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ENHANCED HIGH FRUCTOSE SYRUP PRODUCTION BY A HYBRID FERMENTATION/PERVAPORATION SYSTEM USING A SILICONE RUBBER HOLLOW FIBER MEMBRANE MODULE

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ABSTRACT

In this study, a new mutant of *Saccharomyces cerevisiae* was used for the co-production of fructose and ethanol by selectively converting glucose to ethanol from a sucrose medium. To enhance the fermentation process, a pervaporation unit was coupled to the bioreactor to keep the ethanol concentration in the broth at a low level, thereby reducing the ethanol inhibition on the yeast. The membrane module consisted of silicone rubber hollow fibers assembled in an inside feed/outside vacuum design. Batch fermentation, using a synthetic medium with 31% (w/v) sucrose, without membrane separation of ethanol required about 27 hr for the glucose concentration to decrease to 2% (w/v), with a fructose yield of 99%, and an ethanol yield of 78%. Batch fermentation, using the same medium, with membrane separation of ethanol required about 16.5 hr for the glucose concentration to reach the 2% (w/v) level, with a fructose yield of 96.5% and an ethanol

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yield of 79.5%, if the membrane was initiated 6 hr after the beginning of batch mode. In fed-batch mode, the yeast was able to process the equivalent of a 41% (w/v) sucrose feed in 24 hr, compared to over 40 hr without ethanol removal, with fructose and ethanol yields of 98 and 82%, respectively.

INTRODUCTION

Fructose is widely used as a sweetener in the food industry since it has a much higher degree of sweetness than sucrose or glucose (1). Fructose is commercially available as high fructose corn syrups (HFCS) of three grades: 42, 55, and 90% HFCS, respectively. Solid fructose is manufactured from the crystallization of 90% HFCS (2). To obtain these very high fructose syrups, the glucose content must be reduced. It is difficult to separate glucose from fructose because these sugars are isomers of the same molecular size. For this separation, industry uses chromatographic techniques (3), but these are quite expensive, making the production of solid fructose uncompetitive with other sweeteners.

To solve this problem, new technologies are being developed for the production of high fructose syrups. They involve the conversion of glucose into a product such as ethanol, which can be separated easier than glucose from fructose. The selective conversion of glucose to ethanol has been achieved by fermentation using microorganisms such as *Mucor* sp. m105 and *Fusarium* sp. F5 (4), *Zymomonas mobilis* (5), *Tricholoma nudum* (6), *Pullularia pullulans* (7), and *Saccharomyces cerevisiae* (8–15). The fact that the fermentation produces ethanol as a valuable by-product, in addition to the reduction in separation costs, renders the process more profitable. The microorganisms previously tested presented some difficulties such as a substantial consumption of fructose (*Tricholoma nudum* (6) and *Pullularia pullulans* (7)) or the production of unwanted by-products such as sorbitol (*Zymomonas mobilis* (5)). To rectify these problems, extensive studies were performed using *Saccharomyces cerevisiae* ATCC 36859 and *S. cerevisiae* ATCC 36858 for the selective conversion of glucose to ethanol using glucose/fructose mixtures, or sucrose feeds (8–15). By using these two strains of *S. cerevisiae* almost no fructose consumption was observed in the presence of glucose, and relatively high yields of ethanol were obtained. The yeast *S. cerevisiae* ATCC 36859 did not possess the ability to hydrolyze sucrose; therefore, only glucose/fructose mixtures may be used. On the other hand, *S. cerevisiae* ATCC 36858, studied by Atiyeh and Duvnjak (15), is capable of hydrolyzing sucrose. This mutant also has the advantage of being able to tolerate fairly high sugar concentrations. However, as in the case of many microorganisms, its activity is affected by ethanol and, thus, longer reaction times are required to complete batch fermentation than without this inhibitor.

Therefore, improvements in terms of the time to complete a batch process, while keeping high ethanol and fructose yields, are still needed. One of the ways to accomplish this would be to remove continuously the ethanol from the broth. Pervaporation units coupled to bioreactors for the fermentation of glucose to ethanol have been studied by a number of researchers to enhance the process (17–19). As of yet, no studies have been done for the co-production of fructose and ethanol by fermentation processes combined with pervaporation. Studies of pervaporation have shown that membranes of silicone rubber are alcohol selective, and could therefore be used in conjunction with fermentation processes (20,21,23).

In the present study, the goal was to increase the rate of the co-production of fructose and ethanol from sucrose using *S. cerevisiae* ATCC 36858 by removing the inhibitory ethanol as it is being produced. Therefore, the purpose of the initial part of this project was to produce a small silicon rubber hollow fiber module that would be used to obtain basic pervaporation data using an ethanol–water solution and a suspension of yeast cells in an ethanol–water solution. Those data would serve to design and produce a laboratory silicon rubber hollow fiber module. This module and a commercial module would be coupled to a bioreactor to study their effects on fermentation parameters during the production of ethanol and fructose.

EXPERIMENTAL

Yeast Culture and Inoculum Preparation

Saccharomyces cerevisiae ATCC 36858 was maintained on agar slants. Agar medium consisted of glucose (0.5 g), yeast extract (0.5 g), agar (4.5 g), and distilled water (up to 100 mL). The medium used for the preparation of inoculum consisted of glucose (10 g), yeast extract (30 g), peptone (3.5 g), KH_2PO_4 (2 g), $MgSO_4 \cdot 7H_2O$ (1 g), $(NH_4)_2SO_4$ (1 g), and up to 1 L distilled water. Growth was carried out in 500 mL Erlenmeyer flasks in a shaker at 33°C and 200 rpm for a period of 24–36 hr. The biomass from the flasks was used for inoculation of the production medium. The medium used for the co-production of fructose and ethanol consisted of sucrose (310 g), yeast extract (10 g), peptone (3.5 g), KH_2PO_4 (2 g), $MgSO_4 \cdot 7H_2O$ (1 g), $(NH_4)_2SO_4$ (1 g), and distilled water up to 1 L. All media were sterilized for 15 min at 120°C before inoculation.

