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Enhanced High Fructose Syrup Production by an Hybrid Fermentation/Pervaporation System using a Silicone Rubber Hollow Fiber Membrane Module

By

Isabelle Gagné

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Abstract

Fructose is a monosaccharide of high commercial importance. It is available as high fructose corn syrups (HFCS) and solid fructose, which is obtained by crystallisation from syrups containing 90-95% fructose. To obtain enriched fructose syrups, the glucose content must be decreased. Currently, chromatographic techniques are used, but these are very expensive. New technologies for this separation use microorganisms, which selectively convert glucose to ethanol while fructose remains unconverted. In this study, a mutant of the yeast *Saccharomyces cerevisiae* was used for the selective conversion of glucose to ethanol using feed solutions of sucrose. The disadvantage, as with many other microorganisms, is inhibition by ethanol. To enhance the fermentation process, a pervaporation unit was coupled to the bioreactor to keep the ethanol concentration low by its continuous removal. The study included: membrane testing, hollow fibre membrane module design and building, batch fermentation with and without membrane separation of ethanol, and fed-batch fermentation with membrane separation of ethanol. The module consisted of silicone rubber hollow fibres using an inside feed/outside vacuum design. Batch fermentation using 30% (w/v) sucrose without membrane separation of ethanol required about 27 hours for glucose to be decreased to 2% (w/v), with a fructose yield of 99%, and an ethanol yield of 78%. Batch fermentation using 30% (w/v) sucrose with membrane separation of ethanol required about 16.5 hours for glucose to be decreased to 2% (w/v), with a fructose yield of 96.5% and an ethanol yield of 79.5%, if the membrane was started after 6 hours of batch mode. The process required about 15 hours if the membrane was started after 3 hours of batch mode, with a fructose yield of 92%, and an ethanol yield of 82%. In fed-batch mode the yeast was able to process the equivalent of a 40% (w/v) sucrose feed in 24 hours, compared to well over 40 hours without ethanol removal, with yields of, 98% fructose, and 82% ethanol.

Résumé

Le fructose est un monosaccharide de haute importance industrielle. Le fructose est disponible sous forme de sirop concentré fait à partir de maïs, et il est également disponible sous forme crystalline. La crystallisation s'effectue à partir de sirops contenant de 90-95% de fructose. Pour obtenir ces sirops très concentrés, leur contenu en glucose doit être diminué. Présentement, des méthodes de séparation par chromatographie sont utilisées, ce qui est très dispendieux. De nouvelles technologies de séparation incluent l'utilisation de microorganismes capables de convertir de façon sélective le glucose sans consommer le fructose. Dans la présente étude, une souche mutante de la levure *Saccharomyces cerevisiae* a été utilisée pour la conversion sélective du glucose à partir de solutions de sucrose puisque cette souche possède la capacité d'hydroliser le sucrose, et ne consomme pas le fructose en présence de glucose. Le désavantage, comme avec plusieurs souches de microorganismes, est l'inhibition pas l'éthanol. Dans le but d'améliorer la fermentation, une unité de pervaporation a été couplée au bioréacteur afin d'enlever l'éthanol au fur et à mesure qu'il est produit. L'étude a été divisée en 5 volets: tester la membrane, concevoir et construire l'unité de membrane, fermentation en mode discontinu avec et sans séparation d'éthanol par membrane, fermentation en mode semi-continu avec séparation d'éthanol par membrane. L'unité de membrane est constituée de fibres de silicone utilisant le modèle d'alimentation interne et de suction externe. Les fermentations en mode discontinu de solution de 30% (w/v) sucrose requièrent environ 27 heures pour réduire le contenu en glucose à environ 2% (w/v) avec un rendement de fructose et d'éthanol respectifs de 99% et 78%. Les fermentations en mode discontinu avec membrane requièrent environ 16.5 heures pour réduire le contenu de glucose à environ 2% (w/v) avec un rendement de fructose et d'éthanol respectifs de 96.5% et 79.5%, si la membrane est mise en fonction après 6 heures de fermentation. Le procédé demande environ 15 heures si la membrane est mise en fonction après 3 heures de fermentation avec un rendement de fructose et d'éthanol respectifs de 92% et 82%. Dans le mode semi-continu, la levure peut procéder l'équivalent d'une solution de 40% (w/v) sucrose en 24 heures, comparativement à 40 heures et plus sans membrane, avec un rendement de fructose et d'éthanol respectifs de 98% et 82%.

Acknowledgements

I would like to express my deepest gratitude to Dr Z. Duvnjak and Dr T. Matsuura for their support, guidance and financial assistance throughout this project.

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Nomenclature

A effective membrane area (m^2)

ADH alcohol dehydrogenase

B constant related to the vapor transport (mol/(m s Pa^2))

B_0 biomass concentration at the beginning of the fermentation (g/L)

B_1 biomass concentration at the beginning of the exponential growth phase (g/L)

B_2 biomass concentration at the end of the exponential growth phase (g/L)

B_i constant related to the vapor transport of i (mol/(m s Pa^2))

B_j constant related to the vapor transport of j (mol/(m s Pa^2))

B_j/B_i ratio of the constants related to the vapor transport (mol/(m s Pa^2))

B_t biomass concentration at the end of the fermentation (g/L)

E_t experimental ethanol concentration at time t (% (w/v))

F_t : experimental fructose concentration at time t (% (w/v))

G_t : experimental glucose concentration at time t (% (w/v))

HFCS high fructose corn syrup

HFS high fructose syrup

HPLC high performance liquid chromatography

LTV low temperature vulcanization

M_i molecular weight of species i

M_j molecular weight of species j

m_p mass of permeate (g)

$M_{P,t}$ cumulative mass of ethanol removed as permeate at time t (g)

NAD nicotinamide adenine dinucleotide

P ethanol productivity (g/(L h))

PDMS polydimethylsiloxane (silicone rubber)

PDMS-PS IPN polydimethylsiloxane polystyrene interpenetrating polymer network

PTMSP poly(1-trimethyl silyl-1-propyne)

$P_\bullet$ saturation vapor pressure of the feed mixture (Pa)

P_2 pressure of feed liquid (upstream pressure) (Pa)

P_3 pressure of permeate vapor (downstream pressure) (Pa)

$[Sugar]_t$ concentration of sugar in the reactor at time t (% (w/v))

S_t experimental sucrose concentration at time t, if present (% (w/v))

S_0 initial experimental sucrose concentration (% (w/v))

t_p pervaporation time (hours)

t_s time at which the sucrose first becomes zero in the reactor (hours)

t_1 time at which the exponential phase begins (hours)

t_2 time at which the exponential phase ends (hours)

$V_{P,t}$: cumulative volume removed as permeate at time t (mL)

$V_{R,t}$: actual volume of the reactor at time t (mL)

W total weight flux ($kg/(m^2\ h)$)

X_{EtOH} mass fraction of ethanol

X_{H2O} mass fraction of water

$Y_{i,3}$ mole fraction of i in the permeate vapor

$Y_{j,3}$ mole fraction of j in the permeate vapor

$Y_{i,\bullet}$ mole fraction of i in the saturated vapor

$Y_{j,\bullet}$ mole fraction of j in the saturated vapor

α selectivity

δ membrane pore length

μ biomass specific growth rate (h^{-1})

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1. Introduction

1.1 Project Background and Context

The use of fructose as high fructose corn syrup (HFCS) was commercialized in the early 1970's, and soft drink manufacturers adopted these syrups as their sweetener of choice in the 1980's. This reduced the domination of sucrose as being the main sweetener used in the food industry. The main advantage of using fructose over sucrose is that fructose is 60% sweeter than sucrose, and 150% sweeter than glucose, therefore much smaller amounts are needed to obtain the same degree of sweetness, which in itself leads to a reduction in calorie intake (Blanchard and Geiger, 1984). The other advantages of using high fructose syrups are the price and quality. Syrups of consistent quality can be available at lower prices because the production is now scaled up and mechanised (Blanchard and Geiger, 1984).

To date, three HFCS products are commercially available:
42% fructose/ 52% glucose, 55% fructose/ 42% glucose, and 90% fructose/ 6% glucose. These products are all liquid sweeteners, and in order to see a further expansion in the use of fructose as a sweetener in industry, new methods of production are needed to obtain fructose in a form more suitable for a wider range of applications. If solid fructose were to be used, this would allow for the exportation of corn sweeteners in much larger quantities. The production of solid fructose is achieved by crystallisation, and this process requires that the syrup contains at least 90-95% fructose (Forsberg et al., 1975; Bateman et al., 1984; Ito et al., 1987). To obtain these very high fructose syrups, the 42% and 55% frustose syrups can be used but the glucose content must be reduced. To reduce the glucose content of HFS, glucose and fructose must be separated, but these sugars are isomers of the same molecular size, which makes their separation very difficult. Currently, industries use chromatographic techniques (Coker and Venkatasubramanian, 1987), but these are very expensive, making the production of solid fructose uncompetitive with other sweeteners.

To reduce the problem of having to separate glucose from fructose, new technologies are being developed for the production of high fructose syrups. Novel methods involve the conversion of glucose into a product more easily separated from fructose, such as ethanol. This selective conversion of glucose to ethanol has been achieved by fermentation processes using microorganisms such as *Mucor* sp. m105 and *Fusarium* sp. F5 (Ueng et al., 1982), *Zymomonas mobilis* (Bringer-Meyer et al., 1985; Doelle, 1986; Sunitanalert, et al., 1986; and Kirk and Doelle, 1994), *Tricholoma nudum* (Reusser et al., 1960), and *Pullularia pullulans* (Fan, 1988). The fact that the fermentation produces a valuable by-product (ethanol), which is relatively easily separated from fructose, renders the process more profitable because of the reduction in separation costs, and the increase in generated income. The microorgansims previously tested presented some difficulties such as a substantial consumption of fructose or the production of unwanted by-products. To remedy these problems, extensive studies were performed using mutant strains of *Saccharomyces cerevisiae* (ATCC 36859 and 36858) for the selective conversion of glucose to ethanol using glucose/fructose mixtures, or sucrose feeds (Duvnjak and Koren, 1987; Lamarche, 1988; Koren and Duvnjak, 1989; 1990; 1992; 1993; Koren, 1990; Atiyeh, personal communications; Guénette and Duvnjak, 1995; 1996). By using these mutant strains of *Saccharomyces cerevisiae* no fructose consumption was observed when in the presence of glucose, and high yields of ethanol were obtained. The mutant ATCC 36859, used by Koren, did not possess the ability to hydrolyse sucrose; therefore, only glucose/fructose mixtures may be used. On the other hand, *S. cerevisiae* ATCC 36858, studied by Atiyeh, is capable of hydrolysing sucrose. *S. cerevisiae* ATCC 36858 also has the advantage of being able to tolerate fairly high sugar concentrations (osmotolerant mutant). Its disadvantage, as all yeast, is the inhibition of activity by ethanol. Because of this, longer reaction times are required to complete batch fermentation.

1.2 Objectives and Strategy

Although different microorganisms have been studied extensively for the co-production of fructose and ethanol, *S. cerevisiae* ATCC 36858 has many advantages. Atiyeh extensively studied this yeast in batch fermentations using sucrose feeds (Atiyeh, personal communications). At high initial sucrose concentrations, the fermentation times are very long because of ethanol inhibition as the ethanol accumulates in the broth. 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 continuously remove the ethanol from the broth. By maintaining the ethanol concentration in the broth at a low level, the activity of the yeast would increase, and therefore shorter reaction times would be observed. Pervaporation units coupled to bioreactors for the fermentation of glucose to ethanol have been studied by many to enhance the process; for example Nakao et al., 1987; Gudernatsch et al., 1988; and Kaseno et al., 1998. 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 (Kimura and Nomura, 1982).

In this present study, the goal was to enhance the co-production of fructose and ethanol from sucrose by *S. cerevisiae* ATCC 36858 by removing the ethanol as it is being produced. A pervaporation module coupled to the bioreactor was used for the removal of ethanol. In order to achieve this goal, the study was carried out in six stages. The first stage was to conduct batch fermentation experiments without ethanol removal to have a good basis of comparison. The second stage was to design and build a small test module from available silicone rubber hollow fibres. The third stage was to conduct, with the small test module, pervaporation experiments with ethanol/water solutions at various conditions. These experiments were carried out to obtain performance data of the silicone rubber hollow fibres. The fourth stage was to design and build the module to be coupled with the bioreactor. The fifth stage was to conduct batch fermentation/pervaporation

experiments at different conditions, and the sixth stage was to conduct fed-batch fermentation/pervaporation experiments.

2. Literature Review

In this literature review, the main subjects that will be covered are:

- High Fructose Syrup
- High Fructose Syrup Production
  - Conventional Method
  - Novel Fermentation Method
- Pervaporation
- Membrane Bioreactors

2.1 High Fructose Syrup

Fructose is a monosaccharide (Figure 2.1) of very high commercial importance. It is used as high fructose syrups (HFS), which are liquid sweeteners that contain a mixture of glucose and fructose. Fructose is present in all fruits, and in honey, and it can also come from the hydrolysis of sucrose (Figure 2.1). Commercialization of HFS began in the 70's when soft drink manufacturers started using HFS as their sweetener of choice over sucrose. Since then, the domination of sucrose as the main sweetener in the food industry has been greatly reduced. The advantages of using fructose over sucrose are:

- Fructose is 60% sweeter than sucrose, and 150% sweeter than glucose. This means that less fructose is needed to obtain the same degree of sweetness, which in itself leads to a reduction of calorie intake (Blanchard and Geiger, 1984);
- HFS cost less than sucrose because the production is scaled up and mechanized. This in turn offers consistent product quality (Blanchard and Geiger, 1984).

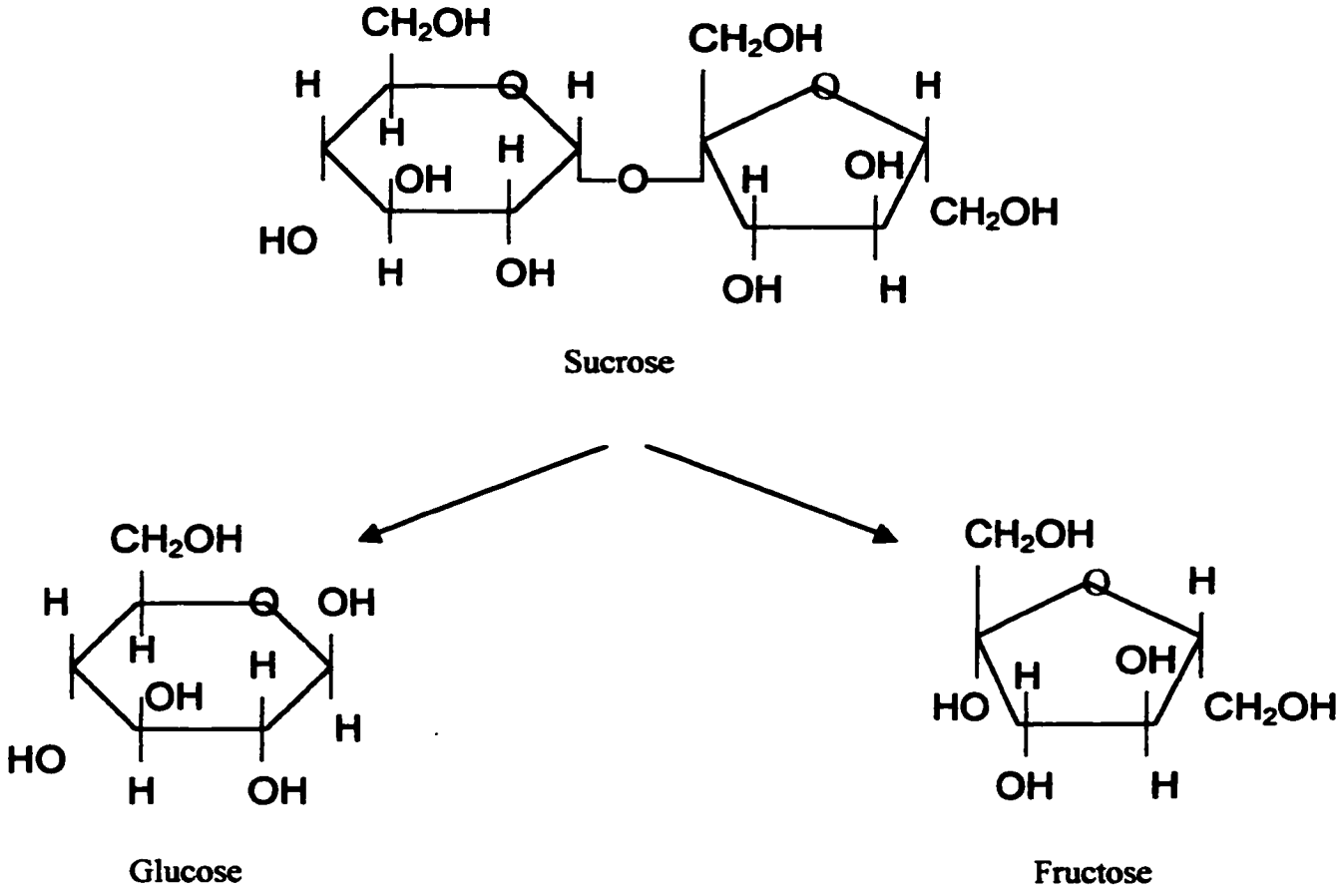


Figure 2.1 Chemical structures of sucrose, glucose, and fructose.

High fructose syrups are commercially available as HFS-42 (42% fructose/53% glucose), HFS-55 (55%fructose/42% glucose), and HFS-90 (90% fructose/8%glucose). Table 2.1 shows the composition of each of those syrups (Hanover and White, 1993).

Table 2.1 Chemical composition of commercially available HFS.

