Ethanol Extraction by Supported Liquid Membrane During Fermentation

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A supported liquid membrane system was developed for the extraction of ethanol during semicontinuous fermentation of *Saccharomyces bayanus.* It consisted of a porous Teflon sheet as support, soaked with isotridecanol. This assembly permitted combining biocompatibility, permeation efficiency, and stability. The removal of ethanol from the cultures led to decreased inhibition and, thus, to a gain in conversion of 452 g/L glucose versus 293 g/L glucose without extraction. At the same time, the ethanol volumetric productivity was enhanced 2.5 times, due to an improvement of yeast viability, while the substrate conversion yield was maintained above 95% of its theoretical value. Besides these improvements in fermentation performances, the process resulted in ethanol purification, since the separation was selective towards microbial cells and carbon substrate, and likely selective to mineral ions present in the fermentation broth. For pervaporation, a concentration of ethanol four times greater was obtained in the collected permeate.

INTRODUCTION

Considerable effort has been given to the production of fermentative alcohols in the past several years, with a goal of developing new resources of liquid fuel and chemical feedstocks. However, industrial application has been delayed by the high cost of production, which depends largely on energy spent for the purification of dilute end-products and on the low productivity of cultures. These two points are directly linked to inhibition phenomena and many attempts have been made to overcome this limitation.

First, conventional unit operations for separation were applied directly to active fermentation broths, particularly for ethanol or acetone/butanol production. Liquid–liquid extraction[1–3] with biocompatible organic solvents, distillation under vacuum,[4,5] and selective adsorption on solids,[6,7] have demonstrated the technical feasibility of the extractive fermentation concept.

More recently, membrane separation processes, which decrease biocompatibility constraints, have been proposed. These include dialysis[8] and reverse osmosis[9] utilizing an aqueous stripping phase, or perstraction with an organic stripping phase, such as dibutylphtalate[10] or tributylphosphate,[11] which were tested for ethanol and butanol extraction.

Lastly, pervaporation experiments on butanol through silicone[12,13] or through an oleylalcohol liquid membrane, supported with hollow fibers,[14] have been reported. These methods minimize the amount of organic solvent involved and permit simultaneous realization of the extraction and recovery phases.

In this work, a supported liquid membrane process is developed for ethanol separation, and studies carried out in actual fermentation conditions are presented. A comparison of the performances of aqueous and gaseous stripping phases, in terms of inhibition removal and ethanol recovery, is proposed.

MATERIALS AND METHODS

Process Equipment and Experimental Procedure

The process combined three operations: fermentation, extraction, and reextraction (stripping), schematically represented in Figure 1. The fermentor was a 1-L stirred tank reactor with pH and temperature regulation. The separation unit, coupled to the fermentor, was a permeation cell with two compartments separated by a supported liquid membrane.

In a previous work, different liquid membrane systems were developed and compared.[15] The most suitable for use in this application consisted of isotridecanol filling the pores of a flat microporous polytetrafluoroethylene (PTFE) membrane. The apparent working surface area was 1.15×10^{-2} m^2 for a culture volume of about 0.5 L. The experimental permeation fluxes are given relatively to this apparent area, although the actual liquid–liquid interface depended on the porosity, which was close to 85% void volume for the different supports. The support used in perstraction experiment was a Fluoropore membrane (Millipore), with thickness of 60 μm and average pore size of 0.5 μm, with a polyethylene weft; in pervaporation, it was an SM11807.293 Sartorius membrane, with thickness of 65 μm and average pore size of 0.2 μm.

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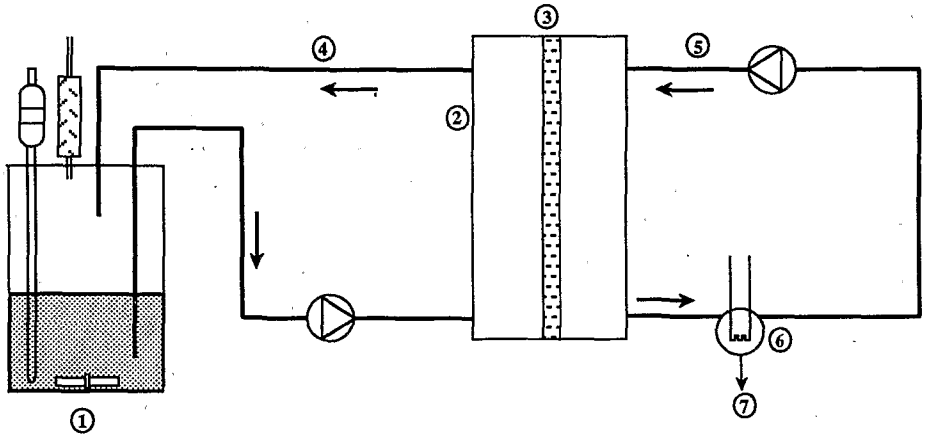