Fructose and Ethanol Production by Batch Fermentation

Batch fermentations were carried out using the production medium in a 600 mL bioreactor with an initial sucrose concentration of 31% (w/v) after

inoculation. The biomass concentration in the production medium was 3.5 g/L, and the initial volume of the broth after inoculation was 150 mL. The broth was constantly stirred and maintained at 33°C. Samples of 3.5 mL were taken aseptically every 3 hr, and then analyzed to determine the biomass, sugars, and ethanol concentrations.

Hollow Fiber Membrane Module Design and Assembly

Silicone rubber hollow fibers designated as NAGASEP M60 were kindly provided by Nagayanagi Industrial Co., Tokyo, Japan. The internal and external diameters of the hollow fibers were 200 and 320 μm, respectively. To conduct preliminary tests of the membrane performance, a small module of 15 fibers, with an effective permeation length of 45 cm (effective membrane area: 42 cm^2), was assembled. The fibers were glued together at both ends into a bundle with commercial bathroom silicone sealant. After the glue hardened, the fiber bundle was inserted into a glass casing and glued to its ends. After preliminary tests with this module, a larger membrane module to be coupled with the bioreactor was designed and built. That module consisted of 630 fibers with an effective permeation length of 22 cm (effective membrane area of 870 cm^2). A commercial module from Nagayanagi Industrial Co. was also tested. This module was also made of NAGASEP M60 hollow fibers and consisted of 750 fibers with an effective membrane area of 900 cm^2. In further text, the module built in the laboratory will be referred to as the "laboratory module" and the commercial one as the "commercial module."

Pervaporation Tests of Ethanol–Water, and Ethanol–Water–Biomass Mixtures

To obtain membrane performance data, preliminary tests were carried out using the 42 cm^2 surface area membrane module. Figure 1 displays the experimental set-up used for pervaporation. The set-up used for fermentation/pervaporation experiments differed only in the membrane module. The feed was pumped through the membrane module with a peristaltic pump. The feed was either a mixture of ethanol and water, or a suspension of ethanol, water, and biomass. The feed entered the inside of the fibers, while vacuum was applied on the outside of the fibers. Pharmed tubing was used to connect the vessel to the membrane module. Downstream pressure was measured with a MKS type 122A pressure transducer and controlled by a MKS type 651 pressure controller (provided by MKS Instruments Canada Ltd., Nepean, Ontario, Canada and Thermo Electric Canada Ltd., Brampton, Ontario, Canada). The feed temperature

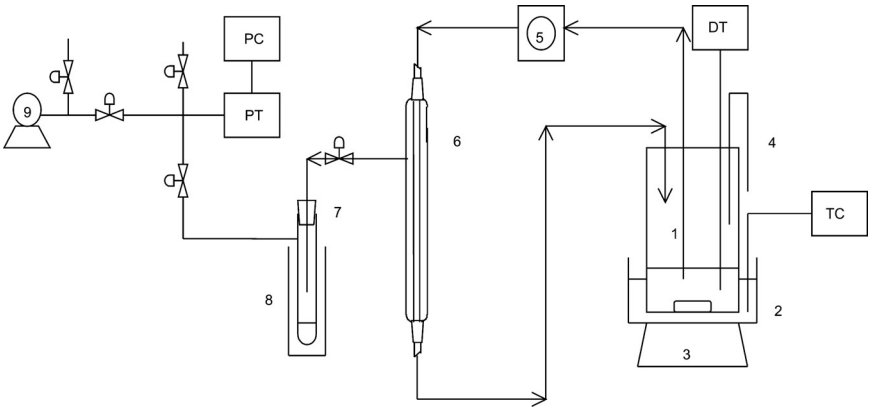

Figure 1. Diagram of the experimental set-up. (1) bioreactor or feed solution vessel for pervaporation tests; (2) temperature control bath; (3) magnetic stirrer; (4) CO_2 vent; (5) peristaltic pump; (6) membrane module; (7) condenser; (8) liquid N_2 bath; (9) vacuum pump; (TC) temperature control unit; (DT) digital thermometer; (PT) pressure transducer; (PC) pressure control unit.

was maintained at 33°C with a PolyTemp temperature controller to simulate fermentation conditions, and the temperature was read using a Thermo Electric digital thermometer. The permeate was collected in a condenser submerged in a liquid nitrogen bath. The condenser was removed from the liquid nitrogen bath approximately every 2 hr and replaced by a new one. The condenser, after being removed from the system, was weighed and the sample was subjected to analysis. Pervaporation tests were performed at various downstream pressures, ethanol concentration in the feed, and biomass in the feed to determine their effects on the total flux, permeate ethanol concentration, and selectivity. The data in Figs. 2 and 3 represent the average values obtained over an 8 hr period.