	HFS-42	HFS-55	HFS-90
Solids (%)	71	77	77
Moisture (%)	29	23	23
Carbohydrates (dry solids basis)			
Fructose (%)	42	55	90
Glucose (%)	53	42	8
Oligosaccharides (%)	5	3	1

The raw materials used to produce HFS throughout the world are sucrose from sugar beets and sugar canes. Other sources used for the production of HFS are starch from rice, wheat, potato, and corn. The most popular raw material used is cornstarch because of its abundance and low cost (Vuilleumier, 1993). Starch is a storage polymer of glucose, which can be depolymerised to units of glucose. With the discovery of glucose isomerase (Akabori et al., 1952), an enzyme that converts glucose into fructose through an isomerization process, it became possible to obtain fructose from starch. When HFS are obtained from cornstarch, they are called HFCS.

2.2 High Fructose Syrup Production

2.2.1 Conventional Method of HFCS Production

Since the most popular raw material used for HFS is corn, this section will describe the conventional method used by the industry to produce HFCS, which is shown in Figure 2.2 (Hanover and White, 1993).

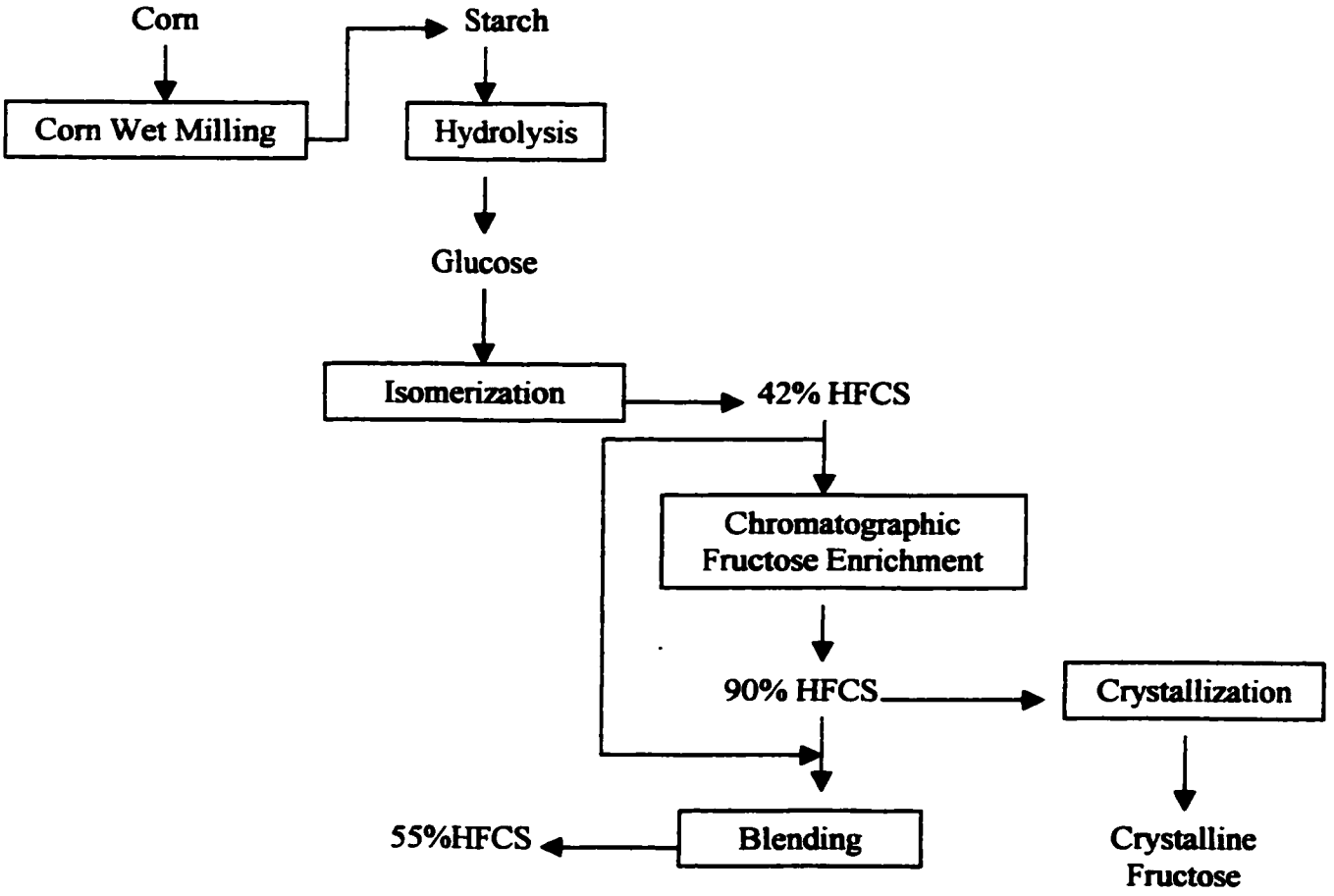


Figure 2.2 Conventional method of HFCS production.

The first step of the process is corn wet milling. The objective of this step is to separate the starch in the corn kernel from other components such as protein, oil, and fibres. Once the starch is obtained, the second step involves the hydrolysis of the starch polymer into its glucose monomers. The hydrolysis is initiated using a dilute solution of mineral acid or by using the enzymes α and β-amylase. Mineral acid and α-amylase make random breaks in the starch producing oligosaccharides of various molecular weights, while β-amylase produces units of maltose, a disaccharide of glucose. The hydrolysis is completed by using glucoamylase enzyme, which hydrolyzes oligosaccharides or dimers further into monomer units of glucose. The third and major step in HFCS production is the isomerization of glucose to fructose. This is accomplished with immobilized enzyme glucose isomerase (Takasaki and Tanabe, 1971) at 60°C. This reaction produces only 42% HFCS because the isomerization is limited by its equilibrium constant. To increase the fructose content of the 42% HFCS, chromatographic techniques are used, which produce 90% HFCS. Since these techniques are very expensive, only a small quantity of the 42% HFCS is treated this way. The rest is blended with 90% HFCS to produce 55% HFCS. This means that only very small amounts remain for crystallization, and therefore only a very small quantity of crystalline fructose is obtained at the end of the process. To eliminate this difficult and expensive chromatographic step, novel methods of separating glucose from fructose have been studied and proposed. These novel methods involve the selective conversion of glucose to ethanol, which is relatively easily separated from fructose. This selective conversion has been studied by using many different microorganisms, and this will be discussed in the next section.

2.2.2 Novel Fermentation Methods for the Production of HFS

Novel fermentation methods for the production of HFS have been studied with the goal of essentially converting glucose into a substance more easily separated from fructose. One way is to convert the glucose into ethanol via fermentation processes. The fact that the fermentation produces a valuable by-product (ethanol), which is more easily separated from fructose than glucose, renders the process more profitable because of the reduction in separation costs, and the increase in generated income. Many researchers have studied the selective conversion of glucose by the use of a variety of microorganisms.

The first to propose this selective conversion of glucose with the goal of producing high fructose syrups were Reusser et al. (1960). In their study, *Tricholoma nudum* was used in sucrose feeds. This microorganism had the ability to hydrolyze sucrose into glucose and fructose, and then it consumed preferentially the glucose to produce cells. The fact that the microorganism was able to hydrolyze sucrose offered the possibility of using sucrose from sugar beets and sugar canes as the raw material for HFS production. The cells produced could easily be separated from the broth by filtration. The major difficulty associated with the use of this microorganism was that when glucose concentrations were low; significant fructose consumption (up to 25%) occurred.

In 1982, a group reported that two mycelia fungal systems (*Fusarium sp.* F5 and *Mucor sp.* M105) were able to preferentially consume glucose over fructose and convert it to ethanol (Ueng et al., 1982). In the case of *Mucor sp.* M105, when subjected to glucose-fructose mixtures, the consumption of glucose was faster than that of fructose, but substantial fructose consumption was observed (around 50%). In the case of *Fusarium sp.* F5, it was shown that this fungus had the ability to hydrolyze sucrose and convert it to fructose and ethanol. In the presence of high glucose concentrations, insignificant fructose consumption was observed. However, as the glucose concentration decreased in the reactor, the fructose consumption increased.

A microorganism that has been studied thoroughly for the simultaneous production of fructose and ethanol from sucrose is *Zymomonas mobilis*, which uses two main enzymes for the purpose of carbohydrate consumption. To consume glucose, it uses glucokinase, and to consume fructose, it uses fructokinase (Doelle, 1982a). Doelle found that the enzyme activity of the fructokinase was inhibited in the presence of glucose (Doelle, 1982b). The hydrolysis of sucrose is very fast compared to the consumption of glucose, and therefore glucose and fructose accumulate in the broth. It was shown that at high sucrose concentrations, there is a point when the microorganism starts consuming fructose to produce sorbitol (Leigh et al., 1984; Viikari, 1984). Because of high fructose consumption, mutants of *Z. mobilis*, which have a very high rate of glucose uptake, were developed (Doelle and Greenfield, 1985a; 1985b; Sunitanalert et al., 1986). With the use of these mutants, they observed less fructose consumption than with the previous strains they had used, and even zero fructose consumption if the pH was controlled, but this reduced the ethanol yield from 90% to 50%. Another mutant, produced by Bringer-Meyer and collaborators, was fructokinase deficient, and could therefore not utilize fructose for ethanol production. When in the presence of glucose and fructose, the mutant consumed glucose at a faster rate than fructose, and the very small amounts of fructose that were consumed produced sorbitol as a by-product (Bringer-Meyer et al., 1985).

Another microorganism capable of selectively converting glucose is *Pullularia pullulans* (Fan, 1988). This microbe, when placed in a glucose-fructose mixture, converts the glucose into a biopolymer that can easily be separated from the fermentation broth by filtration. The disadvantage with this process is that the microbe cannot use sucrose, and up to 50% of the fructose is consumed in the process.

After all these attempts, the attention was turned towards a simple system, that of *Saccharomyces cerevisiae*. A mutant of *S. cerevisiae* was developed, and it was shown that the mutant possessed the ability to selectively convert glucose (Lobo and Maitra, 1977). Lamarche (1988) did preliminary studies on *S. cerevisiae* ATCC 36859 and found that media containing up to 20% (w/v) of both glucose and fructose could be used for the simultaneous production of fructose and ethanol. This yeast however could not be used

with sucrose media, since it did not possess the ability to hydrolyze sucrose. Lamarche also carried out continuous fermentation in an immobilized cell reactor, where the cells were immobilized in Ca-alginate beads. No fructose consumption was observed, but it was demonstrated that the presence of high fructose concentrations in the medium decreased the fermentation rate and no formation of by-products was observed. Ethanol and fructose yields were 100%, and ethanol productivity was 2.2 g/L h in batch fermentation.

Koren and Duvnjak performed extensive studies on *Saccharomyces cerevisiae* ATCC 36859 (Duvnjak and Koren, 1987, Koren, 1990, Koren and Duvnjak, 1989, 1990, 1992, 1993). They performed batch fermentation experiments using glucose, glucose-fructose, and hydrolyzed artichoke juice media. As Lamarche, they demonstrated that this mutant did not consume fructose in the presence of glucose, while producing ethanol with yields of 89%. Also, no sorbitol was produced during the course of the reaction. Koren and Duvnjak also performed experiments using HFCS to reach the goal of reducing the glucose content of the commercially available HFCS (Koren and Duvnjak, 1990). Fed-batch studies were also carried out using HFCS and artichoke juice as substrates in order to produce syrup high in fructose and ethanol concentrations (Koren and Duvnjak, 1992). They obtained a final product containing 238 g/L fructose and 51 g/L ethanol. It was shown that ethanol inhibits the fermentation. The kinetics of this selective fermentation were presented, taking into account both substrate and product inhibition on the yeast (Koren and Duvnjak, 1993).

The most serious disadvantage of *S. cerevisiae* ATCC 36859 was that it did not possess the ability to hydrolyze sucrose. Therefore a new mutant, *S. cerevisiae* ATCC 36858, has been studied by Atiyeh (personal communications), who has demonstrated that this strain has the ability to hydrolyze sucrose into glucose and fructose, and then selectively converts the glucose into ethanol without consuming fructose as long as glucose is still present in the reactor. Figures 2.3 shows the general reactions involved in the fermentation of sucrose by *S. cerevisiae* ATCC 36858 for the simultaneous production of fructose and ethanol.

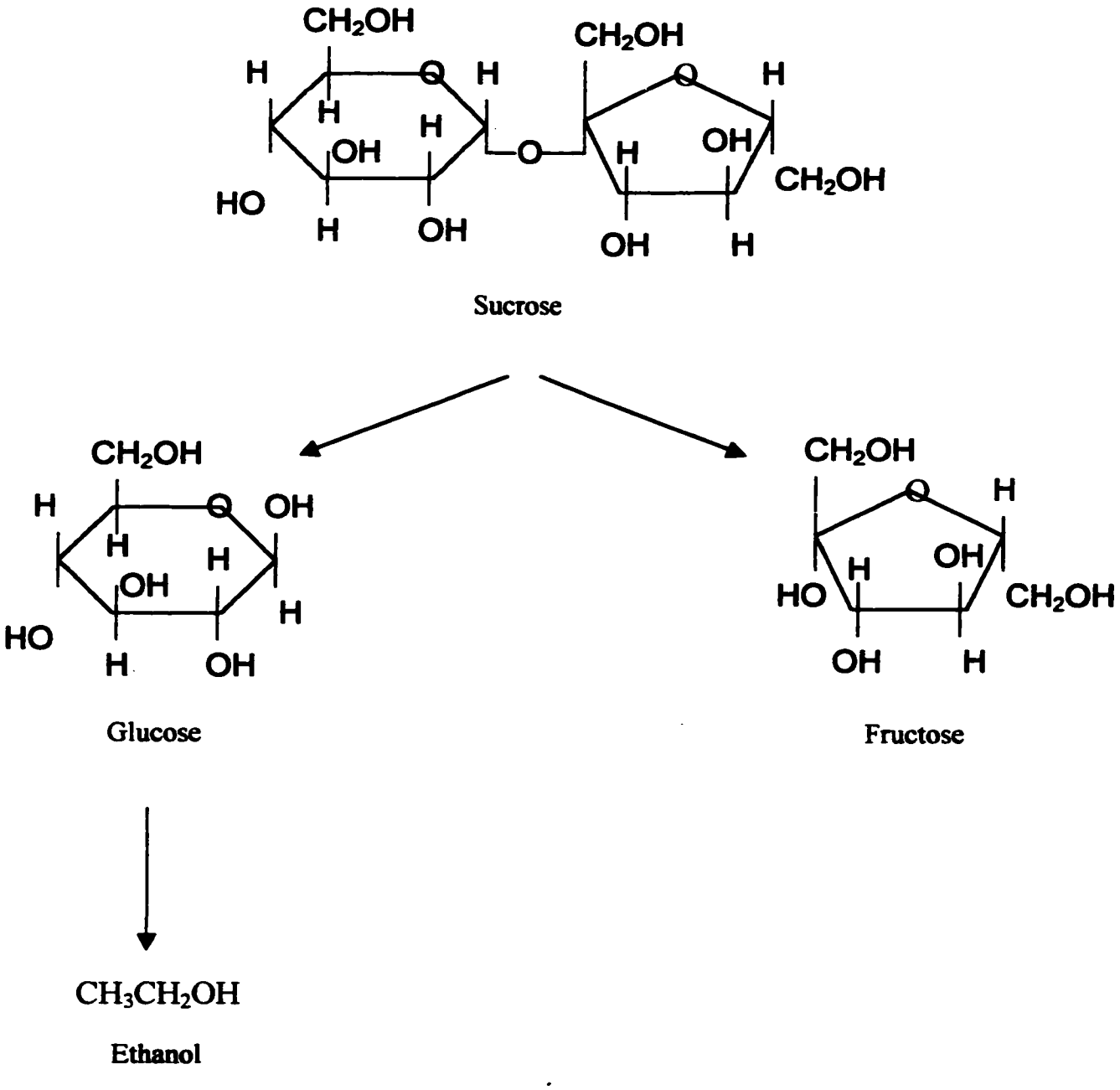
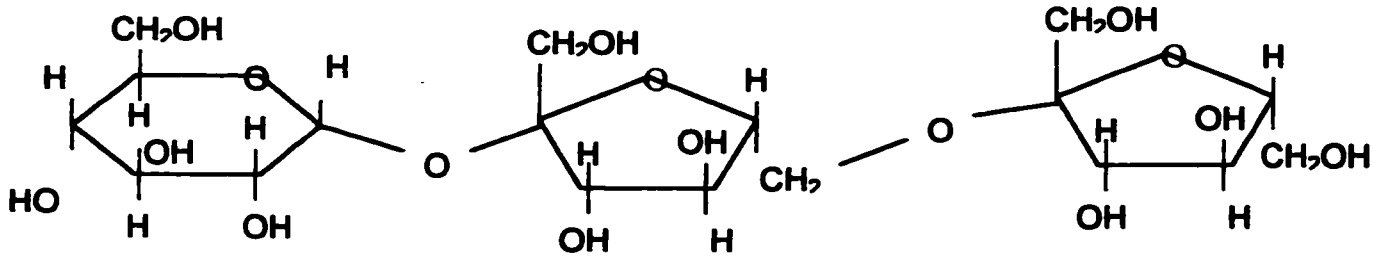


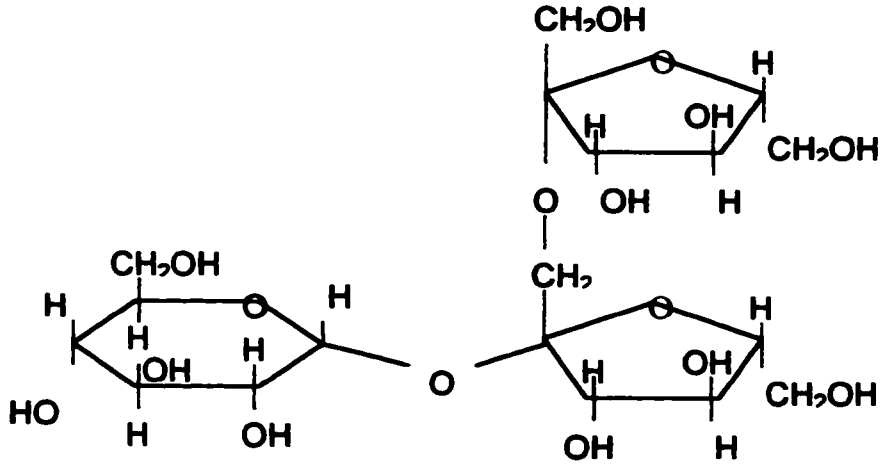
Figure 2.3 General Fermentation Reactions by *S. cerevisiae* ATCC 36858.