Figure 1. Extractive fermentation system: (1) fermentor; (2) permeation cell; (3) supported liquid membrane; (4) extracted phase; (5) gaseous stripping phase; (6) cold trap; (7) condensed permeate.

After soaking under reduced pressure and removal of the excess organic phase, the supported liquid membrane was fitted in a Pleïade cell (Rhone–Poulenc, S.A.), modified to allow the circulation of fluids in each compartment (volume of 0.08 L). The operation was performed aseptically, under sterile laminar air-flux conditions. The separation system was then coupled with the heat-sterilized fermentor. These precautions were found sufficient to prevent contamination.

In working conditions, the permeation cell was fixed on an agitated table placed in a 30°C thermostated chamber, while two peristaltic pumps circulated the fermentation broth and the stripping phase on each side of the membrane. Two types of extractive fermentation experiments were carried out, depending on the nature of the stripping phase.

One type was perstraction, with pure water thermostated at 30°C for the reextraction step. The water was changed every 24–48 h to maintain a high potential of permeation throughout the experiment.

The other type was pervaporation, using air at atmospheric pressure to sweep away the permeate by evaporation. The permeate was then collected in a condenser at −6°C; at regular intervals, this condensate was weighed and ethanol concentration was assayed. In both cases, the extraction was started about 24 h after fermentation had begun.

Conditions of Fermentation

Microorganism

Fermentations were performed using the yeast *Saccharomyces bayanus* studied elsewhere by Mota et al.[16]

Growth Medium

The medium contained (per L) KH_2PO_4, 5 g; $(NH_4)_2SO_4$, 2 g; $MgSO_4 \cdot 7H_2O$, 0.4 g; and yeast extract, 1 g. Cerelose (monohydrated glucose) was used as the carbon substrate. Initial concentrations were approximately 150 g/L glucose; successive additions of crystallized cerelose were carried out during the runs in order to minimize dilution due to feed supply.

Regulation

Fermentation temperature was maintained at 30°C, and pH at 3.8 with 1*N* ammonium hydroxide.

Analysis

Biomass concentration was evaluated after assaying the optical density of dilute broth at 620 nm on an Ultrospec II spectrophotometer (LKB), and conversion in dry weight concentration was calculated from a standard curve. Cell viability was estimated by methylene blue staining.

Ethanol was determined by gas chromatography (Girdel Serie 30), using a flame ionization detector and N_2 as the carrier gas. The column (2 m long, 2 mm i.d.) was packed with Porapak Q (80–100 mesh), and maintained at 220°C. Isopropanol [0.4%(v/v)] was used as an internal standard, and an Icap 50 integrator (Delsi) calculated the peak area. Glucose was determined by colorimetry at 505 nm after enzymatic reaction (Biolyon kits).

RESULTS

The characterization of the processes includes two aspects:

(1) the performances of separation, evaluated by the efficiency of ethanol transfer, the selectivity and the stability of the supported liquid membrane, and the concentration of the recovered ethanol;

(2) their incidence on the performances of fermentation.

Extractive Fermentation with Aqueous Stripping Phase

Separation Parameters

In perstraction experiments, the selectivity of liquid membranes was only checked relative to glucose and magnesium, this being used as a test of stability. Preliminary permeation experiments showed that newly prepared isotridecanol-supported liquid membranes were totally impermeable to glucose and magnesium ion, leading us to assume their impermeability for other hydrophilic solutes, in particular mineral ions. In contrast, when the organic film is altered, which can be due to mechanical or physicochemical causes,[17] glucose and magnesium are rapidly found in the aqueous stripping phase while a sudden and significant water transfer, driven by osmotic pressure, is observed. Such leakage of glucose (or magnesium) through the membrane served to define the loss of stability of a liquid membrane. In the experiment of extractive fermentation with aqueous stripping phase, the liquid membrane remained stable, without maintenance, during 170 h operation. It failed after that time, showing a transfer of stripping water to the fermentor and glucose to the stripping compartment. The experiment was then stopped.