Batch and Fed-Batch Fermentation Coupled With Pervaporation

The 42 cm^2 surface area module in the set-up shown in Fig. 1 was replaced by the laboratory or commercial modules and the set-up was used in batch and fed-batch fermentation/pervaporation tests. All experiments were carried out in the production medium. After inoculation of the sterile medium, the system was left in batch mode for 3 or 6 hr to allow the biomass to grow well and to produce some ethanol. After that, the membrane unit was initiated. The flow of the fermentation broth was adjusted to 2.5 mL/min, which corresponds to a residence time of 1 hr in the reactor. In all experiments, the downstream pressure and the bioreactor

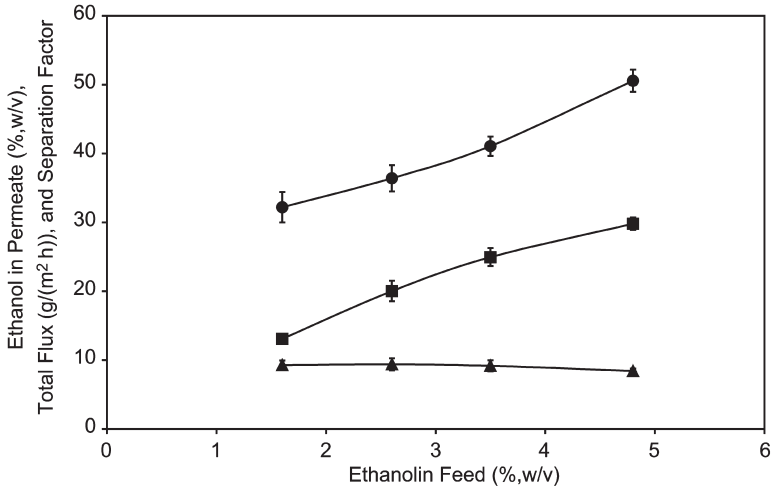

Figure 2. Effect of feed ethanol concentration on the membrane performance for pervaporation through silicone rubber hollow fibers. Downstream pressure, 1 torr; temperature, 33°C; circulation flow rate, 0.2 mL/min. (▲) separation factor; (●) total flux; (■) ethanol in permeate.

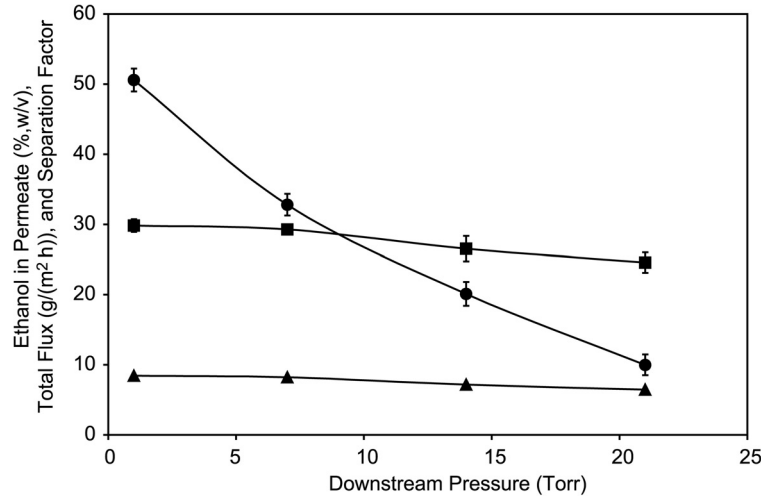

Figure 3. Effect of downstream pressure on the membrane performance for pervaporation of a 4.8% (w/v) ethanol feed solution through silicone rubber hollow fibres. Temperature, 33°C; circulation flow rate, 0.2 mL/min. (▲) separation factor; (●) total flux; (■) ethanol in permeate.

temperature were maintained at 1 torr and 33°C, respectively. When the condenser was changed, a sample of 3.5 mL was taken from the reactor and analyzed to determine the biomass, sugars, and ethanol concentrations. Various runs were performed using the laboratory module as well as the commercial module. In the case of fed-batch fermentation, after 14.5 hr of the process, the pervaporation was stopped and 22 mL of 60% (w/v) sucrose in the production medium was added to the bioreactor. The system was then left in batch mode without pervaporation for additional 10 hr to increase the concentration of ethanol in the broth.

Analysis

Ethanol concentration was determined by a spectrophotometer using alcohol dehydrogenase (22). Concentrations of sucrose, glucose, fructose, kestoses, and glycerol were determined by a high performance liquid chromatograph (Waters, Model 600) using a differential refractometer (Waters, Model R 410) as the detector with a column (Sugar Pac I, 6.5 × 300 mm) also supplied by Waters Associates (Milford, MA). A solution of 50 mg calcium ethylenediaminetetraaceticacid (CaEDTA) per liter of deionoized water was used as the mobile phase flowing at a rate of 0.5 mL/min. A spectrophotometer was used to measure the biomass concentration by measuring the absorbance at 600 nm.

RESULTS AND DISCUSSION

Pervaporation Tests of Ethanol–Water Mixtures

To design the membrane module to be coupled with the bioreactor, pervaporation tests of ethanol–water mixtures with and without biomass at various operating conditions were performed using the 42 cm^2 membrane surface area test module.

Effect of Ethanol Concentration in the Feed

To determine the effect of the ethanol concentration in the feed on the pervaporation performance of the used hollow fibers, a downstream pressure of 1 torr, a temperature of 33°C, and a feed circulation rate of 0.2 mL/min were applied. The results show (Fig. 2) that the total flux as well as the ethanol concentration in the permeate increased, whereas the separation factor decreased only slightly, with an increase in the feed ethanol concentration. The values and trends of the obtained results are in agreement with those previously reported for

the pervaporation of ethanol–water mixtures through silicone rubber membranes by Kimura and Nomura (19) and Nakao et al. (18). They demonstrated that the total flux increased from about 15 to 25 g/(m^2 hr) as the ethanol concentration in the feed increased from 0 to 5% (w/v).