Although Atiyeh found no production of sorbitol when initial sucrose concentrations were 30% (w/v), some by-products were formed during the fermentation. Figure 2.4 shows the by-products formed in the fermentation. Some glycerol is produced, but always at levels lower than 1% (w/v) (Atiyeh, personal communications). Another category of by-products is a mixture of kestoses identified by Atiyeh, which consists of 61.5% 6-kestose, 8.8% 1-kestose and 29.7% neokestose. Kestoses are oligosaccharides made of one unit of glucose and two units of fructose. This mixture of kestoses is produced at the beginning of fermentation when sucrose hydrolysis is taking place, and then the kestoses are broken into their monosaccharide units as the glucose content in the reactor decreases. The maximum level of kestoses is approximately 1.2% (w/v).

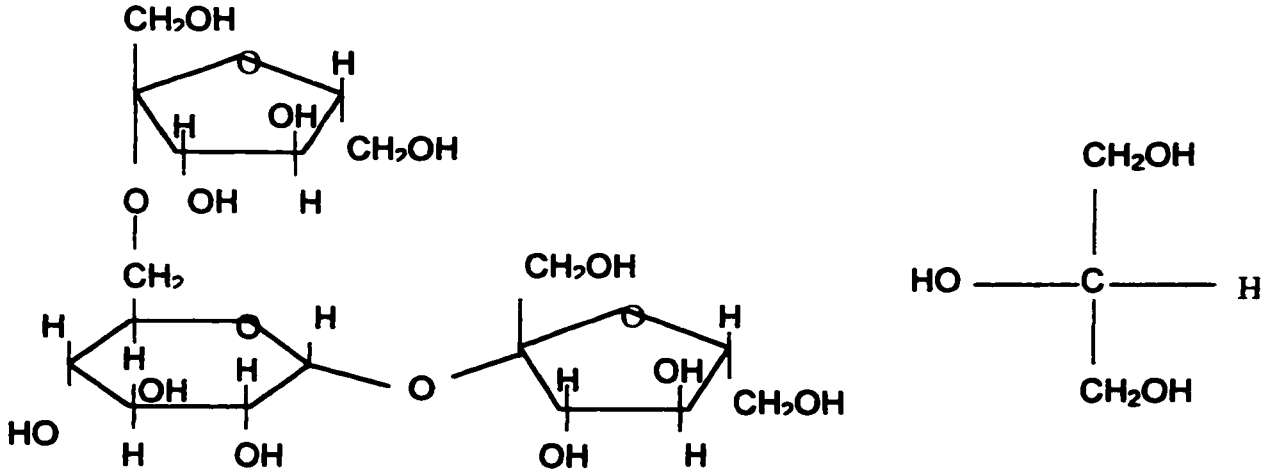
In batch fermentation, using a sucrose synthetic medium of 34.4% (w/v) initial sucrose and an initial biomass concentration of 7.52 g/L, Atiyeh obtained the following results: biomass specific growth rate of 0.062 h^{-1}, ethanol productivity of 2.31 g/(L h), fructose yield of 95.6%, and ethanol yield of 82% (determined based on sugar consumption). He has also shown that the ethanol productivity decreases from 2.79 g/(L h) to 0.56 g/(L h) when the initial ethanol content in the reactor increases from 0% (w/v) to 5% (w/v) (Atiyeh, personal communications). As with any other yeast, ethanol inhibition is evident, and the purpose of the present study was to decrease the ethanol inhibition by continuously removing the ethanol from the broth using a pervaporation unit coupled to the bioreactor.



6-kestose



1-kestose



neokestose

glycerol

Figure 2.4 By-Products in the fermentation of sucrose by *S. cerevisiae* ATCC 36858.

2.3 Pervaporation

Since the goal of this study was to decrease the ethanol inhibition on the yeast by removing the ethanol from the bioreactor with a pervaporation module, the theory behind pervaporation will be discussed. Pervaporation was chosen over other membrane separation systems for its combination of advantages, which will be presented in the section herein.

2.3.1 Basic Aspects

Pervaporation is a membrane separation process for azeotropic mixtures, aromatic and aliphatic hydrocarbons, and close-boiling-point compounds that involve the evaporation of volatile components from liquid mixtures through a selective membrane by the application of vacuum. Pervaporation therefore relies on differences in membrane permeability and on differences in the vapor pressures of the components to be separated (Böddeker and Bengtson, 1991). Membranes can be of two types: water selective (permeates water preferentially) or organic compound selective (permeates the organic compound preferentially). A few transport mechanism models have been applied to pervaporation, the most well known being that of the solution-diffusion model (Mulder and Smolders, 1984), which involves the following steps:

- the sorption of the components from the liquid phase at the membrane surface;
- the diffusion of the sorbed components through the polymer matrix;
- and the evaporation from the polymer into the vapor phase on the permeate side of the membrane.

Another model for the transport mechanism is that of the pore model, which was proposed by Okada and Matsuura (1991a, 1991b). This model assumes that there are a bundle of straight cylindrical pores penetrating across the active surface layer of the membrane perpendicular to the membrane surface. It is also assumed that the entire membrane is in an isothermal state. The exact theory will be discussed in details in section 4 (theoretical aspects), but the general idea is that the pores are filled with liquid

from the pore inlet to a certain distance, and that the rest of the pores are filled with vapor. More specifically, a portion of the pore contains the permeant that is in equilibrium with an imaginary liquid phase, and another portion of the pore contains the permeant that is in equilibrium with a vapor phase. The liquid-filled portion of the pore is defined as the portion of the pore where the permeant flows by liquid transport, and the vapor-filled portion of the pore is the portion of the pore where the permeant flows by vapor-phase transport.

The main advantages offered by pervaporation when compared to other methods of liquid separation like distillation are (Dutta and Sikdar, 1996-97):

- Energy efficient process because only the fraction of the feed that permeates is vaporized;
- Pervaporation does not require energy consuming reflux like distillation;
- The process is simple to operate and control, and there is a low maintenance cost;
- Can be used in small or large-scale operation.

The main disadvantages of pervaporation are (Dutta and Sikdar, 1996-97):

- The separation process is slow, and therefore cannot always replace distillation processes;
- Membranes that have high flux and selectivity are not always available for a specific separation;
- Membrane fouling.

The efficiency of a pervaporation process depends mainly on the properties of the polymer material used for membrane preparation. The next section will therefore discuss polymer selection for the preparation of pervaporation membranes.

2.3.2 Polymer Selection for Pervaporation Membrane Preparation

Depending on the material chosen for the membrane, pervaporation can either be water selective (removes water preferentially), or it can be selective at removing a volatile organic compound. It has been experimentally shown that membranes made of glassy (amorphous) polymers permeate water preferentially, and that membranes made of elastomeric (rubbery) polymers permeate the organic compound preferentially (Bell et al., 1988). In glassy polymer membranes, the permeability is determined by the diffusivities of the permeate, meaning that smaller molecules permeate preferentially. This is due to the fact that in glassy polymers, there is very little movement of polymer segments. This makes the diffusion path longer than the membrane thickness, and the selectivity is determined by the size of the permeating molecules, which have to go through the existing spaces between the rigid polymer segments (Bell et al., 1988). In rubbery polymer membranes, the permeability is determined by the solubility behavior of the permeating molecules. In rubbery polymers, macromolecules are in a coiled state, and the polymer segments between the cross-links are continuously in vibrational or rotational movement. The permeability is determined by the ability of the permeating molecules to decrease intermolecular attractions between the polymer segments and to increase the number and the dimensions of the moving polymer segments (Bell et al., 1988). Therefore, to selectively remove organic compounds from liquid mixtures, elastomeric (rubbery) polymers must be used instead of glassy polymers. The next section will emphasize on the pervaporation of ethanol/water mixtures through various types of membrane materials.

2.3.3 Pervaporation of Ethanol/Water Mixtures

The separation of ethanol from ethanol/water mixtures by pervaporation finds its importance in the potential application of recovering ethanol from fermentation broths. This particular application will be discussed in detail in the section of membrane bioreactors. Furthermore, the ethanol/water system is generally used as the system of

reference for pervaporation performance (Böddeker and Bengtson, 1991). This section will therefore give a brief review of ethanol selective membranes studied over the last few years. Most of the membranes used in ethanol/water pervaporation are geared towards dehydration of the mixture, i.e. the removal of water from the ethanol/water mixture. In this case, the membranes are water selective (Yamagishita et al., 1994; and Rückenstein and Chen, 1991). When ethanol concentration is low in a mixture, especially when it is in a fermentation broth, ethanol selective membranes are needed to recover and concentrate the ethanol.

Not many membranes are ethanol selective, but the most studied material has been silicone rubber (crosslinked polydimethylsiloxane (PDMS)) in the form of flat sheets and hollow fibres. Table 2.2 shows the results of ethanol/water pervaporation studies using different ethanol selective membrane materials. In 1982, Kimura and Nomura reported membrane performance of silicone rubber for the pervaporation of ethanol/water mixtures. They have shown that the flux increases almost linearly with the increase of ethanol concentration in the feed. In 1983, Mulder et al. studied many different membrane materials and amongst those tested, they found that only PDMS permeated ethanol preferentially. When using PDMS membranes, a general trend observed is that the total flux increases with decreasing membrane thickness (Böddeker and Bengtson, 1991). Other general trends are: the total flux decreases when the downstream pressure is increased (less vacuum is applied), the total flux increases and the selectivity remains constant as the ethanol concentration in the feed increases. Table 2.2 shows the results of other studies done using PDMS membranes. As a general observation, both the flux and the selectivity tend to be low when using PDMS membranes.

In order to improve the organic selectivity of PDMS membranes, researchers have modified the material by adding various fillers. One group added SiO_2 to the membrane material, which stabilizes the mechanical properties of the membrane (Okamoto et al., 1987). With this modified filled PDMS, they observed an improvement in the selectivity (1%) and in the flux (1%). To further improve the performance of PDMS membrane,

Hennepe et al. (1987) incorporated hydrophobic silicalite. When they increased the silicalite content of the membrane, the ethanol flux increased, whereas the water flux remained constant, which resulted in an overall increase in selectivity. Unlike pure PDMS, filled PDMS membranes show an increase in selectivity with an increase in ethanol feed concentration.

Table 2.2 Results of Ethanol-Water pervaporation studies performed with various ethanol selective membrane materials.

Membrane Material	Thickness (μm)	Temp. (°C)	Permeate Pressure (mbar)	Ethanol Feed Concentration (%, wt)	Ethanol Permeate Concentration (%, wt)	Selectivity	Flux ($g/(m^2 h)$)	Reference
PDMS	180	25	0.7	4.2	31.5	10.5	28	Kimura et al.
	180	25	0.7	20	71	9.6	48	1982
PDMS	30	30	0.1	5	19	4.5	146	Mulder et al.
								1983
Aromatic PA	-	43	80	4.5	11.6	2.8	5500	Schissel et al.
composite		43	80	18.7	51.9	4.7	3300	1984
PDMS	500	25	1	10	50	8.9	11	Changluo et al.
								1987
PDMS	120	30	8	5	37	11	50	Nakao et al.
		30	8	10	49	9	50	1987
PDMS	100	22.5	1	5	29	7.6	24	Hennepe et al.
								1987
PDMS	140	22.5	1	10	51	9.3	27	Okamoto et al.
Filled with SiO_2								1987

Table 2.2 (cont.)								
PDMS	100	22.5	1	5	45	14.9	36	Hennepe et al.
40 wt% silicalite								1987
PDMS	100	22.5	1	5	47	16.5	51	Hennepe et al.
60 wt% silicalite		22.5	1	3.6	37	16	45	1987
		22.5	1	7.5	67	25	72	
PTMSP	30-50	30	0.7	10	55	11.2	300	Ishira et al.
		30	0.7	20	65	7.3	350	1986
PDMS-PS IPN	15-20	60	-	10	-	5.5	160	Liang et al.
								1996
PTMSP/PDMS	-	30	5	5	-	17-6 in 7	600-200 in	Kang et al.
Semi IPN						days	7 days	1994
PTMSP/PDMS	-	30	5	5	-	12	250	Kang et al.
Sorbed								1994
PTMSP/PDMS	-	30	5	5	-	6	100	Kang et al.
Sorbed and								1994
crosslinked								
PTMSP	-	30	5	5	-	18-8 in 7	850-250 in	Kang et al.
						days	7 days	1994

PTMSP (poly(1-trimethyl silyl-1-propyne) is another ethanol selective membrane material which was studied (Ishira et al., 1986). This material showed higher flux than PDMS while maintaining the same selectivity as PDMS. The high flux is thought to be due to the large free volume caused by the bulkiness and flexibility of the trimethyl silyl group. The disadvantage of PTMSP is the deterioration of transport properties with time, which is due to the relaxation or physical aging of PTMSP chains (Kang et al., 1994). Kang et al. studied the combination of PTMSP and PDMS as a membrane material in order to retard the physical aging process of PTMSP, since PDMS does not show a decrease in transport properties with time. They studied three modifications: PTMSP combined with PDMS (Semi IPN (interpenetrating polymer network) membrane) (I Series), PTMSP with PDMS sorbed membrane (S Series), and PTMSP with PDMS sorbed and crosslinked membrane (X Series). With the I series, PDMS is introduced in the PTMSP chain by crosslinking PDMS by a semi-IPN technique. As with pure PTMSP, the flux and selectivity both decreased with time. The flux and selectivity decreased with an increase in an amount of PDMS, because PDMS's flux and selectivity are lower than those of PTMSP. With the S series, the decrease of flux with time was no longer observed. It was thought to be due to the fact that PDMS filled into the free volume that is present in the PTMSP phase. This PDMS filler restricts the aging process normally seen with PTMSP. In the X series, again, transport properties do not decrease with time. The flux and selectivity of modified PTMSP are lower than PTMSP, but still higher than PDMS, therefore the right combination of both materials will result in properties that are superior to those of individual materials.

Over the last decade, many more membrane materials have been developed and studied for the pervaporation of ethanol-water mixtures (Chen et al.; 1998; Miyata et al., 1997; Nomura et al., 1998; Ulutan and Nakagawa, 1998; and Wang et al., 1999). In the study presented herein, the chosen membrane material was silicone rubber (crosslinked PDMS) even though reports showed this material to have low flux and selectivity. The reason for this choice was because PDMS is the most well known ethanol selective membrane and because hollow fibers can be used to remedy the disadvantage of low flux since they allow for a large membrane surface area/volume ratio. Also, the performance

properties of PDMS such as flux and selectivity were shown to be stable with time i.e. they do not decrease with time, unlike PTMSP, which is an important aspect to consider when subjected to biomass. In the following section, a literature review on the various combinations of membrane bioreactors studied over the past few years will be presented.

2.4 Membrane Bioreactors

2.4.1 Introduction

The idea of applying membrane separation processes in biotechnology is growing rapidly, especially in the case of alcohol fermentation, which represents one of the most important resources of renewable energy. Over the past decades, studies were performed on membrane bioreactors for the recycling of biomass and the removal of ethanol produced by fermentation processes. The reason why ethanol removal by membrane has received so much attention in the past years is due to the fact that membrane separation processes consume less energy than traditional separation processes such as distillation, and they can be used in a continuous mode. Also, by removing ethanol from the broth as it is being produced, researchers have shown that the fermentation can be improved. Some of the methods, other than membrane bioreactors, which have been tested for the continuous removal of ethanol from fermentation broths, are vacuum fermentation (Aiba et al., 1968; Cysewski and Wilke, 1978), extractive fermentation (Wang et al., 1981), on-line adsorption with hydrophobic adsorbent (Campbell et al., 1983; Luong, 1982), and perstraction (membrane-aided solvent extraction) (Matsumura and Maruki, 1986). Traditionally, ethanol fermentation has been performed in batch mode. Batch fermentations are not efficient, mainly because of the down time between batches and because of the low productivity during the start-up period of the batch (Cheryan and Mehaia, 1986). Efforts have been made to achieve continuous fermentation instead of batch fermentation. The main disadvantage associated with continuous fermentation is that cell washout is observed if the dilution rate is higher than the growth rate of the microorganism. Membrane bioreactor designs have been studied to eliminate the problem

of cell washout in continuous fermentation, and to remove at the same time inhibitory products from the broth. The combination of fermentation and membrane separation can be done in two ways:

- immobilize the microorganism within a hollow fibre membrane (hollow fibre membrane bioreactor);
- couple a membrane unit to the fermentor in a semi-closed or closed loop (membrane recycle bioreactor).

This review will mainly but not exclusively use the classic fermentation of glucose to ethanol by *S. cerevisiae* as examples in the literature since this is the fermentation that is more closely related to this project.

2.4.2 Hollow Fibre Membrane Bioreactor

In this design of membrane bioreactor, the microorganisms are immobilized (trapped) on the shell side of a hollow fibre membrane module and the feed is pumped through the lumen side of the module, as shown in Figure 2.5. This is a design specific to continuous fermentation and the reactor essentially operates as a plug flow reactor. The membrane is permeable to the substrate and the products, but is impermeable to the microorganism. The substrate diffuses through the membrane, and reaches the microorganisms on the shell side where the substrate is converted to product. Then the product diffuses back to the lumen side and is recovered.