The efficiency of ethanol transfer, measured by its transmembrane flux, depends on the diffusional resistances and the concentration gradients caused by the liquid membrane and the aqueous boundary layers near liquid–liquid interfaces.

The resistance of aqueous boundary layers is directly related to the hydrodynamics of the fluids in the permeation cell compartments. The circulation flow rate of the broth was fixed at 9 L/h and that of the stripping phase was changed in the course of experiment from 8.1 to 16.2 L/h. Table I indicates ethanol concentration in broth, flux, and global mass transfer coefficient of ethanol during successive extraction cycles. The global mass transfer coefficient of ethanol (k_E) is defined by:

$$J_E = k_E(\overline{C}_E^F - \overline{C}_E^S) = k_E \overline{\Delta C}_E$$

where $\overline{C}_E^F$ and $\overline{C}_E^S$ are the average concentrations of ethanol in the broth and stripping phase, respectively.

Table I. Supported liquid membrane process with aqueous stripping phase.

Cycle of extraction (h)	0–23	23–73	73–120	120–144	144–170
Ethanol concentration range in the broth (g/L)	42–71	71–87	87–84	84–75	75–61
Stripping phase flow rate (L/h)	8.1	8.1	8.1	16.2	16.2
Flux of ethanol (g/m^2 h)	16.5	24.2	35.2	75.0	46.8
Ethanol mass transfer coefficient 10^6 k_E (cm/s)	8.9	10.3	14.7	33.9	24.4

The more important result is that the efficiency of ethanol transfer was sufficient to limit the ethanol accumulation in broth under 87 g/L throughout the run, thus reducing inhibition. However, hydrodynamic conditions had not been optimized. The sharp enhancement of the permeation rate (k_E doubled from 15×10^{-6} cm/s to about 30×10^{-6} cm/s) when the circulation flow rate of the stripping phase was increased from 8 to 16 L/h, is likely due to a reduction of the aqueous boundary layer resistance at the stripping interface.

The ethanol transfer coefficient can be easily evaluated in the hypothesis of negligible aqueous boundary layer resistances, by the expression[15]:

$$k_E = \varepsilon m_E D_E / e$$

where porosity, ε, and thickness, e, of the microporous support are data from the manufacturer, the partition coefficient ($m_E = 0.22$) is determined by liquid–liquid extraction experiments and the diffusivity of ethanol in the organic phase ($D_E = 1.1 \times 10^{-6}$ cm^2/s) is estimated by Wilke and Chang's correlation.[18] It leads to a calculated value for k_E of 34×10^{-6} cm/s, very close to the maximal experimental value (33.9×10^{-6} cm/s) obtained during the fourth cycle. However, since it can be supposed that aqueous boundary layers were actually present and decreased the global ethanol transfer coefficient, this high experimental value would be better interpreted by a decrease of the liquid membrane thickness, due to erosion. This would explain the slow increase of k_E during the first three cycles (Table I) and, eventually, the destabilization of the liquid membrane.

Effects on Fermentation

The positive effects of ethanol removal are shown in Figures 2 and 3, where the cell mortality ratio and the total production of ethanol, respectively, are compared for extractive and reference fermentations.

Although the total biomass formed was not significantly increased ($C_x = 10$ g/L vs. $C_x = 9.5$ g/L dry matter for reference), the viability of the cells was improved and permitted maintaining the culture at a higher specific production rate ($\nu_E = 0.13$ g/g h). This caused an important increase of the total ethanol production (about 210 g/L after seven days vs. 129 g/L as a reference), as well as an enhancement of its average production rate (1.2 against 0.5 g/L h), the conversion yield remaining unchanged ($Y_{E/G} = 0.46$ g/g). By this method, the culture exploitation was extended, while ethanol was recovered on-line, with a yield ρ_E of 71%, in a purified but diluted form.

