] Solvent productivity increased by a factor of two,

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and the total solvent yield increased by 23% due to a reduction of acid production and a reuse of cells in the fed-batch operation.

Butanol productivity increased by 4 times in a cell recycled, 4 stage-mixer settler cascade system using cross-flow microfiltration modules and n-decanol saturated with butyric acid. Decanol was practically insoluble in the fermentation medium, thus the contact of the cell-free medium with the solvent phase in the cascade did not interfere with cell growth and product formation.[26]

In experimental and theoretical work, total solvent productivity during butanol fermentation increased by more than 40% in a hydrophobic hollow fiber based tubular fermentor-extractor using cells immobilized on wood chips and 2-ethyl-1-hexanol as extractant.[128]

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Even though liquid-liquid extraction is an established unit operation process in chemical engineering, it is not an attractive choice in extractive alcohol fermentation because of solvent toxicity to microorganisms. Nontoxic solvents were not useful because of large requirement. Perstraction using solid membranes or liquid membranes solve this problem. Perstraction shares most of advantages and disadvantages of pervaporation including clean product separation, overcome of phase equilibrium limitation, and limited transmembrane flux. However, perstraction experiences an additional disadvantage because of alcohol recovery from extractant.

4.2.2. Reverse Osmosis

All reverse osmosis membranes tested to date are alcohol-rejecting (i.e., preferentially permeate water).[77] One exception is n-hexadimethylsilylated poly[1-(trimethylsilyl)-1-propyne) (PTMSP) membranes which exhibit very low ethanol-to-water selectivity.[133] Without major improvements in separation performance, these membranes are not likely to find practical application for concentrating ethanol.[77] So far, reverse osmosis applied to

extractive alcohol fermentations uses water permeable membranes such as cellulose acetate and rejects alcohols. Percent separation or percent rejection is defined as follows.

$$\frac{\text{Alcohol conc. in the feed - alcohol conc. in the permeate}}{\text{Alcohol concentration in the feed}} \times 100$$

Reverse osmosis does not involve energy-intensive phase change processes such as vaporization and condensation, and showed the most promise as a separation method for aqueous ethanol solution.[12,13,55] However, because of the high osmotic pressure of an ethanol-water mixture, the present-day thin film composite desalination membranes (e.g. polyamide membrane) can be used only for partial concentration of beer solutions from 7.6% to 30% alcohol concentration.[93]

The characteristics of styrene-grafted cellulose acetate membranes was studied for separation of ethanol from ethanol-water mixture by reverse osmosis.[12] Permeation flux was 1.8-2.0 L/m^2-h at 1200 psig with 82-93% separation.

An irradiated styrene-grafted cellulose acetate membrane was used to separate ethanol from molasses based fermentation broth.[13] Separation efficiency of 92% was observed at 1200 psig. The permeation flux obtained with the molasses broth as feed was lower than the value obtained with aqueous ethanol feed. For example, at 1200 psig, the permeate flux was 0.99 L/m^2-h for fermentation broth.

Polyamide membrane was used to separate butanol during acetone-butanol fermentation.[39] A butanol rejection rate of 98% was possible at recoveries of 20 - 45%. The flux through the reverse osmosis membrane was reduced for the fermentation liquor to about one-third the flux of an aqueous mixture because of the added constituents. Flux ranged from 3 to 36 L/m^2-h. Additional RO data are available in the literature.[77]

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5. SUMMARY

Separation techniques used for simultaneous separation of products during ethanol and butanol-acetone fermentation can be classified by product removal into gas phase, liquid phase, and solid phase. Non-membrane based separation techniques are vacuum fermentation, gas stripping, liquid-liquid extraction, aqueous two-phase system, and adsorption. Two of the most established techniques, liquid-liquid extraction and adsorption, suffer major disadvantages. Liquid extractants which are nontoxic to microorganisms are required at large quantities because of their poor distribution coefficients. The required amount of solid adsorbents is also large. Membrane based techniques (pervaporation and perstraction) have enjoyed more attention recently because they can increase product selectivity dramatically as compared to normal phase equilibrium processes. Gas stripping shares an advantage of clean product separation with pervaporation and perstraction. A disadvantage of membrane based techniques is the low product flux which can be overcome by reducing membrane thickness and by increasing contact area. New membrane manufacturing technologies, liquid membranes and hollow fibers have been used for this purpose. Since perstraction requires alcohol recovery from extractants, pervaporation seems to be the most promising technique, but for a larger scale operation, gas stripping is probably a more attractive process because of its relative simplicity. Since genetic improvement of microorganisms' tolerance to alcohols is relatively minor, simultaneous fermentation and separation together with high cell density culture will be a major way of improving alcohol productivity.

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