When compared to membrane recycle bioreactors, which will be described next, hollow fibre reactors have not been very successful. The problem is either lower productivities or low substrate utilization. Ethanol fermentation from glucose by *S. cerevisiae* has been done using this type of design (Inloes et al., 1983; Mehaia and Cheryan, 1984). Results obtained by these groups are shown in Table 2.3. The reason for the poor performance of these hollow fibre membrane bioreactors is that the biocatalyst is separated from the substrate and product stream by the membrane, which acts as a

physical barrier. The rate-limiting step of the process then becomes the diffusion of the substrate into the shell side to reach the biomass (Cheryan and Mehaia, 1985).

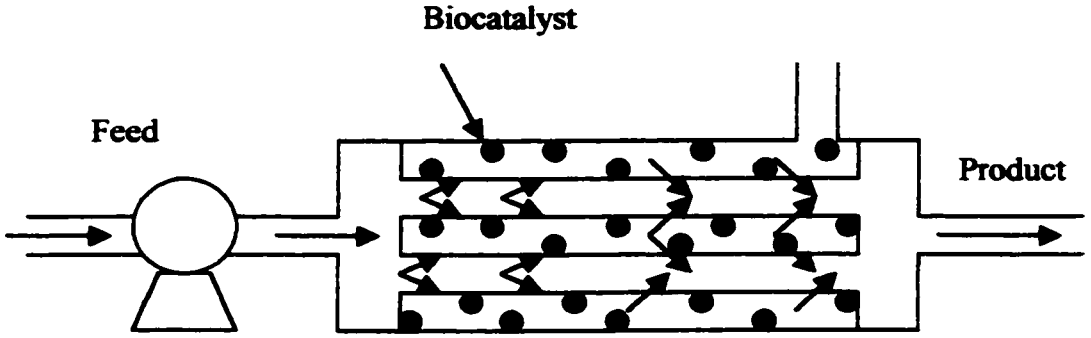


Figure 2.5 Schematic diagram of a hollow fibre membrane bioreactor.

2.4.3 Membrane Recycle Bioreactor

Figure 2.6 shows a typical set-up of a fermentor coupled with a membrane module. This system can either be operated in a continuous or a batch mode (without the feed stream), depending on the type of membrane used in the module. The difference between the hollow fibre and the membrane recycle bioreactor is that the latter has its membrane outside of the reactor unit. The first studies on membrane recycle bioreactors used mainly filtration membranes of various materials in the module (Hoffman et al., 1985; Nishizawa et al., 1983; Cheryan and Mehaia, 1984; Janssens et al., 1984; Rogers et al., 1982; Ohlsen et al., 1984). Table 2.3 also shows the results obtained by some of these groups. The improvement of using a membrane bioreactor is the increase in ethanol productivity in all cases when compared to batch fermentation. When studying the membrane recycle bioreactor, both flat-sheet membranes and hollow fibres have been used.

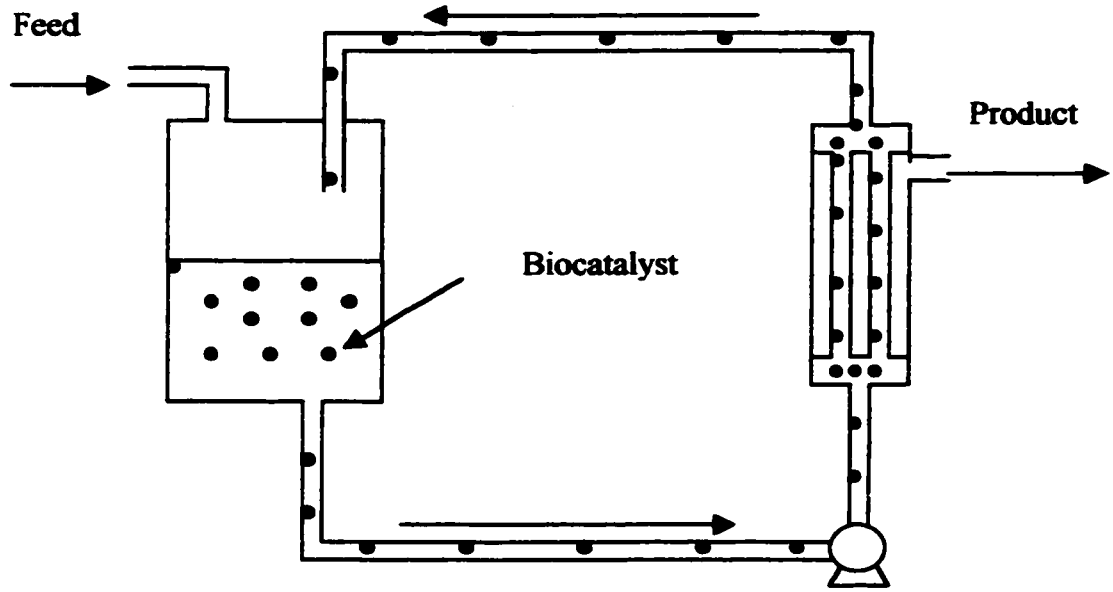


Figure 2.6 Schematic diagram of a membrane recycle bioreactor.

When using ultrafiltration or microfiltration membranes, only the membrane retains the microorganism and everything else permeates, therefore only continuous fermentation can be used. Substrate waste and big volume reduction would be observed in a batch mode. Because of the continuous recycling of the biomass, the advantage of this system is the elimination of cell washout usually observed in continuous fermentation.

Table 2.3 Membrane Bioreactor Performance

System	Initial Sugar (g/L)	Micro-organism Strain	Final EtOH (g/L)	Sugar Conversion (%)	Ethanol Productivity (g/ (L h))	Reference
Batch	100	*S. cerevisiae*	49	99	2.5	Mehaia et al. 1984
Hollow Fibre	89	*S. cerevisiae*	12	27	26	Inloes et al. 1983
Bioreactor	100	*S. cerevisiae*	40	85	10	Mehaia et al. 1984
Membrane	100	*S. cerevisiae*	49	99	100	Cheryan and
Recycle	200	*S. cerevisiae*	65	65	130	Mehaia 1984
Bioreactor[a]	150	*S. cerevisiae*	65	99	20	Nishizawa et al. 1983

[a] *with ultrafiltration membrane*

Over the last few years, research has been geared towards the use of pervaporation membranes in the coupled module instead of ultrafiltration or microfiltration membranes because of the potential advantages offered by pervaporation (Kaseno et al., 1998, Cho et Hwang, 1991). Pervaporation offers a unique separation that fulfills a specific separation requirement posed by fermentation. Figure 2.7 describes the separation offered by pervaporation membranes in the case of fermentation conditions.

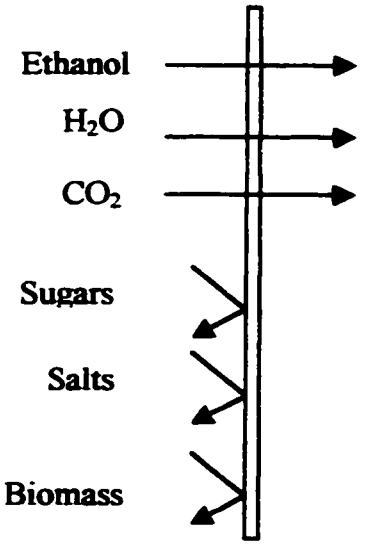


Figure 2.7 Separation offered by pervaporation membrane during fermentation.

Ideally, it would be best if only ethanol and CO_2 would permeate through the membrane in order to recycle biomass and unconverted sugars to the bioreactor. In such a case, the system could be operated in batch mode as well as continuous. In batch mode, the small volume of ethanol removed compared to the total volume of the broth would only cause a very small decrease of the total volume of the broth. It would be of a great benefit if, while simultaneously removing the product, this very product could be concentrated in the same separation step. Of all the membrane separation processes, pervaporation is the only one that satisfies all of these requirements; if of course the membrane used is ethanol selective, in which case, the ethanol would be removed and concentrated at the same time. No membrane is completely ethanol selective, meaning that there will always be water permeating through. The goal is to use membranes that permeate smaller amounts of water in order to avoid concentrating the broth in terms of sugars. Other advantages offered by pervaporation is that the process avoids the high mechanical, thermal or chemical stresses exerted on the biomass by other processes like reverse osmosis, distillation or solvent extraction (Mulder and Smolders, 1986).

The proposal to apply pervaporation in a membrane bioreactor was made by Mulder et al., in 1981. The first experimental data were reported by Groot et al. (1984a, 1984b). This group studied the separation of n-butanol produced in glucose/xylose fermentation. They used a pervaporation unit made of silicone rubber, which has been shown to be selective to alcohols. They observed a decrease in product inhibition, and an increase in product yield when continuous product recovery by pervaporation was achieved.

A study was made on the removal of ethanol during the fermentation of glucose to ethanol by *S. cerevisiae* using different membrane materials (Nakao et al., 1987). They compared silicone rubber (SR), polypropylene (PP) and polytetrafluoroethylene (PTFE). Among these, they found that PTFE offered the highest flux, with a good selectivity. The membrane bioreactor was more efficient in many ways than fermentation alone. Both ethanol productivity and ethanol yield increased (Nakao et al., 1987).

A study on continuous fermentation/pervaporation was performed using a hollow fibre module made of a polysulfone porous substrate that was coated with PDMS (Gudernatsch et al., 1988). They studied the fermentation of glucose to ethanol by *S. cerevisiae*. They reported constant flux and enrichment factor throughout the whole fermentation run, which lasted about 30 days. They did not report fibre blocking by the biomass. They observed that the flux decreased with increasing the downstream pressure. Their composite membrane offered a very high flux compared to pure PDMS membranes, but the enrichment factor decreased (Gudernatsch et al., 1988).

Since then, other studies have been done on the combination of fermentation and pervaporation. These studies were mostly for the production of butanol or ethanol using various microorganisms, and most of them were in continuous operation (Mori and Inaba, 1990; Sodeck et al., 1988; Larrayoz and Puigjaner, 1987; Cho and Hwang, 1991).

An attempt was made to couple pervaporation to fed-batch fermentation of glucose to ethanol *by S. cerevisiae* (Kaseno et al., 1998). They used a microporous polypropylene membrane, which is alcohol selective. They also demonstrated an increase in performance of the fermentation process. When they adjusted the ethanol removal rate to 84.4% of the produced ethanol, the concentration of ethanol in the broth remained almost constant and the apparent rate of ethanol production was 2 times higher than in fermentation without pervaporation. They carried fed-batch experiments for 72 hours without any fouling of the membrane or blockage of the hollow fibers. They obtained ethanol concentrations in the permeate of approximately 21-32% (w/v).

3. Data Analysis and Modelling

3.1 Fermentation Data Analysis

It is not always evident which methods are used for the calculations of yields, growth rates, and other experimental parameters, since all of these calculations can be based on different elements or points in time in the process. This section describes how all calculations were made in this work and what they are based on. All the fermentations performed (with or without membrane) will be compared to one another using the following parameters: fermentation time, ethanol yield, fructose yield, ethanol productivity, biomass specific growth rate, biomass yield, sucrose hydrolysis rate, final glucose and fructose concentration in the broth, final ethanol concentration in the broth, and in the permeate in the case of fermentation/pervaporation experiments. Figure 3.1 shows the theoretical yields for the general fermentation reactions. At any point in time during fermentation, all the parameters can be calculated, but the point of interest and comparison in this work will usually be the end of the fermentation.

$$\text{Sucrose} + \text{Water} \rightarrow \text{Glucose} + \text{Fructose} \rightarrow \text{Ethanol} + \text{Fructose} + CO_2$$

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6 \rightarrow 2C_2H_6O + C_6H_{12}O_6 + 2CO_2$$

$$342\text{g/mol} + 18\text{g/mol} \rightarrow 180\text{g/mol} + 180\text{g/mol} \rightarrow 2(46\text{g/mol}) + 180\text{g/mol} + 2(44\text{g/mol})$$

$$1\text{g} + 0.0526\text{g} \rightarrow 0.526\text{g} + 0.526\text{g} \rightarrow 0.269\text{g} + 0.526\text{g} + 0.257\text{g}$$

Figure 3.1 Theoretical yields of glucose, fructose, and ethanol from the fermentation of sucrose by *S. cerevisiae* ATCC 36858.

Figure 3.1 indicates that 0.526g of glucose, and 0.526g of fructose should be obtained theoretically from 1g of sucrose, and that from 1g of glucose, 0.51g of ethanol should be obtained.

The fructose yield was calculated based only on sucrose hydrolysis:

$$Fructose\ \ Yield\ \ (\%) = \frac{F_t}{(S_0 - S_t) \bullet 0.526} \bullet 100 \qquad (1)$$

where F_t : fructose concentration at time t (% (w/v))

S_0 : initial sucrose concentration (% (w/v))

S_t : sucrose concentration at time t, (% (w/v))

$(S_0-S_t)\bullet 0.526$: theoretical fructose from sucrose hydrolysis (% (w/v)).

The kestoses formed in the process were not taken into account because of the fact that in batch fermentation, kestoses levels are zero at the end of the process, and in fermentation/pervaporation, kestoses levels are below 0.8%w/v, as will be demonstrated in the results and discussion section. Therefore, the kestoses formed were considered negligible for the purpose of fructose yield calculations.

The ethanol yield calculation was based on sugar consumption:

$$Ethanol\ Yield\ (\%) = \frac{E_t}{[((S_0 - S_t) \bullet 0.526) - G_t] \bullet 0.51 + [((S_0 - S_t) \bullet 0.526) - F_t] \bullet 0.51} \bullet 100 \qquad (2)$$

where E_t : ethanol concentration at time t (% (w/v))

G_t, F_t : glucose and fructose concentration at time t (% (w/v)).

This calculation does not take into account the amount of sugar that is converted to glycerol because the levels of glycerol rarely go beyond 1% (w/v). Therefore glycerol

formed was considered negligible for the purpose of ethanol yield calculations. This calculation also does not take into account that a part of the glucose is used by the biomass for maintenance and reproduction.

The ethanol productivity was calculated as follows:

$$P \ (g/(Lh)) = \frac{E_t}{t} \tag{3}$$

where E_t : ethanol concentration at time t (g/L)

t : time at any point of comparison during the fermentation (hours).

Using equation (3), the ethanol productivity can be calculated at any time during the fermentation, but for comparison in this study, the ethanol productivity was an average calculated over the entire fermentation time.

The biomass specific growth rate was calculated during the exponential phase of the growth curve:

$$\mu \ (h^{-1}) = \ln\left(\frac{B_2}{B_1}\right) \bullet \left(\frac{1}{t_2 - t_1}\right) \tag{4}$$

where B_2 : biomass concentration at the end of the exponential phase (g/L)

B_1 : biomass concentration at the beginning of the exponential phase (g/L)

t_2 : time at which the exponential phase ends (hours)

t_1 : time at which the exponential phase begins (hours).

The biomass yield was calculated based on total carbohydrate consumption:

$$Biomass\ \ Yield\ \ (g/g) = \frac{B_t - B_0}{((S_0 - S_f) \bullet 0.526 - G_f) + ((S_0 - S_f) \bullet 0.526 - F_f)} \qquad (5)$$

where B_t : biomass concentration at the end of the fermentation (g/L)

B_0 : biomass concentration at the beginning of the fermentation (g/L)

S_f, G_f, and F_f : final sugar concentrations at the end of fermentation (g/L).

The sucrose hydrolysis rate was calculated according to the following equation:

$$Sucrose\ \ Hydrolysis\ \ Rate\ \ (g/Lh) = \frac{S_0}{t_s} \qquad (6)$$

where S_0 : Initial sucrose concentration (g/L)

t_s : Time at which the sucrose becomes zero in the reactor (hours).

3.2 Pervaporation Data Analysis

In order to evaluate the performance of pervaporation membranes, two parameters are commonly used. They are the total flux and selectivity.

The total flux was calculated according to the following equation:

$$Total\ \ Flux\ \ (g/m^2h) = \frac{m_p}{t_p \bullet A} \qquad (7)$$

where m_p : mass of permeate (g)

t_p : pervaporation time (hours)

A : effective membrane area (m^2).

The separation factor was calculated according to the following equation:

$$\alpha = \left[X_{EtOH} / X_{H_2O} \right]_{permeate} \Big/ \left[X_{EtOH} / X_{H_2O} \right]_{feed} \qquad (8)$$

where X_{EtOH} : mass fraction of ethanol

X_{H2O} : mass fraction of water

If no separation is accomplished, the separation factor will be unity.

3.3 Pervaporation Modeling

As mentioned previously, most studies on pervaporation have been based on the solution-diffusion model, but the chosen model for this work is the pore model proposed by Okada and Matsuura (1991a). This model presents pervaporation in a pore as a combination of liquid transport and vapor transport in series. According to the mechanism feed liquid enters a pore from the side which is faced to the feed, evaporates in the pore into a vapor phase that comes out of the pore into vacuum at the other side of the membrane. The big difference between the solution-diffusion and the pore model is that in the solution-diffusion, no phase change occurs in the membrane, whereas the pore model is based on the phase change observed in pervaporation.

The development of the transport equations starts from the assumption that there are a bundle of straight cylindrical pores of length δ penetrating across the active surface layer of the membrane. It is also assumed that all the pores are in an isothermal condition. As shown in figure 3.2, the pore is partially filled with liquid from the pore inlet to a distance δ_a along the cylindrical axis. The rest of the pore whose length is δ_b is filled with vapor.