Extractive Fermentation with Gaseous Stripping Phase

The same approach was followed for characterizing the extraction patterns and their influence on the fermentation.

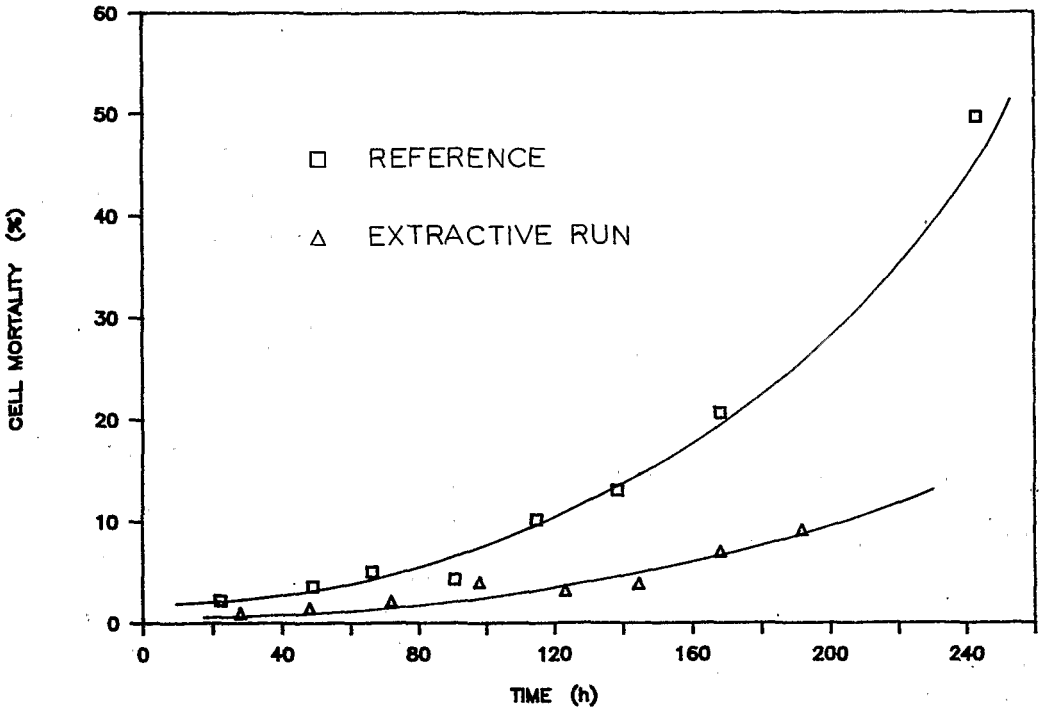

Figure 2. Yeast mortality ratio vs. time.