Effect of Downstream Pressure

To examine the effect of the downstream pressure on the permeation performance of the small hollow fiber module, the pressure was varied between 1 and 21 torr. The obtained results show (Fig. 3) that the total flux of the ethanol–aqueous mixture through the membrane decreased substantially, while the ethanol concentration and the separation factor decreased slightly with an increase in the downstream pressure. These results are in agreement with those of Gudernatsch et al. (16) who observed a decrease of the total flux when the downstream pressure was increased from 8 to 30 torr. On the other hand, Gudernatsch et al. (16) observed a more severe decrease in selectivity with their composite silicone membrane.

Effect of Biomass in the Feed

Considering that the aim of this study was to use membrane modules to separate ethanol from the fermentation broth, it was of interest to determine the effect of biomass in an ethanol–water mixture on the pervaporation performance of the hollow fibers, and a possible blockage of the hollow fibers by biomass. Hence, a pervaporation test was carried out with a feed concentration of 48 g/L ethanol and 10 g/L yeast biomass. No fiber blockage was observed. It was noticed that the presence of biomass caused a decrease of the ethanol–water flux by about 10%, while the ethanol concentration in the permeate and the separation factor increased by about 2.5 and 6%, respectively. When the yeast cells suspension in the ethanol–water mixture was circulated through the fibers, some of the cells deposited on the membrane surface forming a thin film causing the fouling of the membrane. This might have provoked the decrease in the flux, and the increase in both the permeate concentration and the selectivity.

Batch Fermentation Without Membrane Separation

Figure 4 shows the results of a typical fermentation process with *S. cerevisiae* ATCC 36858 performed in the 600 mL bioreactor. As sucrose was hydrolyzed, the concentrations of glucose and fructose increased, and the yeast started to consume

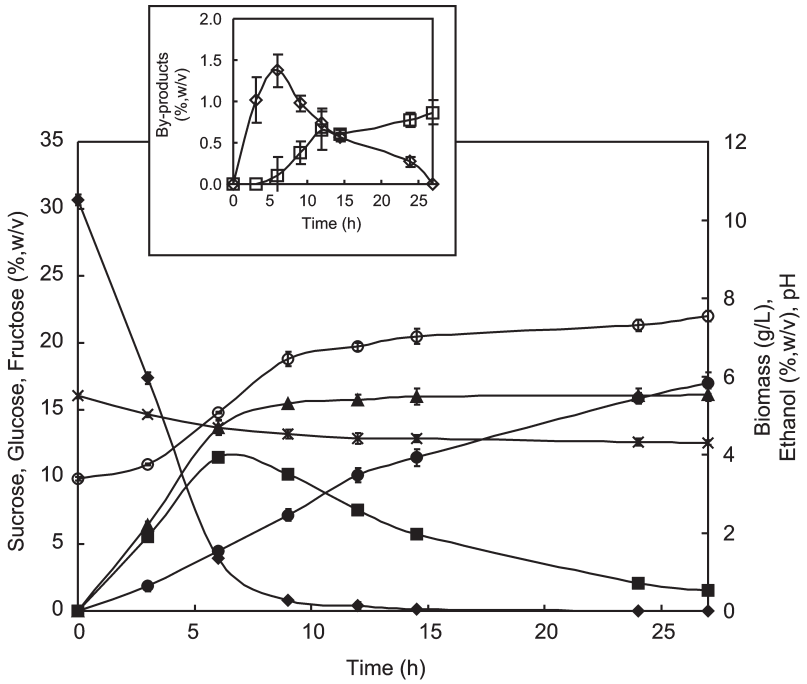

Figure 4. Results of batch fermentation performed in a small capacity bioreactor. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L; temperature, 33°C. (◆) sucrose; (■) glucose; (▲) fructose; (●) ethanol; (○) biomass; (×) pH; (◇) kestoses; (□) glycerol.

glucose. Glucose reached a maximum value after a period of time. The value is below its theoretical value of 16.1% (w/v) because the yeast cells were converting the sugar into ethanol and biomass before sucrose was completely hydrolyzed. The fructose reached its maximum value once all of the sucrose was hydrolyzed. In the presence of glucose, the yeast did not consume fructose and, thus, it remained in the broth. As the glucose was consumed, the ethanol concentration increased until it reached its maximum value. Two by-products were also produced in this process: kestoses and glycerol. By the end of the process, kestoses were metabolized, while glycerol stayed in the broth. The biomass increase followed the typical growth curve observed in fermentation processes.

Fermentation was carried out for 27 hr. At the end, the glucose, fructose, and ethanol concentrations (w/v) were 1.53, 16.11, and 5.83%, respectively (Table 1). These values represent fructose and ethanol yields of 99.9 and 78.3%, respectively. The ethanol productivity was 2.16 g/(L hr), and the biomass specific

Table 1. Fermentation Parameters

	Batch	Batch Fermentation/ Pervaporation I[a] (Laboratory Module, 6 hr)[b]	Batch Fermentation/ Pervaporation II[a] (Commercial Module, 6 hr)[b]	Batch Fermentation/ Pervaporation III[a] (Commercial Module, 3 hr)[b]	Fed-Batch[a] Fermentation/ Pervaporation
Initial biomass concentration (g/L)	3.38	3.10	3.28	3.32	3.36
Final biomass concentration (g/L)	7.55	9.86	9.66	10.90	7.69
Initial sucrose concentration (%,w/v)	30.7	31.10	31.10	31.10	31.0 + 10.0
Fermentation time (hr)	27	16.5	16.5	16.5	24.5
Final glucose concentration (%,w/v)	1.53	2.10	1.4	1.15	3.38
Final fructose concentration (%,w/v)	16.11	15.71	15.76	14.48	21.38
Final ethanol concentration (%,w/v)	5.83	5.92	6.28	7.23	7.84
Specific growth rate of biomass (hr^{-1})	0.07	0.08	0.08	0.08	0.06
Biomass yield (g/g)	0.29	0.35	0.32	0.36	0.34
Ethanol productivity [g/(L hr)]	2.16	3.59	3.81	4.43	3.20
Ethanol yield (%)	78.3	78.6	79.5	82.84	82.75
Fructose yield (%)	99.9	96.4	96.5	88.45	98.67
Fructose/glucose content (%)	91.3/8.7	88.2/11.8	91.8/8.2	92.8/7.2	86.4/13.6
Sucrose hydrolysis rate [g/(L hr)]	21.04	21.99	21.97	24.69	21.30

[a] Data are normalized.