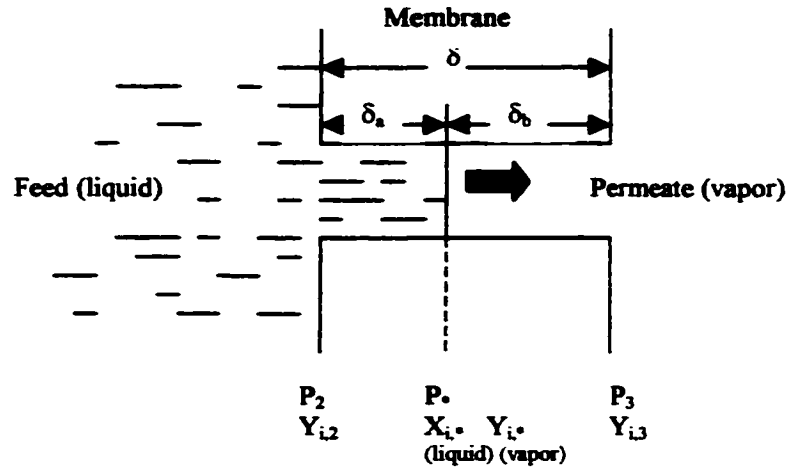


Figure 3.2 Schematic diagram of pervaporation occurring in a membrane pore.

Evaporation takes place at the liquid-vapor boundary. The pressure of feed liquid and permeate vapor are given as P_2 and P_3, respectively, and at the phase boundary the pressure must be the saturation vapor pressure that is denoted by $P_\bullet$. The above diagram can be valid only when $P_3 < P_\bullet$. When $P_3 \geq P_\bullet$, the entire pore is filled with liquid. It is assumed that the Poiseuille flow prevails in the liquid transport while the surface flow prevails in the vapor transport. Surface flow means that a layer of adsorbed vapor formed at the surface of the pore which flows under a pressure gradient. The vapor transport is governed by the surface flow rather than the Knudsen flow that takes place in the center of the pore; the space unoccupied by the adsorbed layer.

For a two component system, consisting of species i and j, the following equations to calculate the mole fraction of i in the permeate and the total flux have been derived by Okada and Matsuura (1991a). These equations assume that no separation takes place in the liquid phase in the pore and species i and j permeate through the membrane independently.

The mole fraction of species i in the permeate can be calculated by the following equations:

$$Y_{i,3} = \frac{\left(P_{i,\bullet}^2 - P_{i,3}^2\right)}{\left(P_{i,\bullet}^2 - P_{i,3}^2\right) + \left(B_j / B_i\right)\left(P_{j,\bullet}^2 - P_{j,3}^2\right)} \tag{9}$$

and

$$P_{i,\bullet} = P_{\bullet} Y_{i,\bullet} \tag{10}$$

$$P_{j,\bullet} = P_{\bullet} Y_{j,\bullet} \tag{11}$$

$$P_{i,3} = P_3 Y_{i,3} \tag{12}$$

$$P_{j,3} = P_3 Y_{j,3} \tag{13}$$

and where $Y_{i,3}$: mole fraction of i in the permeate vapor

$Y_{j,3}$: mole fraction of j in the permeate vapor

P_3 : downstream pressure (Pa)

$P_\bullet$: saturation vapor pressure of the feed mixture (Pa)

$Y_{i,\bullet}$: mole fraction of i in the saturated vapor

$Y_{j,\bullet}$: mole fraction of j in the saturated vapor

B_i : constant related to the vapor transport of i (mol/(m s Pa^2))

B_j : constant related to the vapor transport of j (mol/(m s Pa^2)).

The total weight flux can be given as:

$$W = \left[\frac{B_i}{\delta}\left(P_{i,\bullet}^2 - P_{i,3}^2\right) + \frac{B_j}{\delta}\left(P_{j,\bullet}^2 - P_{j,3}^2\right)\right]\left(M_i Y_{i,3} + M_j Y_{j,3}\right) \tag{14}$$

where M_i : molecular weight of i

M_j : molecular weight of j

W : total weight flux (kg/m^2 h)

δ : membrane thickness.

In this work, pervaporation tests have been performed using ethanol/water mixtures to evaluate the performance of the silicone rubber membrane. The results will be analyzed according to the pore model.

3.4 Fermentation/Pervaporation Data Analysis

When performing fermentation/pervaporation experiments, data analysis must be done carefully because the volume of liquid in the reactor decreases with time as the ethanol and water are removed by the membrane module, and samples for analysis are removed. In order to determine the performance of the membrane bioreactor, the results will be presented in two ways. The first way will be referred to as "actual reactor data" and "permeation data". These are actual values of concentration inside the reactor and the permeate respectively at each point in time. The second way the data will be referred to is as "normalized data". This data was calculated as though at each point in time the permeate would have been put back into the reactor. In this way, the total amounts of ethanol and fructose that have been produced can be determined. The broth in the reactor becomes, in reality, more concentrated in terms of sugar and mineral concentrations with time due to the removal of water and alcohol. The normalized data corresponds to a situation where no concentration effect would have taken place. Unfortunately, the volume removed for sample analysis cannot be considered in the calculation of normalized data because it cannot be assumed that the sample receives the same treatment as the broth after each sample is removed (no pervaporation in the sample). And the fact that samples are removed from the broth changes the extent of the concentration effect brought about by pervaporation, therefore, samples are not considered in the normalized data calculation.

The normalized concentrations of sugars can be calculated by equation 15 for any point in time during the fermentation:

$$[Sugars] = \frac{[Sugar]_t \bullet V_{R,t}}{V_{R,t} + V_{P,t}} \qquad (15)$$

where $[Sugar]_t$: concentration of sugar in the reactor at time t (% (w/v))

$V_{R,t}$: actual volume of the broth in the reactor at time t, which is equal to the initial volume minus the cumulative volume removed as samples minus the cumulative volume removed as permeate at time t (mL)

$V_{P,t}$: cumulative volume removed as permeate at time t (mL).

The normalized concentrations of ethanol were calculated by equation 16 for any point in time during the fermentation:

$$E_t = \frac{(([EtOH]_t \bullet V_{R,t})/100) + M_{P,t}}{V_{R,t} + V_{P,t}} \times 100 \qquad (16)$$

where E_t : concentration of ethanol in the reactor at time t (% (w/v))

$M_{P,t}$: cumulative mass of ethanol removed as permeate at time t (g).

Experiments were performed in duplicate or triplicate for each specified condition, and the statistical analysis of these was done using the student-t test. Therefore, all error bars on figures represent the standard deviation for a set of experiments.

4. Experimental Method

4.1 Yeast Cultures Maintenance

Saccharomyces cerevisiae ATCC 36858 was maintained on agar slants (medium I). The yeast was transferred to fresh sterile slants every two months to maintain high metabolic activity.

- Medium I (Solid medium for agar slants)

  - 4.5 g malt agar
  - 0.5 g glucose
  - 0.5 g yeast extract
  - up to 100 mL distilled water

4.2 Inoculum Preparation

For all experiments conducted, the biomass used in the fermentations was obtained from the agar slants using the following method. A loopfull of cells from the slants was transferred asceptically into 100 mL of sterile liquid medium (medium II) contained in a 500 mL Erlenmeyer flask. Depending on the amount of biomass needed, 4 to 24 flasks were prepared at once. The flasks were then placed in a rotary shaker set at 200 rpm and 33°C for 24-30 hours to ensure that the cells were in the exponential growth phase. Then, the flasks were placed at 4°C to allow the biomass to precipitate. After 24 hours, the liquid supernatant was decanted, and the concentrated biomass could then be used to inoculate reactors to carry out fermentation experiments.

- Medium II (medium for inoculum preparation)

  - 10.0 g glucose
  - 30.0 g yeast extract
  - 3.5 g peptone
  - 2.0 g KH_2PO_4
  - 1.0 g $MgSO_4 \cdot 7H_2O$
  - 1.0 g $(NH_4)_2SO_4$
  - up to 1 L distilled water

4.3 Fructose and Ethanol Production by Batch Fermentation

Batch fermentations were carried out at initial sucrose concentration of 30% (w/v) after inoculation in medium III. These were done in 500 mL Erlenmeyer flasks containing 100 mL of sterile medium III. The medium was asceptically inoculated with biomass previously prepared as described in section 4.2 and the flasks were placed in a rotary shaker set at 200 rpm and 33°C. The amount of biomass added varied to obtain a concentration of approximately 3.5 g/L after inoculation. Samples of 3.5 mL were taken every 3 hours using a sterilised pipette, and then analysed to determine the biomass, sugars, and ethanol concentrations. All tests were done at least in duplicate.

- Medium III (medium for batch fermentation)

  - Various amounts of sucrose
  - 10.0 g yeast extract
  - 3.5 g peptone
  - 2.0 g KH_2PO_4
  - 1.0 g $MgSO_4 \cdot 7H_2O$
  - 1.0 g $(NH_4)_2SO_4$
  - up to 1 L distilled water

Batch fermentations were also carried out in a small capacity bioreactor (maximum volume of 600 mL) with an initial sucrose concentration of 30% (w/v) after inoculation in medium III. The initial volume of broth after inoculation was 200 mL. The amount of biomass added varied to obtain a concentration of approximately 3.5 g/L after inoculation. The broth was constantly mixed using a Fisher Scientific magnetic stirrer. The bioreactor temperature was maintained at 33°C with a PolyTemp temperature controller, which controlled the temperature of a water bath where the bioreactor was sitting (figure 4.1). The temperature inside the reactor was read using a Thermo Electric digital thermometer. Samples of 3.5 mL were taken every 3 hours using a sterilised pipette, and then analysed to determine the biomass, sugars, and ethanol concentrations. All tests were done at least in duplicate. Figure 4.1 shows the schematic diagram of the experimental set-up used during batch fermentation in the small capacity bioreactor.

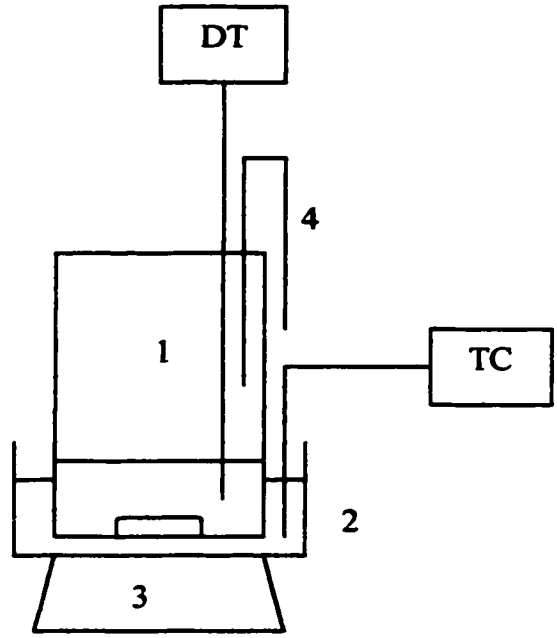


Figure 4.1 Schematic diagram of the experimental set-up used during batch fermentation in the small capacity bioreactor: (1) Bioreactor; (2) Water bath; (3) Magnetic stirrer; (4) CO_2 vent; (TC) Temperature control unit; (DT) Digital thermometer

4.4 Analysis

4.4.1 Ethanol

Ethanol concentration was determined by enzymatic analysis using alcohol dehydrogenase (Bernt and Gutmann, 1974). In this method, ethanol is oxidized by nicotinamide adenine dinucleotide (NAD) and alcohol dehydrogenase (ADH) to form reduced nicotinamide adenine dinucleotide (NADH) and acetaldehyde according to the following reaction:

$$\text{Ethanol} + \text{NAD} + \text{ADH} \Leftrightarrow \text{Acetaldehyde} + \text{NADH} + H^+$$

The concentration of NADH produced is directly proportional to the amount of ethanol in the sample, and NADH can be measured spectrophotometrically at 340 nm. The reaction equilibrium is pushed completely to the right at alkaline pH's.

To perform the analysis, the following reagents are needed:

- Buffer
    - 10.0 g $Na_4P_2O_7 \cdot H_2O$
    - 2.5 g semicarbazide hydrochloride
    - 0.5 g glycine
    - up to 250 mL distilled water
    - adjust pH to 8.7 with 4N KOH
    - dilute to 300 mL

- Nicotinamide adenine dinucleotide (NAD)
    - 50.0 mg in 3 mL of distilled water

- Alcohol dehydrogenase (ADH)
    - 5.0 mg in 1 mL of distilled water

A calibration curve was made using ethanol solutions containing between 0.0018% (w/v) and 0.0113% (w/v) ethanol. Therefore, the dilution of samples was necessary to bring ethanol concentration to the range stated above. After dilution, 0.2 mL of the diluted sample was transferred into a test tube, which contained 3 mL of buffer and 0.1 mL of NAD. Lastly, 0.02 mL of ADH was added to each test tube. The tubes were then mixed thoroughly and incubated at 37°C for 25-28 minutes. The absorbance of the solution was measured at 340 nm against a reference using a Beckman DU-640 spectrophotometer. The reference was prepared by adding 0.2 mL of distilled water to the test tube instead of 0.2 mL of sample.

4.4.2 Sugars

Concentrations of sucrose, glucose, fructose, kestoses and glycerol were determined with a Water's high performance liquid chromatography (HPLC). Control of the system, data acquisition, and data analysis were accomplished by computer using the Millennium 2010 Chromatography Manager software version 2.10. The HPLC's system components were: a Water's 717 Autosampler, a Water's 600E System Controller and a Water's 410 Differential Refractometer. The column used was a Water's Sugar Pak I (6.5x300 mm). The column was packed with a microparticulate cation exchange gel in calcium form, and was used to separate sugars and alcohols according to molecular weight. A solution of 50 mg calcium ethylene diamine tetra acetic acid (CaEDTA) per liter of deionoized water was used as the mobile phase. The HPLC settings were: column temperature, 70°C; sample injection volume, 20 µL; mobile phase flow rate, 0.5 mL/min.

To regenerate the column the flow direction of the mobile phase was reversed while the column temperature was maintained at 90°C. Flow rate was maintained at 0.5 mL/min for a period of 4 hours. After regeneration, the column was equilibrated for a minimum of 2 hours in the normal flow direction using the 50mg/L CaEDTA solution.

Standard curves for pure sucrose and for a mixture of glucose/fructose were prepared using sugar solutions containing between 0.1% (w/v) and 0.4% (w/v) sugars. The concentration of glycerol in standard glycerol solutions were between 0.05% (w/v) and 0.2% (w/v). Therefore, the samples taken from the fermentation broth were diluted to bring down the sugar and glycerol concentrations to the ranges stated above. Before injection, all samples were thoroughly filtered to ensure that no particles were injected in the column.

4.4.3 Biomass

Biomass concentration was determined by spectrophotometrical analysis. Absorbance was measured at 600 nm against a reference (water) with a Beckman DU-640 spectrophotometer. A calibration curve was prepared using the dry weight method. In this method, a known volume of a sample from the broth is centrifuged, the supernatant is removed and the biomass is resuspended in distilled water and recentrifuged. This washed and centrifuged biomass is transferred to a preweighed aluminum dish and dried at 105°C for 24 hours. The dish is then weighed, and biomass concentration is given as grams of biomass in 1L of the sample. The calibration curve was made for a range of biomass concentrations from 1-15 g/L.

4.5 Hollow Fibre Membrane Module Design and Assembly

Silicone rubber hollow fibres designated as M60 were provided by NAGASEP, Japan. Fibre dimensions were: inside diameter of 200 μm, outside diameter of 320 μm, and a thickness of 60 μm. An inside feed/outside vacuum design (Figure 4.2) was used to eliminate the risk of fibres collapsing if vacuum was applied on the inside of the fibres. To conduct preliminary tests of membrane performance, a small module of 15 fibres was assembled with an effective permeation length of 45 cm. The effective membrane area was 42 cm^2. The fibres were glued together at both ends into a bundle. Various glues, including epoxy resin, liquid anaerobic vulcanising silicone rubber, and commercial bathroom silicone sealant, were tested. The chosen glue to build all modules was the commercial bathroom silicone sealant. The module assembly will be discussed later in detail. After the glue hardened, the fibre bundle was inserted into a glass casing and glued to both ends of the glass casing using the same commercial sealant. After the preliminary testing was done, a module to be coupled with the bioreactor was designed based on the goal of maintaining the ethanol concentration in the bioreactor at 1.5 %(w/v). The final module included: 630 fibres with an effective permeation length of 22 cm (Effective membrane area = 870 cm^2). It was assembled in the same way as the test module. A commercial module from NAGASEP, Japan, was also tested. This module was also made of M60 hollow fibres. It was assembled in a polycarbonate casing with LTV silicone rubber as the glue. This module contained 750 fibres of 14 cm in length. The effective membrane area was therefore 900 cm^2. The module built in the laboratory will be referred to as the "laboratory module" from now on, and the commercial one as the "commercial module" hereafter.

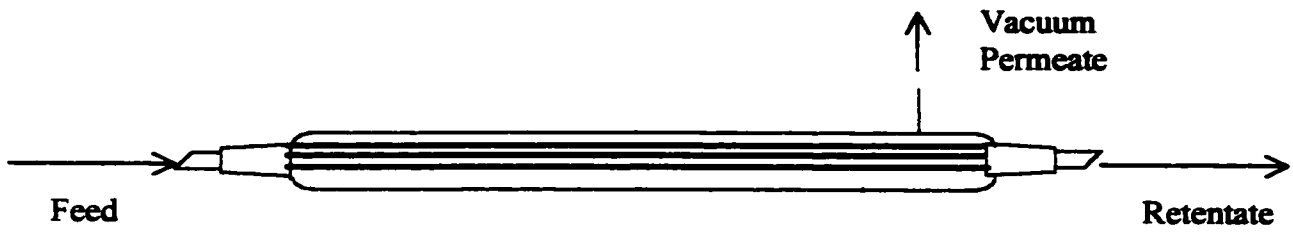


Figure 4.2 A schematic diagram of a membrane module: inside feed/outside vacuum.