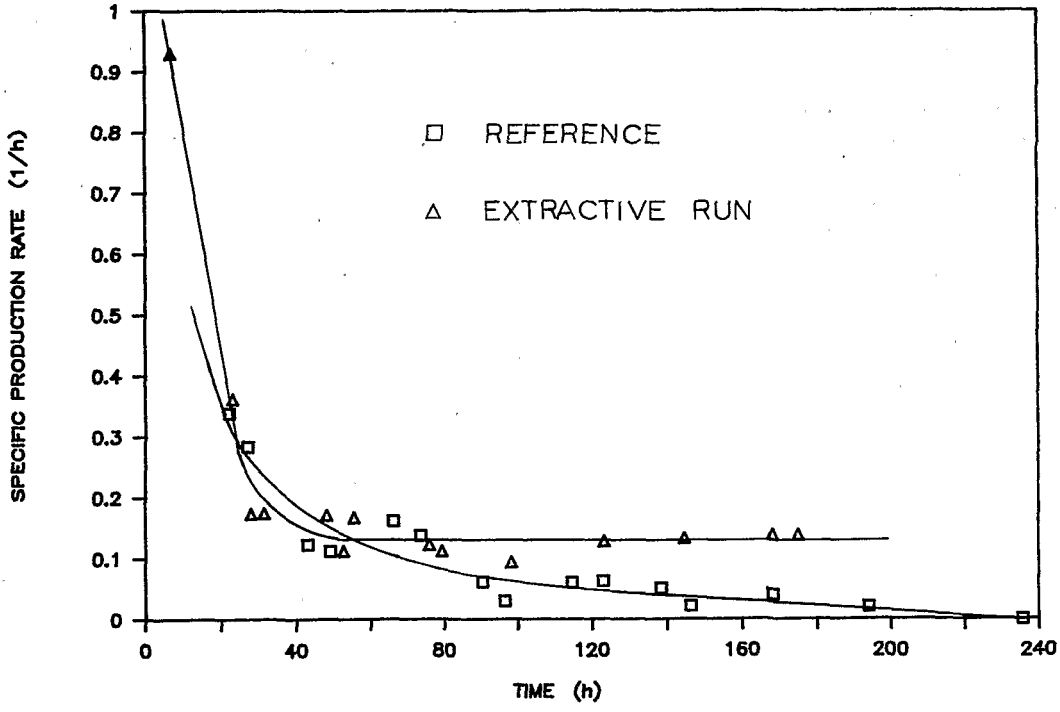

Figure 3. Specific production rate of ethanol vs. time.

Separation Parameters

No problem of destabilization of the liquid membrane was encountered during the 14 days of continuous operation, although no special maintenance was provided.

The ethanol and total permeate fluxes were determined from periodic weighing and concentration assaying of the solution collected in the condenser (Table II). Again, it has been observed that an increase of the stripping air flow could enhance them, but the ethanol flux remained inferior to that obtained with the aqueous stripping phase at similar broth concentrations. These performances still made possible to limit the accumulation of ethanol in the broth, under 107 g/L, and to maintain longer the culture activity.

Table II. Performances of ethanol pervaporation through a supported liquid membrane.

Ethanol concentration in broth (g/L)	Air flow rate (L/h)	Total flux of permeation (g/m^2 h)	Ethanol flux (g/m^2 h)
105	9	28	12.8
105	18	39	16.3

Figure 4 compares the ethanol concentration in the broth and the permeate during the course of fermentation. The ethanol concentration was about four times higher in the permeate, while extraction remained strictly selective towards biomass and glucose.

The selectivity of the isotridecanol membrane for the ethanol and water separation is $\alpha = [y_E(1 - x_E)]/[x_E(1 - y_E)]$, where x_E and y_E are the weight fractions of ethanol in the broth and in the stripping phase condensate, respectively. It remained between 5.5 and 11 throughout the 330 h extractive fermentation. The variation of this parameter with time is not related to changes of the liquid membrane, but mainly to the evolution of the broth ethanol concentration. As shown in Figure 5, the selectivity decreases linearly with x_E in this range of weight fraction. The selectivity observed in other permeation experiments with ethanol/glucose synthetic solutions, was preserved when operating with fermenting broth.

Figure 5 also shows results of extractive fermentation using the same teflon microporous support (Sartorius), but not impregnated by an organic liquid phase. In this case, selectivity, α, was lower, strengthening the interest of isotridecanol film interposition.

The literature reports results with other systems of separation, particularly with silicone rubber membranes, on synthetic alcohol solutions;[19] in this system, the value of α lies between 8.9 and 8.3 for the range 0.01–0.1 molar fraction of ethanol in the liquid phase (0.025–0.22 weight fraction) at 25°C. The selectivity of our isotridecanol liquid membrane (at 30°C) was slightly higher for the lower ethanol concentrations [about 10 for $x_E = 0.025$ (g/g)], and rather inferior for higher concentrations [about 6 for $x_E = 0.11$ (g/g)].

The pervaporation technique, when performed with solid polymeric membranes, leads to lower fluxes of permeation. The ethanol flux at 30°C, estimated from the permeability of silicone rubber tubes[19] is between 1.6 g/m^2 h for $x_E = 0.025$ (g/g) and 5.9 g/m^2 h for $x_E = 0.1$ (g/g), 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 and for a similar level of ethanol (Table II). It was yet a little higher with a nonimpregnated Teflon support (flux estimated to 24 g/m^2 h for similar conditions).

Fermentation Performance

As shown in Figure 4, the ethanol concentration in the broth did not rise about 107 g/L. This limited the inhibition phenomena and extended in time the culture activity. The biomass concentration was practically unchanged in regard to reference patterns, but the total production of ethanol was increased up to 169 g/L, produced at an average rate of 0.69 g/L h, and with a conversion yield $Y_{E/G} = 0.49$ g/g.