[b] Six and 3 hr indicate the initiation periods of pervaporation.

growth rate was 0.07 per hr. These results are in the typical ranges obtained for fermentation by *S. cerevisiae* ATCC 36859 (11). They were used as the basis of comparison with the results obtained in the fermentation/pervaporation systems.

Batch Fermentation Coupled with Membrane Separation

All the fermentation/pervaporation experiments were compared to the batch fermentation without pervaporation. It should be noted that two kinds of data are presented for each batch fermentation/pervaporation experiment. In one, the actual concentrations of sugars and ethanol as determined in the bioreactor are used. In the other, "normalized concentrations" are used. The "normalized data" refer to the data that were calculated as though at each point of time the permeate would have been put back into the reactor. Therefore, the normalized data correspond to a situation where no concentration effect would have taken place due to the removal of water and ethanol from the bioreactor.

It should also be noted that the batch experiment was terminated when the glucose concentration was 1.5%(w/v). The fermentation parameters for the batch fermentation/pervaporation experiments are those obtained when the normalized glucose concentration was close to 1.5% (w/v). This enables the comparison of the results obtained in the systems with and without pervaporation.

Batch Fermentation/Pervaporation I

This set of experiments was done using the laboratory membrane module. The module was initiated after the process had been in batch mode for a period of 6 hr. Figures 5 and 6 show the actual and normalized reactor data as a function of time, respectively. Table 1 shows the fermentation parameters used for comparison with batch fermentation without pervaporation. The data in Figs. 5 and 6 follow the same trends as those shown for the batch system without pervaporation. The system with pervaporation was successful in continuously removing part of the ethanol from the fermentation broth, and its concentration was 3.6% (w/v) at the end of the process (Fig. 5). This value is 62% of the ethanol concentration value that was recorded in the batch fermentation without pervaporation. A consequence of the ethanol removal is a decrease in inhibition of the yeast activities by a higher ethanol concentration. Thus, the fermentation/pervaporation I system required only about 60% of the time to reduce the glucose content to about 2% (w/v) that was close to that at the end of the batch system without pervaporation. The removal of ethanol in this process was reflected in an increased ethanol productivity of the bioreactor coupled to the

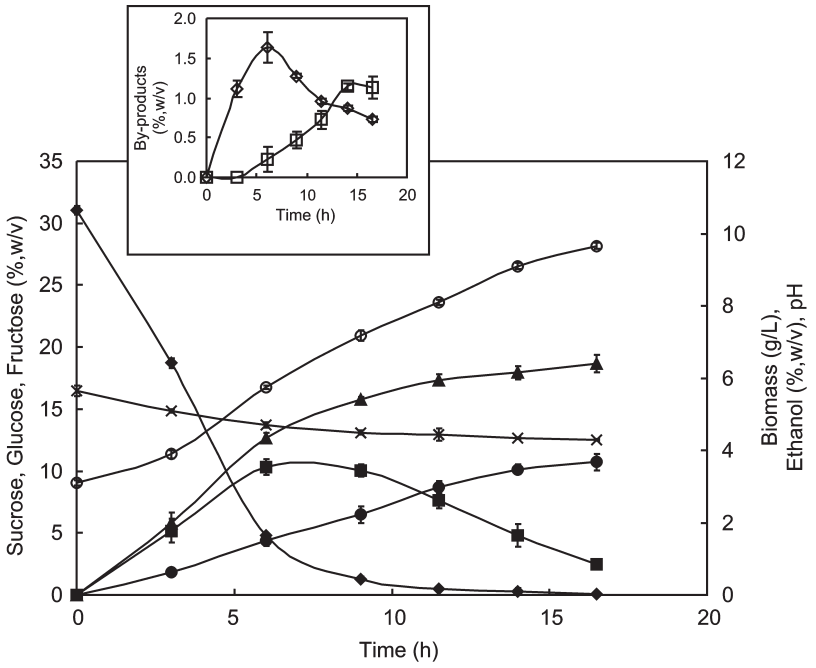

Figure 5. Actual reactor data of batch fermentation/pervaporation I using the laboratory pervaporation module. Initial sucrose concentration, 31%w/v; initial biomass concentration, 3.1 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 6 hr of batch fermentation. (◆) sucrose; (■) glucose; (▲) fructose; (●) ethanol; (○) biomass; (×) pH; (◇) kestoses; (□) glycerol.

pervaporation membrane module (Table 1). The fermentation/pervaporation parameters presented in Table 1 are based on the normalized data (Fig. 6).