4.6 Pervaporation Tests of Ethanol/Water, and Ethanol/Water/Biomass Mixtures

To obtain membrane performance data, preliminary tests were carried out using the small 42 cm^2 membrane module. Figure 4.3 describes the experimental set-up used for pervaporation (the same set-up was used for fermentation/pervaporation experiments). The feed solution in vessel 1 on Figure 4.3 was pumped through the module with a Watson Marlow peristaltic pump. The feed solution was either a mixture of ethanol and water or a mixture of ethanol, water and biomass. The tubing used for circulation was 1/32" silicone rubber tubing of 90 cm in length from the feed vessel to the module and of 65 cm in length from the module exit to the vessel. The feed entered the inside of the fibres, and vacuum was applied on the outside of the fibres with a Welch DuoSeal vacuum pump model 1400. Downstream pressure was measured with an MKS type 122A pressure transducer and controlled by an MKS type 651 pressure controller. Feed temperature 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 condensed and collected in a condenser submerged in a liquid nitrogen bath. The condenser was removed from the liquid nitrogen bath every two hours and replaced by a new one. The condenser, after being removed from the system was weighed, the empty condenser having been previously weighted when empty. The sample collected in the condenser was then subjected to analysis.

Test conditions were as follows:

- Feed ethanol concentration, 5 % (w/v); circulation flow rate, 0.2 mL/min; temperature, 33°C; total permeation time, 6 hours; downstream pressures, 1 torr, 7 torr, 14 torr and 21 torr;

- Downstream pressure, 1 torr; circulation flow rate, 0.2 mL/min; temperature, 33°C; total permeation time, 6 hours; ethanol concentrations in the feed, 1.5 % (w/v), 2.5% (w/v), 3.5 % (w/v) and 5 % (w/v);

- Downstream pressure, 1 torr; temperature, 33°C; total permeation time, 6 hours; feed ethanol concentration, 5% (w/v); circulation rate, 2 mL/min; feed biomass concentration, 8 g/L.

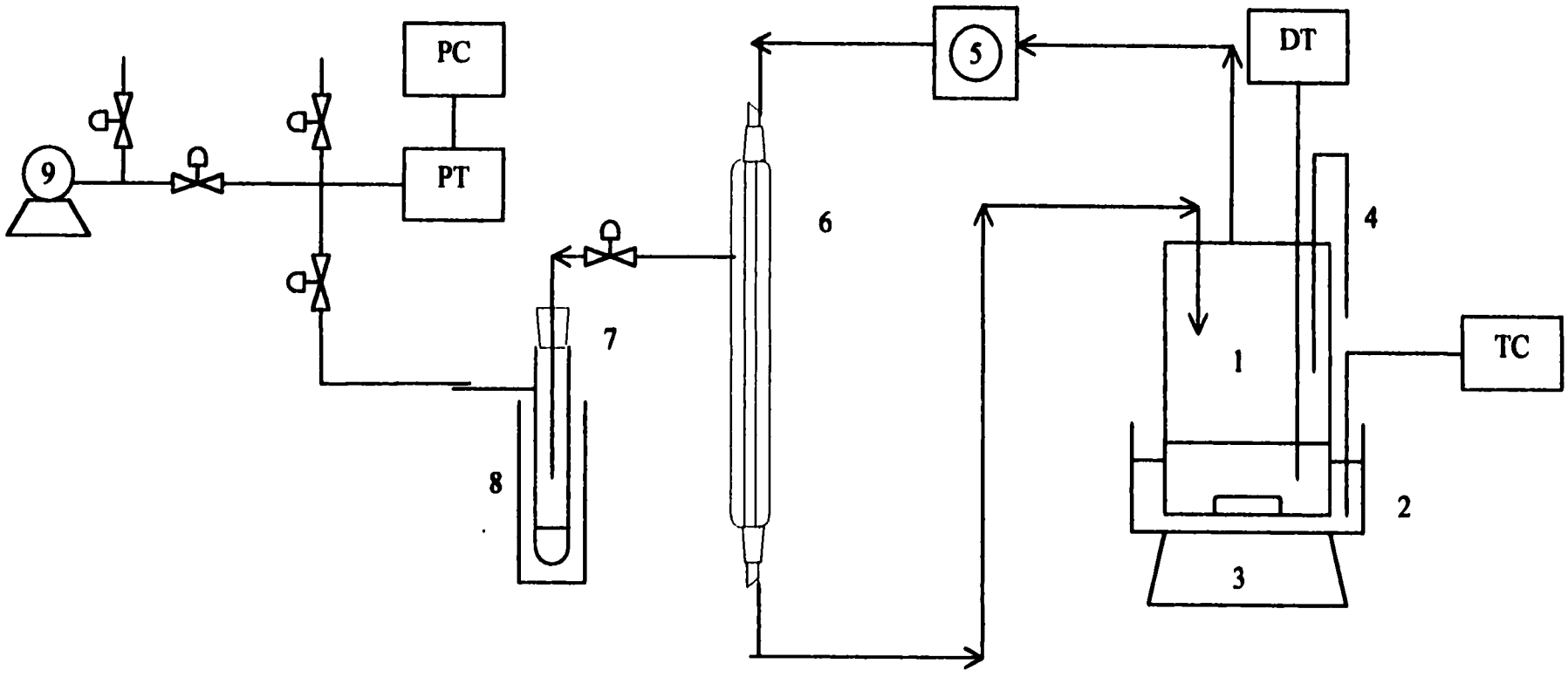


Figure 4.3 Schematic diagram of the pervaporation experimental set-up: (1) Bioreactor or vessel for membrane testing; (2) Temperature control bath; (3) Magnetic stirrer; (4) CO_2 vent for bioreactor; (5) Peristaltic pump; (6) Membrane module; (7) Condenser; (8) Liquid N_2 bath; (9) Vacuum pump; (TC) Temperature control unit; (DT) Digital thermometer; (PT) Pressure transducer; (PC) Pressure control unit.

4.7 Batch and Fed-Batch Fermentation Coupled with Pervaporation

The same set-up as described in Figure 4.3 was used to perform batch fermentation/pervaporation as well as fed-batch fermentation/pervaporation. A small capacity bioreactor was used instead of using just a vessel. All experiments were carried out with an initial sucrose concentration and an initial biomass concentration of 30% (w/v) and 3.5 g/L respectively after inoculation. The fermentation medium used was medium III. The total volume in the bioreactor after inoculation was 150 mL in all experiments. After inoculation of the sterile medium, the system was left in batch mode for a few hours to allow the biomass to enter exponential growth, and to allow a certain amount of ethanol to be produced. The membrane unit was initiated, then the circulation of the fermentation broth was started at a flow rate of 2.5 mL/min corresponding to a residence time of 1 hour in the reactor. Simultaneously, the vacuum pump on the permeate side of the pervaporation system was started. The bioreactor was connected to the inlet of the membrane module with a 0.635cm silicone tubing of 96 cm in length and the module outlet was connected to the bioreactor with the same tubing of 69cm in length. In some experiments, Pharmed tubing was used. The lengths of the latter tubing were the same as those of the 0.635 cm silicone tubings. In all experiments, the downstream pressure and the bioreactor temperature were maintained at 1 torr and 33°C respectively. The permeate vapor was collected in a condensed form in a condenser submerged in a liquid nitrogen bath. Every 2.5-3 hours, the condenser was removed and replaced by a new condenser. After removal, the condenser was immediately weighed. The sample in the condenser was then subjected to analysis. At the same time the condenser was changed, a sample of 3.5 ml was taken from the reactor and analysed to determine the biomass, sugars, and ethanol concentrations. All tests were done at least in duplicate.

The following experiments were performed:

a) Laboratory module, 30% (w/v) sucrose, 150 mL, membrane initiated after 6 hours of batch fermentation, silicone rubber tubing for circulation;
b) Laboratory module, 30% (w/v) sucrose, 150 mL, membrane initiated after 6 hours of batch fermentation, Pharmed tubing for circulation;
c) Commercial module, 30% (w/v) sucrose, 150 mL, membrane initiated after 6 hours of batch fermentation, Pharmed tubing for circulation;
d) Commercial module, 30% (w/v) sucrose, 150 mL, membrane initiated after 3 hours of batch fermentation, Pharmed tubing for circulation;

To perform fed-batch fermentation coupled with pervaporation, the same method was used as described for batch fermentation. The experimental conditions of the fed-batch experiments were exactly the same as variation d) stated above. After 14.5 hours of fermentation, the pervaporation was stopped and 22 mL of 60% (w/v) sucrose in medium III was added to the bioreactor. The system was then left in batch mode without pervaporation for an additional 10 hours.

5. Results and Discussion

5.1 Membrane Module Design Considerations and Assembly

Before building the smallest module, glue compatibility was tested on a very small bundle of hollow fibres. The first trial at gluing together the small hollow fibres bundle was done using epoxy resin. At first glance, it seemed that the fibres held together and that a mechanically stable bundle had been formed. When inserted into a casing and tested under vacuum conditions, the feed leaked in between the fibres, and was therefore accumulating on the shell side of the module. It was concluded that epoxy resin is not compatible with the silicone rubber fibres, even though some commercial modules of silicone rubber are glued with epoxy resin. In our experiments, it seemed that the epoxy could simply be pealed off very easily from the fibre.

The next glue that was tested was commercial silicone rubber bathroom sealant. This glue was very viscous and made it hard to manipulate with the very small fibres. The challenge was to make sure that the glue filled the space between fibres to ensure that the feed could not flow in between the fibres and accumulate on the shell side of the module. While being manipulated, the fibres are very static, and therefore did not stay in place very long. To eliminate that problem, an anti-static surface would be needed. Nonetheless, when building a very small bundle, it was feasible to have glue between each fibre, but the resulting bundle diameter was quite large. The glue was compatible with the membrane, but to build a large module (700 fibres and more), it was decided to test another type of silicone rubber glue, which was less viscous, and could therefore be manipulated more easily with the fibres. This other type of silicone rubber glue was anaerobic (cured under nitrogen atmosphere), and therefore added the problem of putting the bundle in a nitrogen atmosphere for 24 hours. But, this glue was not even compatible with the membrane. It could be pealed off from the fibres just as epoxy resin.

After all these attempts, it was determined that the best glue was the commercial silicone rubber bathroom sealant. When building the module of 700 fibres, special care was taken to ensure that the glue applied evenly in between each of the fibres. During the first attempt to build this large module, about 2 cm in length of glue was used to hold the bundle together. Once the bundle was glued inside the glass casing, it was tested for leaks. When leaks occurred, the method used to repair them was to put a drop of glue on top of the bundle where the leak was suspected to be. This created the problem of blocking some fibres, and in the long run, those drops of silicone glue would be removed with the applied flow rate, and the leak would occur again. The module with 2 cm in length of glue was not strong: when applying vacuum, the glue would move in the casing. Therefore, another 1cm lengthwise of glue was added for fibre support. Afterwards, whenever leaks occurred in the bundle, glue was added inside the bundle of glue by means of injection with a syringe. This way, no fibres would get blocked and if the glue was inserted in the correct place, the leak was repaired. The laboratory module was stable for a period of about 10 runs, and then started leaking. Whenever one leak was repaired, another one would appear, and so on. It was concluded that the silicone glue was breaking apart due to the vacuum, and was becoming impossible to repair. In order to complete the project, a commercial module made of the same membrane material and dimensions as the laboratory module was bought.

5.2 Pervaporation Tests of Ethanol/Water Mixtures

In order to design as precisely as possible 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 a small test module of $42cm^2$.

5.2.1 Effect of Ethanol Concentration in the Feed

The first set of experiments was conducted to determine the effect of varying the ethanol concentration in the feed on the pervaporation performance. All runs were performed under the same operating conditions, i.e. a downstream pressure of 1 torr, a feed temperature of 33°C, and a circulation rate of 0.2 mL/min. Figure 5.1 shows the experimental data as well as the model predictions obtained from the pore model described in section 3.3. The experimental data show that the total flux as well as the ethanol concentration in the permeate increased, whereas the selectivity (separation factor) decreased slightly with an increase in feed ethanol concentration. The experimental total flux increased from 32.21 g/(m^2 h) to 50.57 g/(m^2 h), and the experimental ethanol concentration in the permeate increased from 13.0% (w/v) to 29.8% (w/v) as the ethanol in the feed was increased from 1.6% (w/v) to 4.8% (w/v). As for the separation factor, it decreased from 9.2 to 8.4 as the ethanol in the feed was increased from 1.6% (w/v) to 4.8% (w/v). These values are well in the range of previously reported data for the pervaporation of ethanol/water mixtures through silicone rubber membranes (Kimura et al., 1982, Nakao et al., 1987). These groups also observed the same trends, i.e. total flux and permeate concentration increase and a slight decrease in separation factor with an increase in ethanol concentration in the feed. When the ethanol and water flux are given separately as shown in table 5.1, the ethanol flux increases more rapidly than the water flux with an increase in the ethanol concentration in the feed. If the water flux would remain constant, and only the ethanol flux would increase, then the separation factor would increase with an increase in the feed ethanol concentration, and

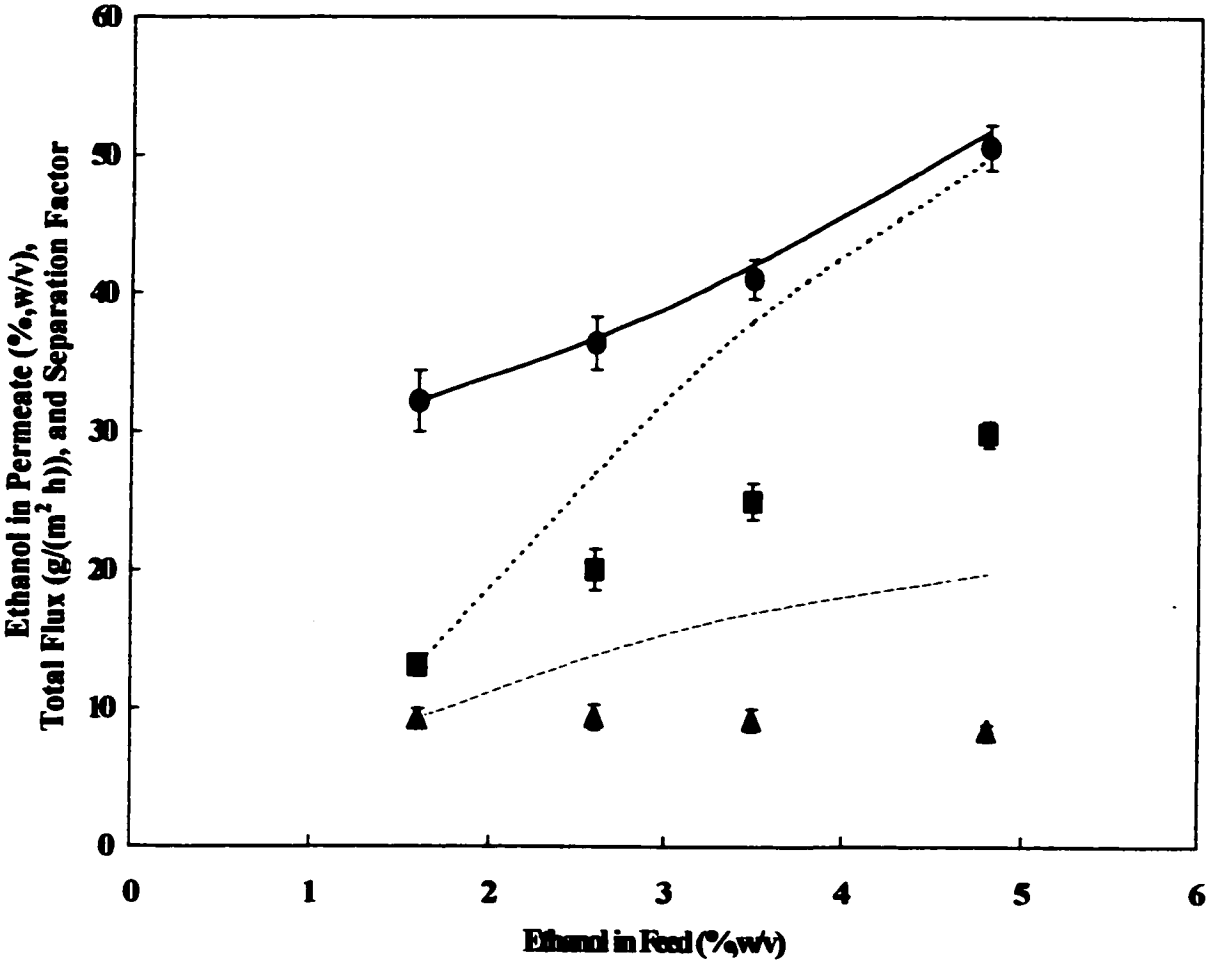


Figure 5.1 Effect of feed ethanol concentration on the membrane performance for pervaporation through silicone rubber hollow fibres. A downstream pressure, 1 torr; temperature, 33°C; circulation flow rate, 0.2 mL/min. Symbols are experimental data, and lines are model predictions according to the pore model. (▲,------) Separation Factor; (●, —) Total Flux; (■, ·····) [EtOH] Permeate.

the permeate would be more concentrated in ethanol than actually observed. One factor that would explain this increase in water flux is the swelling of the membrane which becomes stronger at larger concentrations of ethanol in the feed (Bueso et al., 2000). This makes the membrane more permeable to water and, as a result, less selective to ethanol. This shows that for very dilute solutions, pervaporation through silicone rubber is more suitable than for concentrated solutions. This swelling effect will be described in more details in conjunction with the modeling.

Table 5.1 Experimental total, water, and ethanol flux as a function of ethanol feed concentration.