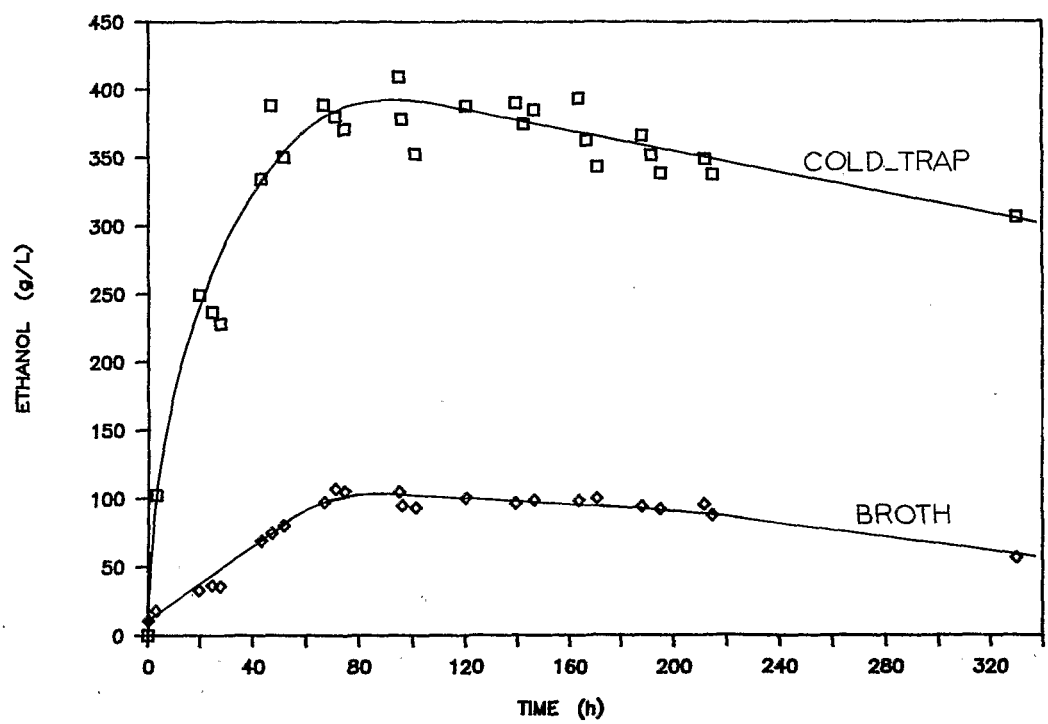

Figure 4. Evolution of ethanol concentration in broth and condensed permeate vs. time.