Data displayed in Table 1 show that the initial and final conditions (normalized concentrations for the system with pervaporation) are approximately the same in the batch and batch/pervaporation systems. The sucrose hydrolysis rates are also almost identical with values of 21–22 g/(L hr). This was expected since most of the hydrolysis took place in the first 6 hr of fermentation, during which period the membrane module was not activated in the fermentation/pervaporation I system. The normalized final ethanol concentration in the broth is 5.92% (w/v) compared to 5.83% (w/v) in batch fermentation without pervaporation. These correspond to ethanol yields of 78.6 and 78.3%, respectively. The normalized fructose concentration at the end of this fermentation is lower [15.71% (w/v)] than in the batch fermentation without pervaporation [16.11% (w/v)]. These correspond to respective fructose yields of

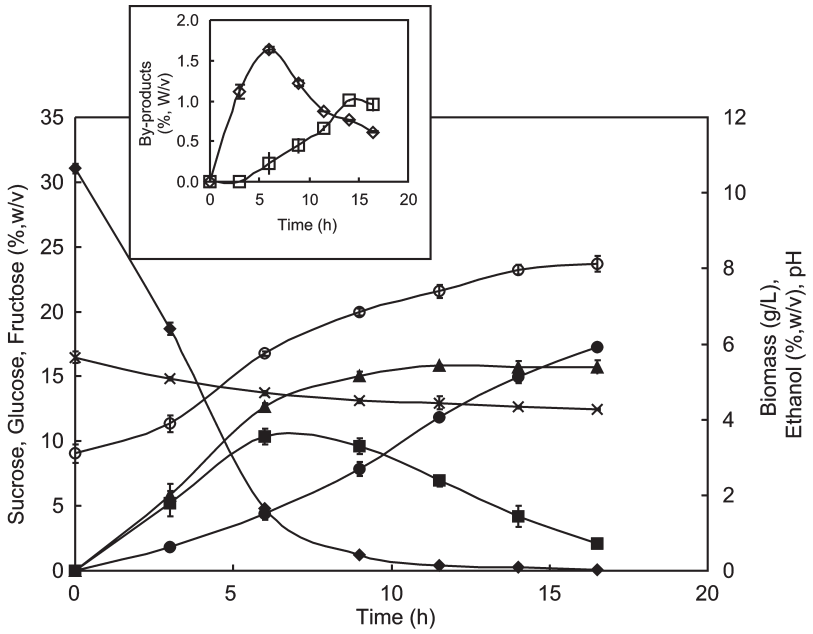

Figure 6. Normalized reactor data of batch fermentation/pervaporation I using the laboratory pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.1 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 6 hr of batch fermentation. (◆) sucrose; (■) glucose; (▲) fructose; (●) ethanol; (○) biomass; (×) pH; (◇) kestoses; (□) glycerol.

96.4% with pervaporation and 99.9% without pervaporation. One factor leading to a lower fructose yield, when pervaporation is applied, is that some by-products (kestoses) are still present in the bioreactor after the 16.5 hr of fermentation, whereas in the batch fermentation without pervaporation, the kestoses level was almost zero. Since a molecule of kestoses is made of two molecules of fructose and one molecule of glucose, this would account for a lower fructose yield. The fructose/glucose ratio at the end of the process is 88.2:11.8%, which is suitable for the production of crystalline fructose.

Figure 7 shows the membrane performance as a function of the fermentation time. As shown in Fig. 5, the actual ethanol concentration in the reactor was increasing. This means that the membrane did not remove all the ethanol produced, and the ethanol concentration increased from 1.6 to 3.6% (w/v) from the time the pervaporation was initiated to the end of the fermentation. Therefore, according to the earlier pervaporation tests, the total flux should increase with time, but in fact it decreased. The ethanol–water flux in the

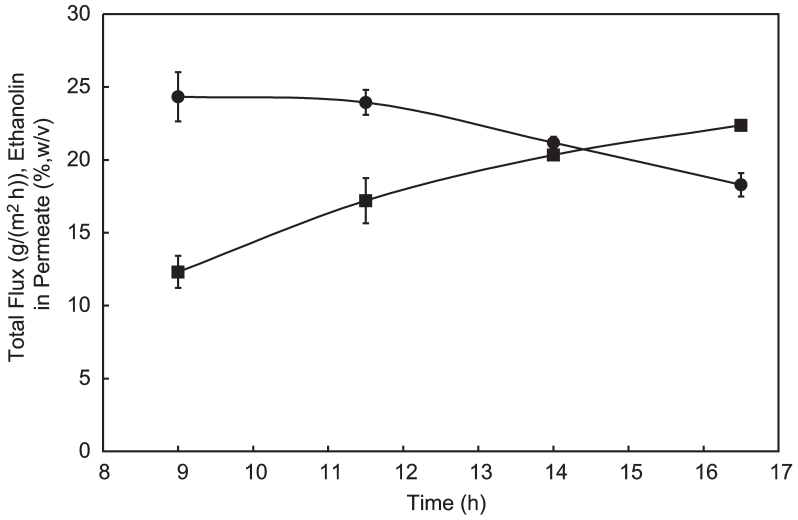

Figure 7. Membrane performance of the laboratory pervaporation I module coupled to a bioreactor. Membrane was initiated after 6 hr of batch fermentation. (●) total flux; (■) ethanol in permeate.

fermentation/pervaporation system varied from 24 to 18 g/(m^2 hr) compared to the flux that varied for comparable ethanol concentrations from 32 to 42 g/(m^2 hr) when a pure ethanol–water mixture was used in the 42 cm^2 active area module. The reason why the flux was lower in the former system was the presence of biomass. This decrease is also in agreement with the already-mentioned results of the pervaporation tests carried out with 42 cm^2 module using a suspension of the yeast cells in an ethanol–water solution. During the fermentation/pervaporation process, the concentration of biomass in the reactor increased, and therefore more cells deposited on the membrane surface, which caused the flux to decrease with time. The decrease in the flux can also be explained by the presence of sugars in the broth, the phenomenon that was reported by Wood et al. (23). They performed pervaporation tests using a silicone rubber membrane with solutions of water–sugar and water–ethanol–sugar, and reported a drop of 30% in total flux as the concentration of sugar was increased in the feed solution by 5–15% (w/v).