Ethanol in Feed (%,w/v)	Total Flux ($g/(m^2\ h)$)	Ethanol Flux ($g/(m^2\ h)$)	Water Flux ($g/(m^2\ h)$)
1.6	32.21	4.21	28.00
2.6	36.42	7.30	29.12
3.5	41.07	10.25	30.82
4.8	50.57	15.08	35.49

The pore model was used to analyze the experimental pervaporation data. Initially, equation 9 was used to determine the value of B_j/B_i from the experimental data. In the equation, i represents ethanol and j represents water. Table 5.2 shows the values of B_j/B_i calculated using each single data point. As it can be seen, B_j/B_i increases with an increase of ethanol in the feed. This B_j/B_i increase parallels the increase in water flux as shown in Table 5.1. No single value of B_j/B_i can accurately predict all the experimental data. Therefore, the B_j/B_i value obtained from the experimental data corresponding to 1.6% (w/v) feed ethanol concentration was used to calculate theoretical curves of permeate concentration, total permeate flux and separation factor. The B_j/B_i used to determine the theoretical curves was therefore 0.092. Parameters B_j/δ, and B_i/δ were obtained from the experimental total permeate flux that corresponds to feed ethanol concentration of 1.6% (w/v) using eq. 14. B_j/δ, and B_i/δ so obtained were 1.72×10^{-14} and

$1.87x10^{-13}$ respectively. The latter B_j/δ, and B_i/δ were then used to calculate total permeate fluxes for other feed ethanol concentrations.

Table 5.2 Ratio of constants related to the vapor transport of species j and i (B_j/B_i) calculated from each single data points using eq. 9.

Ethanol in Feed (%,w/v)	B_j/B_i
1.6	0.092
2.6	0.14
3.5	0.185
4.8	0.26

From Figure 5.1, we can see a large discrepancy between the experimental data and the theoretical curves for permeate concentration and selectivity. For the flux, on the other hand, very good agreement was obtained with a B_j/B_i value of 0.092. Okada stated that the criteria for the justification of the proposed model was agreement in tendencies (Okada and Matsuura, 1991), and this is observed except for the case of the separation factor. It should also be noted that eqs. 9 and 14 are only valid when the liquid evaporation takes place at the pore inlet, which has been specifically shown to be valid in the ethanol/cellulose system, but might not be the case in the ethanol/PDMS system. The model equations are also based on the assumption that the pore size remains constant in the entire feed composition range and also in the direction of the permeant flow. This assumption may not be true particularly when the swelling of the membrane occurs at higher feed ethanol concentrations. Bueso et al. (2000) have shown the swelling behavior of pervaporation membranes, specifically for PDMS-PMHS composite membranes. They reported that as the ethanol concentration in the feed is increased, more swelling occurs in the membrane, which means that the distance between polymer chains becomes larger, and therefore the membrane will lose its selectivity. The swelling increased rapidly with feed ethanol concentration until it reached 30% (w/v) and then leveled off. The degree of

swelling with pure water was very low, and the equilibrium swelling was reached very rapidly. As the ethanol concentration in the feed was increased, the equilibrium swelling took longer to reach (Bueso et al., 2000). Their experiments showed that the membrane had a maximum quantity of solvent it was capable of absorbing. In the case of this present study, swelling phenomenon might explain why the pore model could not predict the experimental data very well. In the experiments, as the ethanol concentration in the feed was increased, it can be assumed that more swelling took place and therefore, the discrepancy between the data and the model increased. Since the model does not consider swelling behavior, the smallest value of B_j/B_i, which corresponds to the least level of swelling, was used for the modeling. As shown in table 5.3, the pore model predicts that the water flux will decrease with an increase in ethanol in the feed. This would be right if no swelling occurred, but as described using table 5.2, the water flux increased with an increase in ethanol concentration. Adjustments in the model would be necessary to incorporate this effect in order to obtain better agreement between experimental and theoretical data.

Table 5.3 Predicted total, water, and ethanol flux as a function of ethanol feed concentration.

Ethanol in Feed (%,w/v)	Total Flux ($g/(m^2\ h)$)	Ethanol Flux ($g/(m^2\ h)$)	Water Flux ($g/(m^2\ h)$)
1.6	32.21	4.22	27.98
2.6	36.80	9.92	26.88
3.5	42.16	16.02	26.14
4.8	51.76	25.81	25.95

5.2.2 Effect of Downstream Pressure

The second set of experiments was conducted to determine the effect of downstream pressure on pervaporation membrane performance. All runs were performed under the same operating conditions, i.e. a feed ethanol concentration of 4.8% (w/v), a feed temperature of 33°C, and a circulation rate of the feed in the hollow fibres of 0.2 mL/min. Figure 5.2 shows the experimental data as well as the model predictions obtained from the pore model. The experimental data shows that as the downstream pressure increases (less vacuum is applied), the total flux decreases quite rapidly, and the ethanol concentration in the permeate and the selectivity also decrease, but very little. A decrease in flux with an increase in downstream pressure is expected, since the chemical potential on the downstream side increases with an increase in the downstream pressure and, as a result, the chemical potential difference across the membrane, which is the driving force for the pervaporation transport, will decrease. As for the decrease of permeate concentration and selectivity, swelling still has to be considered because this trend is not constant in all experimental situations, as will be seen. As described in the previous section, an operating pervaporation membrane has a swelling gradient in it. It may be assumed that the upstream surface remains at the swelling equilibrium with the feed solution, whereas the cross-membrane surface under the vacuum is swollen only slightly. There appears to be a threshold value of the downstream pressure below which the permeate face of the membrane remains virtually dry, as a result of which both flux and selectivity level off with further decrease in permeate pressure. Above this permeate pressure value, both flux and selectivity decline with an increase in downstream pressure (Dutta and Sikdar, 1996-97). This means that as permeate pressure increases, the membrane selectivity can either increase (Ji et al., 1994), decrease (Brun et al., 1985), or remain constant (Greenlaw et al., 1977), depending on the swelling of the membrane, and the range of pressure used.

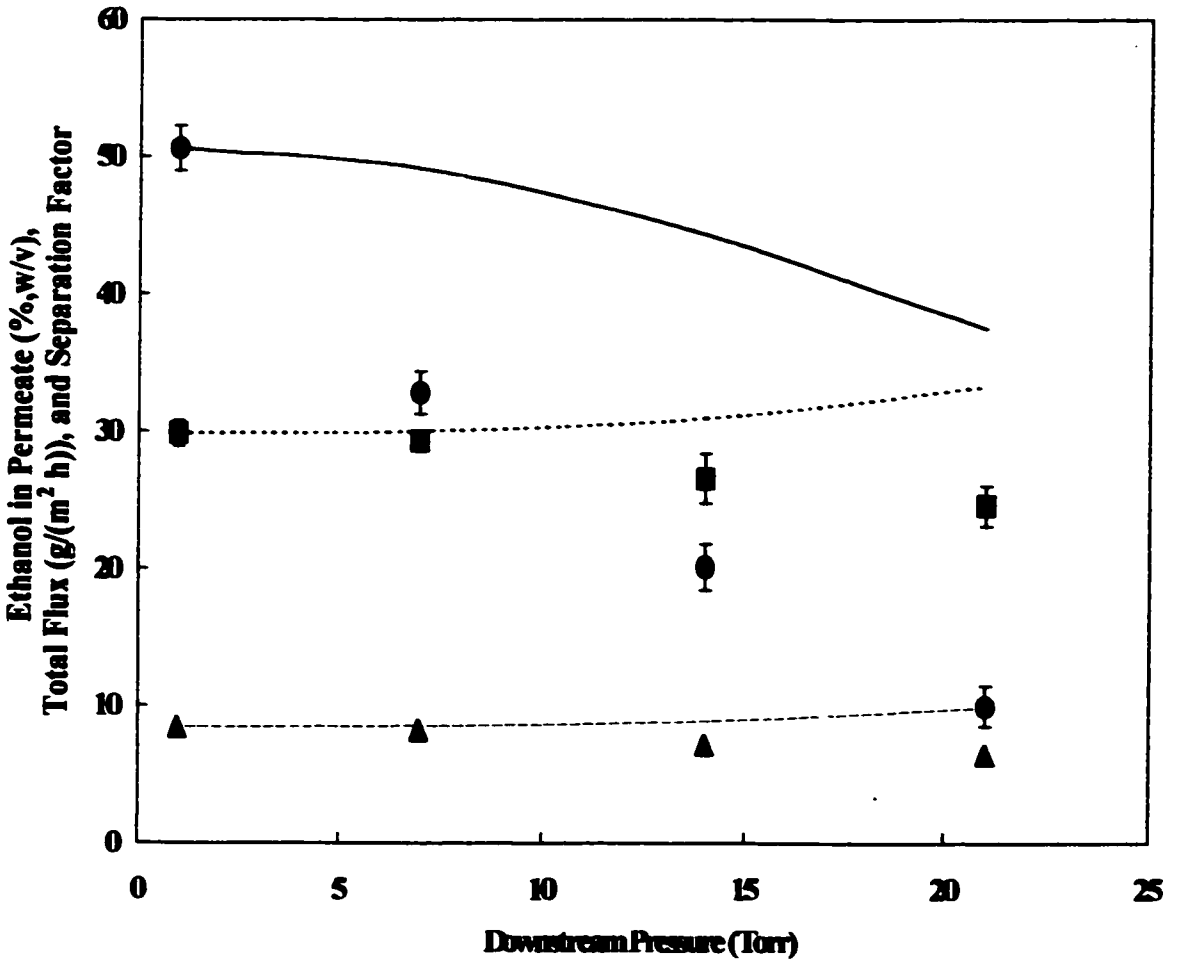


Figure 5.2 Effect of downstream pressure on the membrane performance for pervaporation of a 4.8% (w/v) ethanol solution through silicone rubber hollow fibres. A temperature, 33°C; circulation flow rate, 0.2 mL/min. Symbols are experimental data, and lines are model predictions according to the pore model. (▲,------) Separation Factor; (●, —) Total Flux; (■, ·····) [EtOH] Permeate.

The pore model was used again to produce theoretical curves of the effect of downstream pressure predicted by the model (Figure 5.2). To produce the theoretical curves, a value of B_j/B_i of 0.26 was used because at this point in the experiments, the membrane had been subjected to many feeds of 4.8% (w/v), and it was assumed that the swelling in the membrane was at its maximum equilibrium value. The theoretical flux, as well as the experimental flux decrease with an increase in downstream pressure. This was expected, but Figure 5.2 shows that the model predicts a less dramatic drop in flux than that which was experimentally observed. The selectivity and permeate concentration trends observed and predicted are not the same. The model predicts an increase in both as the permeate pressure increases, but again, this model does not take into account the swelling effect of the membrane. As the downstream pressure approaches the saturation vapor pressure of the mixture, the membrane swells more, leading to lower selectivity. The pore model assumes that liquid evaporation takes place at the pore inlet, but if the downstream pressure approaches the saturation vapor pressure, liquid penetrates more deeply in the pore, which makes the model invalid.

5.2.3 Effect of Biomass in the Feed

The third set of experiments was carried out to determine the effect of the presence of biomass in the feed on the pervaporation membrane performance and to determine the feasibility of circulating biomass inside the hollow fibre. All runs were performed under the same operating conditions, i.e. a feed ethanol concentration of 4.8% (w/v), a downstream pressure of 1 torr, a feed temperature of 33°C, a biomass concentration of 10 g/L, and a circulation rate 0.2 mL/min. Figure 5.3 shows the comparison between pervaporation data with and without biomass in the feed. During the experiments, no fibre blockage was observed, and therefore it was concluded that it was possible to circulate the whole fermentation broth through the bore side of the hollow fibres without any concern of blocking the fibres and increasing the pressure drop. No blockage was observed in the sense of flow rate, i.e the flow rate of the feed inside the hollow fibres was never zero. The main features of these experimental results are that in the presence of biomass, the flux decreased, but the ethanol concentration in the permeate

and the separation factor remained almost unchanged. Furthermore, a slight increase was observed in both ethanol concentration in the permeate and separation factor. When the broth was circulating inside the fibres, yeast cells deposited on the membrane surface creating a very thin film, which could also block the membrane pores; this is known as fouling. This might have caused the decrease in flux, and the small increase in permeate concentration and selectivity. A membrane module was designed based on the experimental data given in section 5.2.1 and 5.2.2 together with the available fermentation data. In particular, the data corresponding to the feed ethanol concentration of 1.6% (w/v) were used, taking into account the biomass effect. A module with an effective membrane area of 870 cm^2 was then built according to the design. This module was used in conjunction with the fermentation conditions.

Table 5.4 Design of the laboratory membrane module to be coupled to the bioreactor

Membrane Dimensions	**Effective Membrane Area of one Fiber**
I.D. 0.02cm O.D. 0.032cm Thickness 0.006cm Effective length 22cm	1.38 cm^2
Design	**Module**
Objective Ethanol in the Bioreactor 1.5% (w/v) Ethanol productivity in the bioreactor[1] 0.46 g/h Ethanol flux[1] 3.507 g/(m^2 h) Ethanol in permeate[1] 12.1% (w/v)	Membrane area required 1311.4 cm^2 # fibers required 948

[1] All these values were taken for a broth ethanol concentration of 1.5% (w/v), and they take into account the effect of biomass on the membrane

This table shows that 1311 cm^2 would be required to maintain an ethanol broth concentration of 1.5 %(w/v), but as the module was built, difficulties were encountered and the final module obtained had an effective membrane area of 870 cm^2.

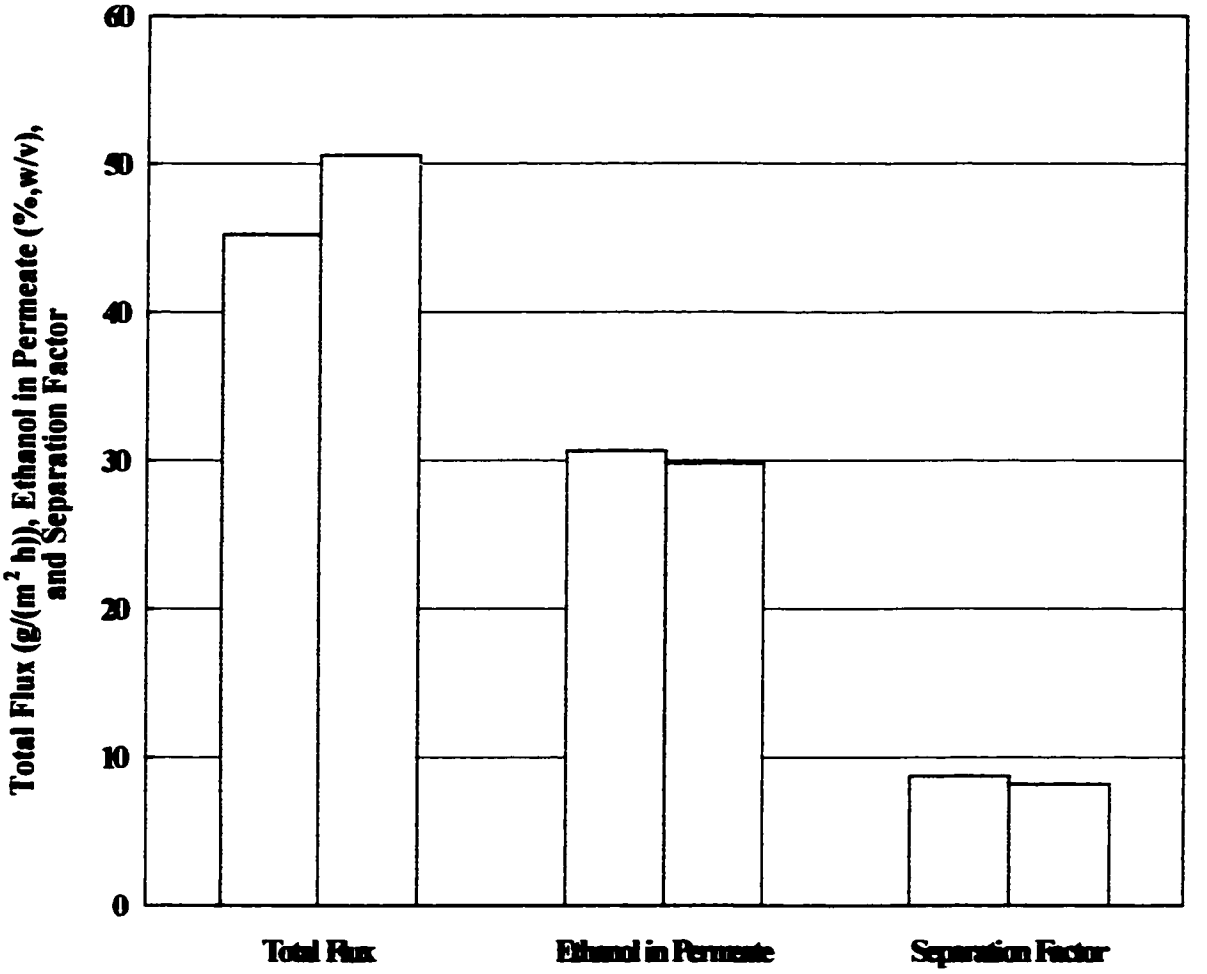


Figure 5.3 Effect of 10 g/L of biomass in the feed on the membrane performance for pervaporation. Feed ethanol concentration, 4.8% (w/v); temperature, 33°C; downstream pressure, 1 torr; circulation flow rate 0.2 mL/min. (⊡) With biomass; (□) Without biomass.

5.3 Batch Fermentation without Membrane Separation

Batch fermentations were performed in Erlenmeyer flasks and in a small capacity bioreactor for comparison purposes with fermentation/pervaporation experiments. Both were done using a high initial sucrose concentration of 30% (w/v), and an initial biomass concentration of 3.3 g/L.