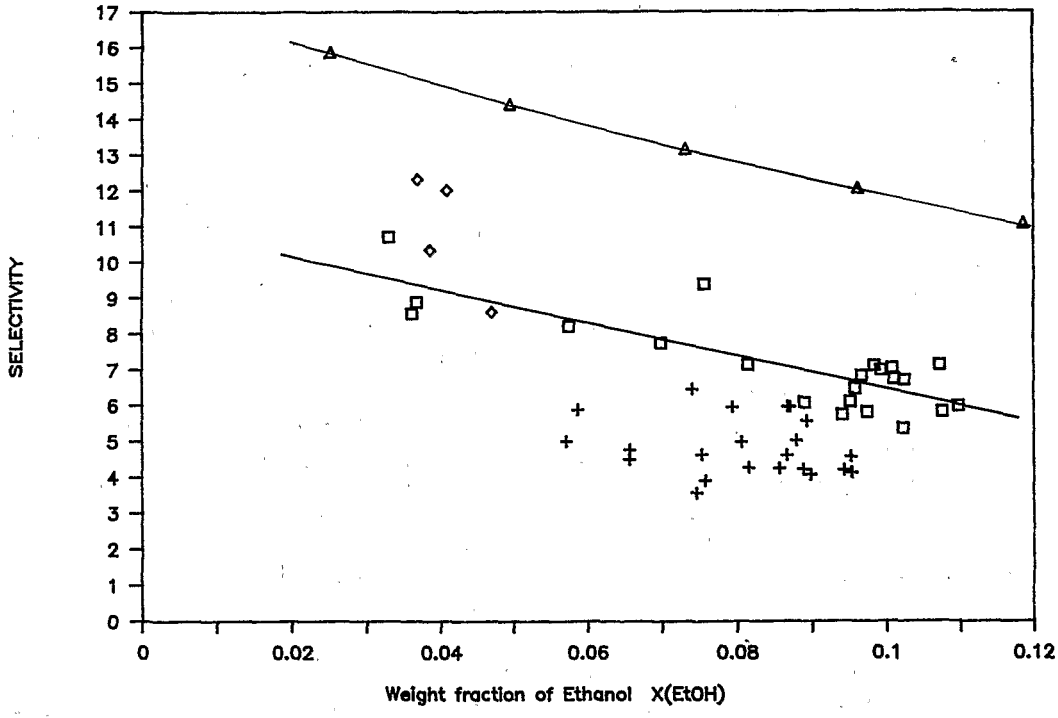

Figure 5. Ethanol/water selectivity vs. ethanol weight fraction in extracted phase. SLM extractions were made (◇) on synthetic ethanol solutions and (□) on fermentation broth. Nonimpregnated microporous support extraction (+) was also made. Theoretical distillation step at 30°C (△) is also shown.

DISCUSSION AND CONCLUSIONS

Separation by supported liquid membrane has proven suitable for fermentation coupling. With small amounts (about 70 mL/m^2) of industrial quality isotridecanol, it has been possible to achieve, in a single operation, the extraction of ethanol from an active culture and its reextraction, without encountering problems of biocompatibility. Although isotridecanol is rather well tolerated by yeasts,[2] an interest of the method is to avoid the dispersion of the extracting solvent in the culture: this allows us to use a toxic mixture as supported liquid membrane while maintaining the culture activity, as show in other extractive fermentation experiments.[20] In addition, the separation can be handled as an autonomous operation unit, next to the fermentor, without interferences on the fermentation control.

Two kinds of experiments have been described: with aqueous or gaseous stripping phases. In both cases, ethanol was removed at a rate sufficient to reduce inhibition. The main results of the two runs are summarized in Table III.

Taking only the permeation fluxes, stripping by an aqueous phase appears more efficient than by air. The fluxes obtained in pervaporation however may be underestimated since a partial condensation of ethanol/water vapor on the liquid membrane/air interface reduces the overall transfer rate. This condensation could be avoided by a local heating of the sweeping gas, in order to provide heat for vaporization and to make up for the energy losses in the cold trap.

In both techniques, the optimization of hydrodynamics should improve the present results. However, a compro-

Table III. Comparison of the extractive fermentation performances.

	Maximal biomass concentration, C_x (g dry w/L)	Total ethanol production, C_E* (g/L)	Average rate of production, $\bar{r}_E$ (g/L h)	Production yield, $\bar{Y}_{E/G}$ (g/g)	Maximal concentration	
					$C^F_{E\,Max}$ (g/L)	$C^s_{E\,Max}$ (g/L)
Reference	9.5	129	0.5	0.44	129	—
SLM system with aqueous stripping phase	10	210	1.2	0.46	87	38
SLM system with pervaporation	9.1	169	0.7	0.49	107	410

mise must be found between the enhancement of the permeation rate and the preservation of the membrane stability and yeast cells integrity.[8]

Concerning this last point, microscopic observations did not reveal an accumulation of cellular debris in our cultures but, in contrast, an improvement of viability. The problems caused by broth compounds such as debris or whole cells, proteins, and polysaccharides, which often block membrane systems by taking up interfaces or by filling pores, were not encountered. This could be expected, since no applied pressure tends to plug solid particles inside pores and since the separation need not carrier mediated transport, sensitive to many competitors.[21]

The stability is an essential parameter for the promotion or prevention of liquid membrane utilization. The fluidity of liquid organic films leads to high diffusion coefficients of solutes and thus high fluxes, compared with permeation through dense polymeric membranes. A drawback is their possible fragility. A study, focused on the stability of supported liquid membranes,[17] was carried out to evaluate quantitatively the parameters determining membrane lifetimes. For example, the drop-point pressure, or the bubble point pressure in the case of pervaporation, define the upper limits of the applied pressure gradient, above which oil filling of the pores is ejected. This requires a fine control of hydrodynamic in each side of the membrane and practically limits the circulation flow rates of aqueous phases. It can thus increase diffusional resistances and reduce the flux.