Batch Fermentation/Pervaporation II

A set of fermentation/pervaporation tests was also performed using the commercial membrane module, which was initiated after the system had been in

a batch mode for a period of 6 hr. The actual reactor data and the normalized data as a function of time are not shown here because their pattern is the same as in the fermentation/pervaporation I tests. This set of tests was compared to fermentation/pervaporation I since the only difference between these two experimental set-ups was the module used for pervaporation. After 16.5 hr, the normalized final glucose concentration was lower when the commercial module was used compared to the laboratory module (Table 1). This indicates that the level of inhibition by ethanol on the yeast was lower since more glucose was consumed in the same amount of time. This can be explained by the actual ethanol concentration in the reactor, which is 2.4% (w/v) with the commercial module compared to 3.6% (w/v) with the laboratory module (Table 2) i.e., the commercial module removed more ethanol during the same period of time. Since the ethanol concentration in the reactor was kept lower, a lower inhibition on the yeast was exerted, leading to more consumption of glucose. Ethanol yield and productivity were slightly higher when the commercial module was used than in the case of the utilization of the laboratory module (Table 1). Considering that the glucose concentration at the end of fermentation was lower with the commercial module system, and the fructose concentration was the same in both systems, the fructose/glucose ratio in the commercial module system was higher than in the latter one. Other process parameters in both systems were approximately the same.

Regarding the pervaporation results, the same trends in this set of tests were noticed as in the batch fermentation/pervaporation I tests. However, more ethanol was removed by the commercial module as indicated by the actual ethanol

Table 2. Actual Pervaporation Data

System	Time After Inoculation (hr)	Ethanol in Broth (%, w/v)	Total Flux (g/m^2 hr)	Ethanol in Permeate (%, w/v)
Fermentation/pervaporation I	6[a]	1.6	24[c]	12[c]
(lab. module)	16.5[b]	3.6	18	23
Fermentation/pervaporation II	6[a]	1.6	28[c]	15[c]
(comer. module)	16.5[b]	2.4	24	30
Fermentation/pervaporation III	3[a]	0.6	26[c]	11[c]
(comer. module)	16.5[b]	2.5	23	28
Fed-batch/pervaporation	3[a]	0.5	23[c]	12[c]
(comer. module)	14.5[b]	2.4	20	28

[a] Initiation of modules.
[b] Pervaporation stopped.
[c] Samples taken 3 hr after initiating modules.

concentration in the reactor. The reasons for the faster removal of ethanol are: (1) the membrane surface area of the commercial module is larger (900 cm^2) and (2) the flux as well as the selectivity of this module are higher than in the laboratory module. The flux decreased with time, from 28 to 24 g/(m^2 hr), and the ethanol concentration in the permeate increased from 15 to 30% (w/v) for the same reasons as described previously (Table 2). It is possible that the actual flux in the laboratory module was higher than that reported, since the membrane area could be smaller than the nominal value of 870 cm^2, due to the difficulty in knowing the exact membrane area because of the assembly method used.

Batch Fermentation/Pervaporation III

The purpose of this set of tests was to examine the effect of the pervaporation process when it was started 3 hr after beginning of fermentation. These tests were performed using the commercial membrane module. The actual and normalized data with time from this process showed the same fermentation/pervaporation patterns as those obtained in the fermentation/pervaporation I and II tests. This set of experiments was compared to fermentation/pervaporation II since the only difference between these two experiments was the length of the fermentation time prior to starting pervaporation. Fermentation parameters from this set of tests (Table 1) differed from those obtained in the set of tests when the pervaporation process was initiated 6 hr after beginning of fermentation. Considering that the removal of ethanol started earlier, its concentration, and the resulting inhibitory effect, was lower during the largest part of the process. This had a positive effect on the growth of biomass and the ethanol productivity. The biomass final concentration and the ethanol productivity were by about 12 and 16% higher than in the tests when pervaporation started 6 hr after beginning of fermentation, respectively. However, it can also be noticed that the fructose yield in this case was lower (Table 1). The greater fructose consumption resulted in a higher ethanol and biomass concentration at the end of the process. An increase in the fructose consumption occurs usually when the glucose content is low. If the process had been stopped after 14.5 hr (when the glucose concentration was about 2%) instead of 16.5 hr, the fructose yield would have been about 92% (data not shown). Data in Table 1 also indicate that the sucrose hydrolysis rate was higher in the process when the pervaporation started earlier. This is a consequence of the lower ethanol concentration in the early stages of the process and, therefore, its lower inhibitory effect on the invertase that was responsible for the hydrolysis of sucrose. The inspection of other fermentation parameters when the membrane module was initiated at 3 and 6 hr after beginning of the process reveals that they are approximately the same. From the obtained data, it can be concluded that

initiating the membrane module 3 hr after beginning of fermentation showed some advantages comparing to the process when the module was initiated 6 hr after inoculation.

The pervaporation results had the same trend as those observed in batch fermentation/pervaporation II. More ethanol was removed when the membrane was initiated earlier in the process, but since more ethanol was also produced because of fructose conversion, the final actual ethanol concentration in both cases was approximately the same. The flux decreased with time from 26 to 23 g/(m^2 hr), and the ethanol concentration in the permeate increased from 11 to 28% (w/v) for the same reasons as described previously (Table 2). The range of ethanol concentration in the permeate is wider in batch fermentation/pervaporation III than in batch fermentation/pervaporation II, because at the point when the pervaporation was started, the actual ethanol concentration in the reactor was only 0.6% (w/v) in the former experiment instead of 1.6% (w/v) in the latter experiment. As seen previously (Fig. 2), the ethanol permeate concentration decreased with a decrease in the feed ethanol concentration.

Fed-Batch Fermentation/Pervaporation

The purpose of the fed-batch tests was to increase the concentration of ethanol in the broth after stopping permeation. This set of experiments was performed using the commercial membrane module. The fermentation process was initially carried out without pervaporation for 3 hr, and then the pervaporation module was activated. After a total of 14.5 hr after inoculation, the pervaporation was stopped, and a fresh sucrose solution of 60% (w/v) was added to the reactor. The system was then left in a batch mode without pervaporation for additional 10 hr. When the added sucrose is combined with the sucrose present in the reactor at the start of the experiment, the total sucrose concentration was 41% (w/v) in the medium. Therefore, the fermentation parameters presented in Table 1 were calculated based on a 41% (w/v) sucrose medium, even though Fig. 8 indicates a 31% (w/v) initial sucrose concentration. Therefore, the comparison of this fed-batch experiment could be made with batch fermentation with a 41% (w/v) initial sucrose concentration. Figure 8 shows the actual reactor data as a function of time, while some normalized data are given in Table 1. Before considering the parameters in Table 1 that were determined at the point of 24.5 hr, it would be worthwhile to look at the fructose and ethanol yields at the time when the pervaporation was stopped before the addition of a concentrated sucrose solution i.e., at 14.5 hr. At this point, the yields were 79 and 91% for ethanol and fructose, respectively, both calculated based on sucrose consumption at 14.5 hr before a new batch of sucrose was added. These results are in agreement with those obtained in fermentation/per-

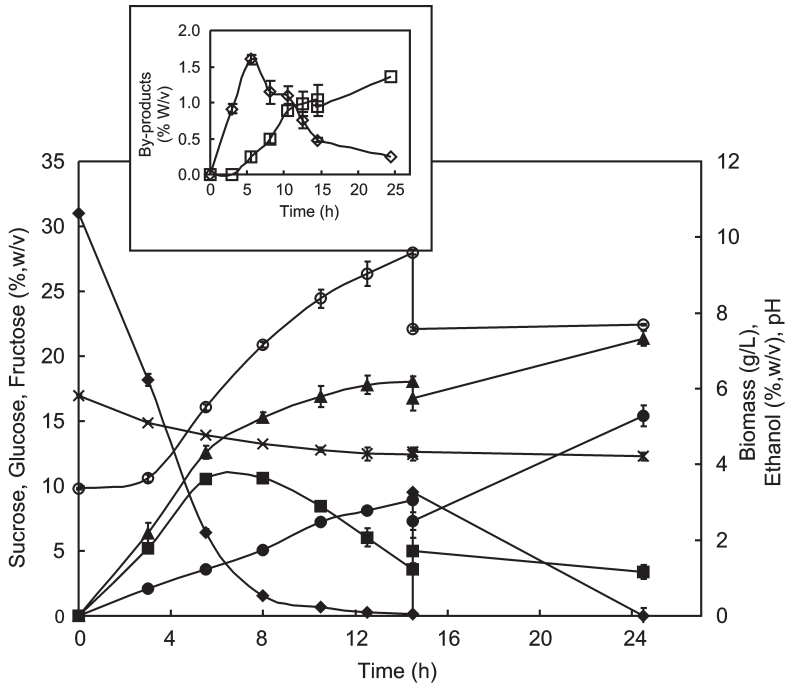

Figure 8. Actual reactor data of fed-batch fermentation/pervaporation using the commercial pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 3 hr of batch fermentation. (◆) sucrose; (■) glucose; (▲) fructose; (●) ethanol; (○) biomass; (×) pH; (◇) kestoses; (□) glycerol.

vaporation III tests. Figure 8 and Table 1 show that the yeast was able to process the equivalent of a 41% sucrose solution. Without attaching the hollow fiber module, well over 40 hr would be required to process the same amount of sucrose (15). Therefore the reaction time was shortened substantially by decreasing the inhibition by ethanol. In this fed-batch fermentation, the normalized final fructose and glucose concentrations are 21 and 3.4% (w/v), respectively, which gives a fructose/glucose ratio of 86:14%. If the fermentation had been allowed to continue for a few more hours, the glucose content would have been reduced further, and the fructose/glucose ratio would have increased. But after 24.5 hr, the actual ethanol concentration in the reactor increased to 5% (w/v), a concentration at which ethanol inhibition is high. It would take too much time to further decrease the glucose content and the advantage of attaching the hollow fiber module would be decreased. Therefore, it would be the best to

restart pervaporation after 24.5 hr. It could be even better to continue pervaporation without stopping it at 14.5 hr, after an additional amount of sucrose was added. All other parameters were in the same ranges as observed with other experiments.

Regarding the pervaporation results, it was noticed that the flux decreased with time, and it was in the range 23–20 g/(m^2 hr), and the ethanol concentration in the permeate increased from 12 to 28% (w/v) for the same reasons as described previously (Table 2). The flux, in this case, was somewhat lower than the 28–24 g/(m^2 hr) range, which was observed when the commercial module was used for the first time. A lower ethanol concentration at the initiation of the module in the fed-batch tests, as well as an insufficient cleaning of the membrane, after a long time of operation, may have caused this flux decline.

CONCLUSIONS

The following can be concluded from the experimental data obtained in this study.

The pervaporation tests performed with ethanol–water mixtures containing yeast biomass showed that it is possible to use hollow fibers of silicone rubber, applying an inside feed/outside vacuum design, without concerns of fiber blockage. The ethanol selectivity and the total ethanol–water flux through the hollow fibers were reasonably satisfactory.

Batch fermentation/pervaporation set-up with a membrane module made of silicone rubber hollow fibers can decrease the process time of production of ethanol and fructose by decreasing the ethanol inhibition of the yeast cells activity and thus, increasing the productivity of the bioreactor.

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