In our extractive fermentation experiments, the loss of stability which occurred in perstraction while the pervaporation system remained stable can be explained by the isotridecanol solubility in water. In perstraction, the successive changes of stripping phase, performed for maintaining a high transmembrane ethanol gradient throughout the experiment, led to contact with the liquid membrane a total of about 3.5 L aqueous volume, including the broth. Assuming its solubility to 100 ppm (maximum value given by the manufacturer), 70% (v/v) of the isotridecanol initially immobilized in pores could have been solubilized, compared with only 10% (v/v) in the pervaporation experiment. This indirectly confirms the conclusion of Deblay et al.,[17] that the stability can be predicted from the knowledge of few parameters and that a proper choice of the supported liquid membrane components and operating conditions can ensure a reliable stability.

An improvement of the perstraction method developed here could consist in supplying for the solubilized solvent, by pre-saturation of the stripping phase or by direct feed of the microporous support during operation.[22] This does not raise particular difficulties since isotridecanol can be considered as fairly water-insoluble and since it is used in a technical, inexpensive quality. In the experiment of perstraction reported here, a supply of 0.35 g isotridecanol should have been sufficient to maintain the stability of the liquid membrane. It must be noted that the utilization of even more soluble solvents is not prohibited: other extractive fermentation experiments were achieved with continuous perstraction through a supported decanol mixture;[20] the stability of such a liquid membrane was maintained without solvent supply throughout a 40-day run.

The last important parameters for a supported liquid membrane system are its selectivity and the recovery form of separated solutes. Due to the microporous support, the selectivity towards biomass always remained complete and ensured its retention in the broth, while the selectivity towards glucose gave evidence of the liquid membrane stability. The selectivity of ethanol/water, assayed only in the pervaporation experiment, is the main interest of this method: it achieved a prepurification step, yielding a concentration of ethanol as high as 410 g/L in the recovered permeate. This selectivity was slightly lower than that given by a theoretical single step of distillation at 30°C in the range of concentrations studied (Fig. 5).

For other components, for example organic acids, often produced in fermentations, the selectivity was not evaluated. However it is clear that different substances were transferred from the broth through the liquid membrane. In terms of inhibition relief, the ability of removing nonvolatile inhibitors[23] can even been considered as an advantage of perstraction over pervaporation. Fermentation tests on the aqueous stripping phases of our perstraction experiment indeed demonstrated the presence of inhibitory substances other than ethanol.

The main features needed for applying supported liquid membranes to extractive fermentation were achieved in the two examples reported, even if perstraction and pervaporation present different interests. Further investigations remain to be done to optimize the process according to biological (ethanol level in the broth) and downstream (ethanol/water separation) constraints, since the ethanol transfer rate increases with ethanol concentration in the broth, while the microbial productivity and the ethanol/water selectivity decrease. The simplest response would be to increase the specific membrane area (23 m^2/m^3 here), for example, by using hollow fiber supports, provided that the membrane thickness remains low. The application of liquid membranes with extended and improved performances to other extractive bioproductions and downstream processes appears as a near and promising stage of development.

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NOMENCLATURE

C	concentration (g/L)
C_E*	total ethanol production of the run (g/L)
D	diffusivity (cm^2/s)
e	membrane thickness (cm)
J	flux (g/cm^2 s)
k	mass transfer coefficient (cm/s)
m	partition coefficient (concentration ratio)
r	rate of production (g/L h)
SLM	supported liquid membrane
t	time (h)
x	weight fraction in the extracted phase (g/g)

y weight fraction in the aqueous stripping phase or in the condensed permeate (g/g)
$Y_{E/G}$ production yield (g ethanol/g glucose)
α selectivity
ε porosity
ν specific production rate (g/g dry w · h)
ρ yield of extraction recovery (g/g produced)

Subscripts

E ethanol
G glucose
X biomass

Superscripts

F fermentation broth
S stripping phase or condensed permeate
$-$ average

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