Figures 5.4 and 5.5 show results of a typical fermentation process by *S. cerevisiae* 58 performed respectively in a flask and in the bioreactor. Both figures show the same trends for all fermentation components. As sucrose (initial concentration of 30.7% (w/v)) is hydrolyzed, concentrations of glucose and fructose increase, and the yeast begins to consume glucose. Glucose reaches a maximum value below its theoretical value of 16.1% (w/v) because it starts being consumed before sucrose is completely hydrolyzed. Fructose increases until it reaches its maximum plateau value once all the sucrose is hydrolyzed. As can be seen, fructose remains unconverted for the remaining time of the fermentation. As glucose is consumed, ethanol concentration increases until it reaches its maximum plateau value corresponding to complete conversion of glucose. The biomass curve follows the typical growth curve observed in fermentation processes.

Table 5.4 shows the comparison of the fermentation parameters obtained from a flask and a bioreactor. In both cases, the initial conditions were identical, therefore a direct comparison can be made between both fermentations. In both cases, the fermentation was stopped after 27 hours, at which time the final glucose concentration in the flask and in the bioreactor were, respectively, 0.18% (w/v) and 1.53% (w/v). This indicates that the reaction is slightly slower in the bioreactor. The biomass specific growth rate and the biomass yield are both lower in the case of the bioreactor, in which μ is 0.07 h^{-1} compared to 0.10 h^{-1} in the flask, and the biomass yield is 0.29 compared to 0.45 in the flask.

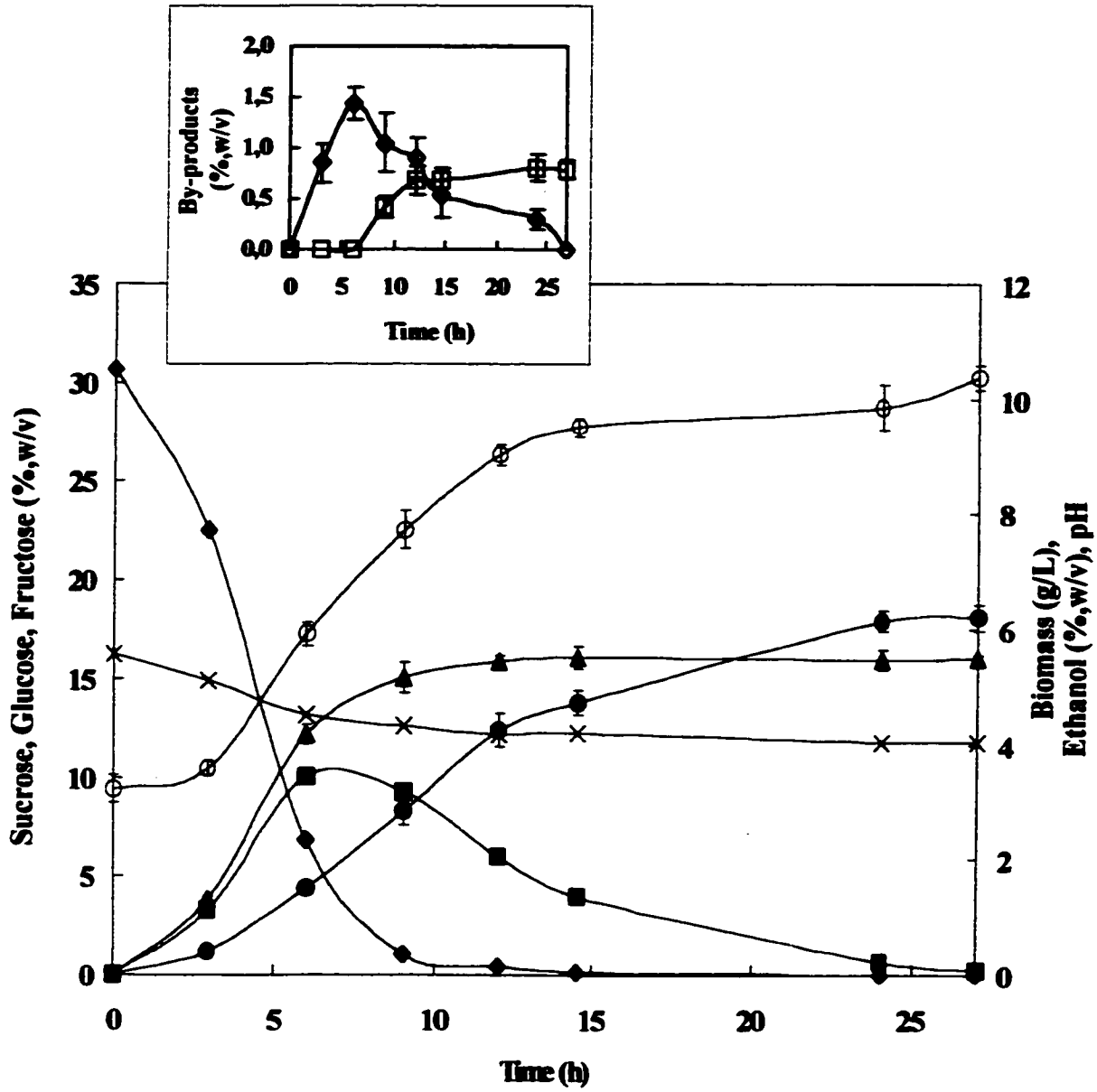


Figure 5.4 Results of batch fermentation performed in a flask. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L; temperature, 33°C. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

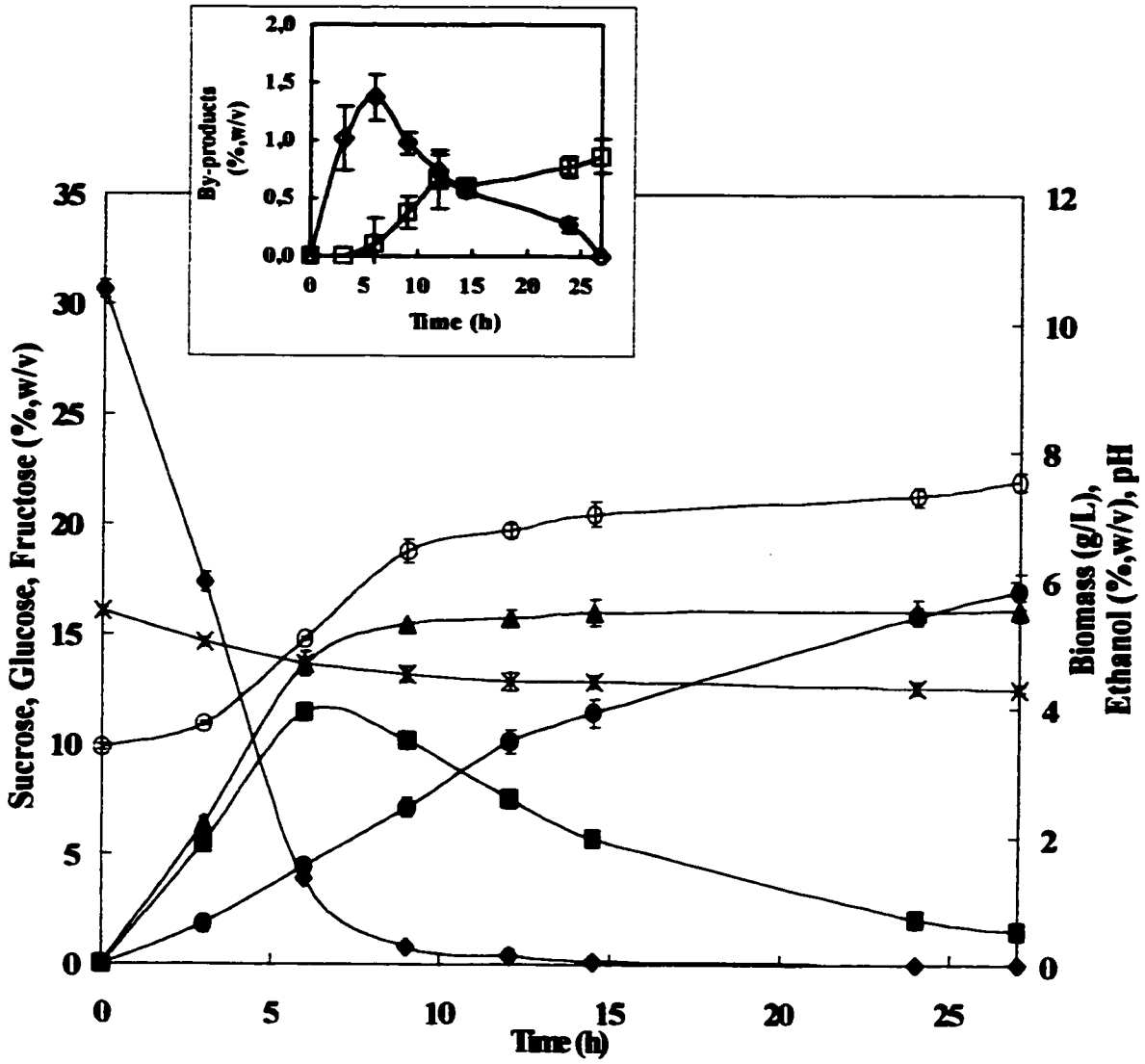


Figure 5.5 Results of batch fermentation performed in a small capacity bioreactor. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L; temperature, 33°C. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

Table 5.4 Comparison of batch fermentation in a flask and bioreactor.

	Flask	Bioreactor
Initial Biomass Concentration (g/L)	3.23	3.38
Final Biomass Concentration (g/L)	10.38	7.55
Initial Sucrose Concentration (%,w/v)	30.7	30.7
Fermentation Time (h)	27	27
Final Glucose Concentration (%,w/v)	0.18	1.53
Final Fructose Concentration (%,w/v)	16.00	16.11
Final Ethanol Concentration (%,w/v)	6.22	5.83
μ (h^{-1})	0.10	0.07
Biomass Yield (g/g)	0.45	0.29
P (g/(L h))	2.3	2.16
Ethanol Yield (%)	75.6	78.3
Fructose Yield (%)	99.1	99.9
Fructose/Glucose Content (%)	98.8/1.2	91.3/8.7
Sucrose Hydrolysis Rate (g/(L h))	21.06	21.04

In both cases, the sucrose hydrolysis rate is the same ($\approx$ 21 g/L h), which indicates that the biomass used as inoculum was at the same level of activity. Since less glucose was converted in the bioreactor during the same period than in the flask, the final ethanol concentration in the bioreactor is smaller than in the flask. Lower ethanol concentration and higher ethanol yield reported in the table seem to contradict each other at first glance, but this is simply because glucose conversion was not completed at the end of the run. If the glucose would be allowed to go down to a final value of 0.18% (w/v), the ethanol yield would decrease slightly because at that point the yeast would use a larger portion of glucose towards cell maintenance than towards ethanol production. This is due to the ethanol inhibition imposed on the yeast. This does not mean, however, that ethanol is not being produced. Flask results show that ethanol is still being produced since the final ethanol concentration is higher than in the bioreactor. Another factor which makes the

ethanol yield lower in the flask is that more biomass was produced in the flask than in the bioreactor. This means more glucose was consumed in the flask towards biomass production than in the bioreactor, which further resulted in lower final ethanol concentration. In both cases, the final fructose concentration is approximately 16% (w/v), and the fructose yield is approximately 99.5%. This confirms that fructose remained unconverted by the yeast when glucose was present. The fructose/glucose content at the end of the experiment was 98.8% of the total sugar is fructose, and 1.2% is glucose in the flask, while 91.3% is fructose and 8.7% is glucose in the bioreactor. As mentioned earlier in the literature review, syrups containing more than 90% fructose may be used to produce crystalline fructose, therefore this fermentation process is possibly a good means of obtaining high fructose syrups. It should also be pointed out that in each vessel kestoses formed initially disappeared at the end of the run (Figures 5.4 and 5.5). Glycerol concentration was as low as 0.8% (w/v), which is the reason why they were not taken into account in the yield calculations.

In conclusion, batch fermentation performed in a flask or in a small capacity bioreactor produced very similar results, as expected. Generally, when fermentation is started with 30% (w/v) sucrose and 3.3g/L of biomass, the yeast will produce a broth containing ≈6% (w/v) ethanol and 16% (w/v) fructose in 27 hours. These reflect ethanol and fructose yields of ≈77% and 99.5% respectively. The ethanol production is ≈2.2g/(L h) and the biomass specific growth rate is ≈0.085h^{-1}. These results are in the typical ranges obtained for fermentation by *S. cerevisiae* ATCC 36859 (Koren, 1990).

5.4 Batch Fermentation Coupled with Membrane Separation

Fermentation/pervaporation experiments will be compared to the batch fermentation performed in the small capacity bioreactor since this was the vessel used to couple to the membrane module. Since batch data ends at 27 hours, at which point, the final glucose concentration was 1.5% (w/v), the point of comparison to determine and calculate all fermentation parameters will be when the normalized glucose concentration in the reactor is between 1-2% (w/v). As mentioned in section 3.4, the normalized data is the recalculated concentrations as though the permeate would be put back in the reactor in order to get final ethanol concentrations as in a batch fermentation.

5.4.1 Batch Fermentation/Pervaporation I

The first set of experiments was conducted using the laboratory membrane module under the following conditions: an initial reactor volume of 150 mL, an initial sucrose concentration of 31% (w/v), an initial biomass concentration of 3.3 g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation, and silicone rubber tubing for broth circulation in the membrane module. The system initially underwent batch reaction for 6 hours before pervaporation was started. Figure 5.6 shows the actual reactor data as a function of time, and Figure 5.7 shows the normalized data as a function of time. The curves follow the same trends as a batch system without membrane separation, but in a shorter period of time. The system was successful in continuously removing the ethanol from the fermentation broth as seen in Figure 5.6 where the actual ethanol concentration is 3.2% (w/v) at the end of fermentation instead of 5.8% (w/v) in batch without membrane separation. The first apparent advantage by looking at Figure 5.6 is the reduced fermentation time, which when coupled

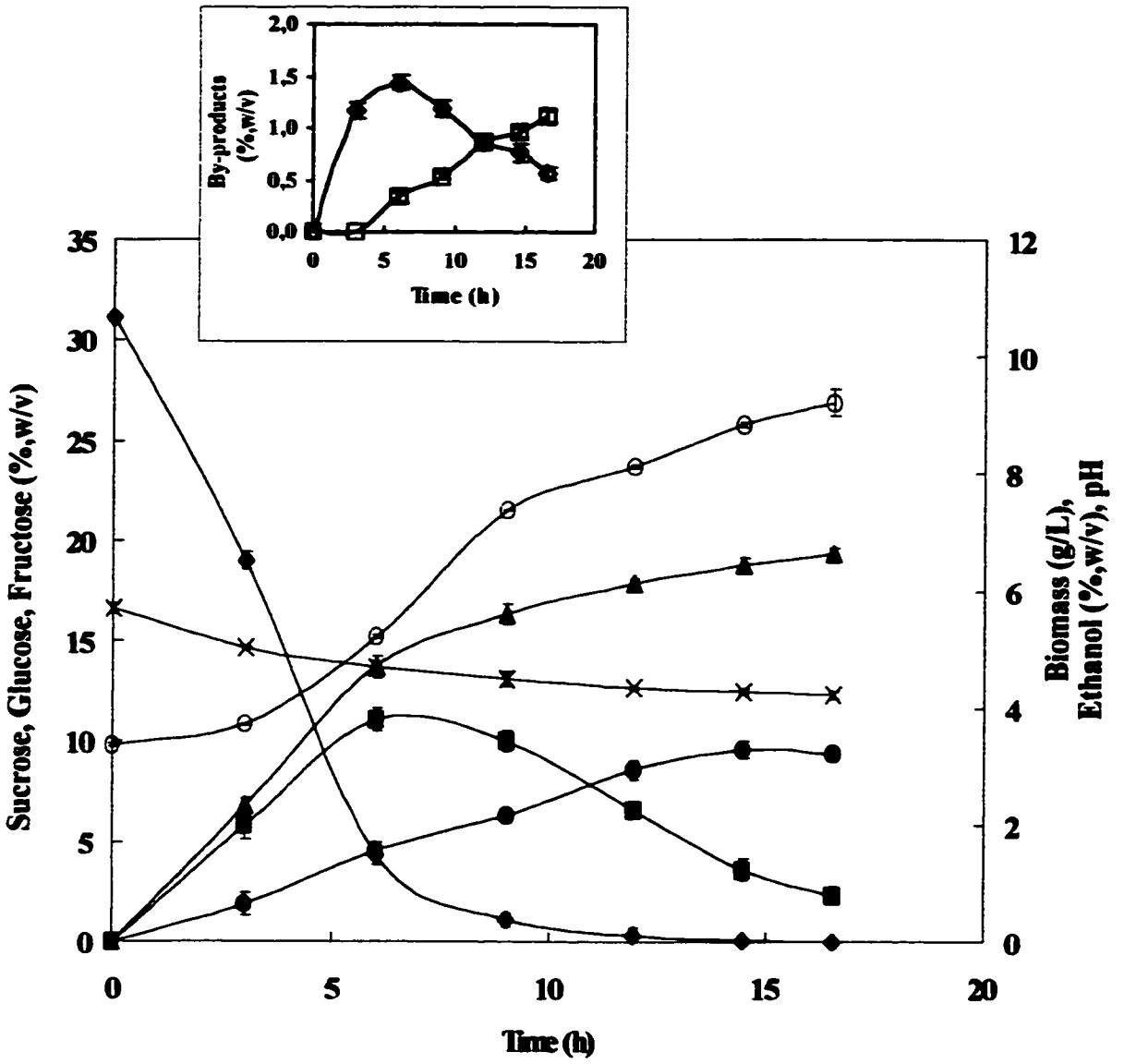


Figure 5.6 Actual reactor data of batch fermentation/pervaporation using the laboratory pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 6 hours of batch fermentation; silicone rubber tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

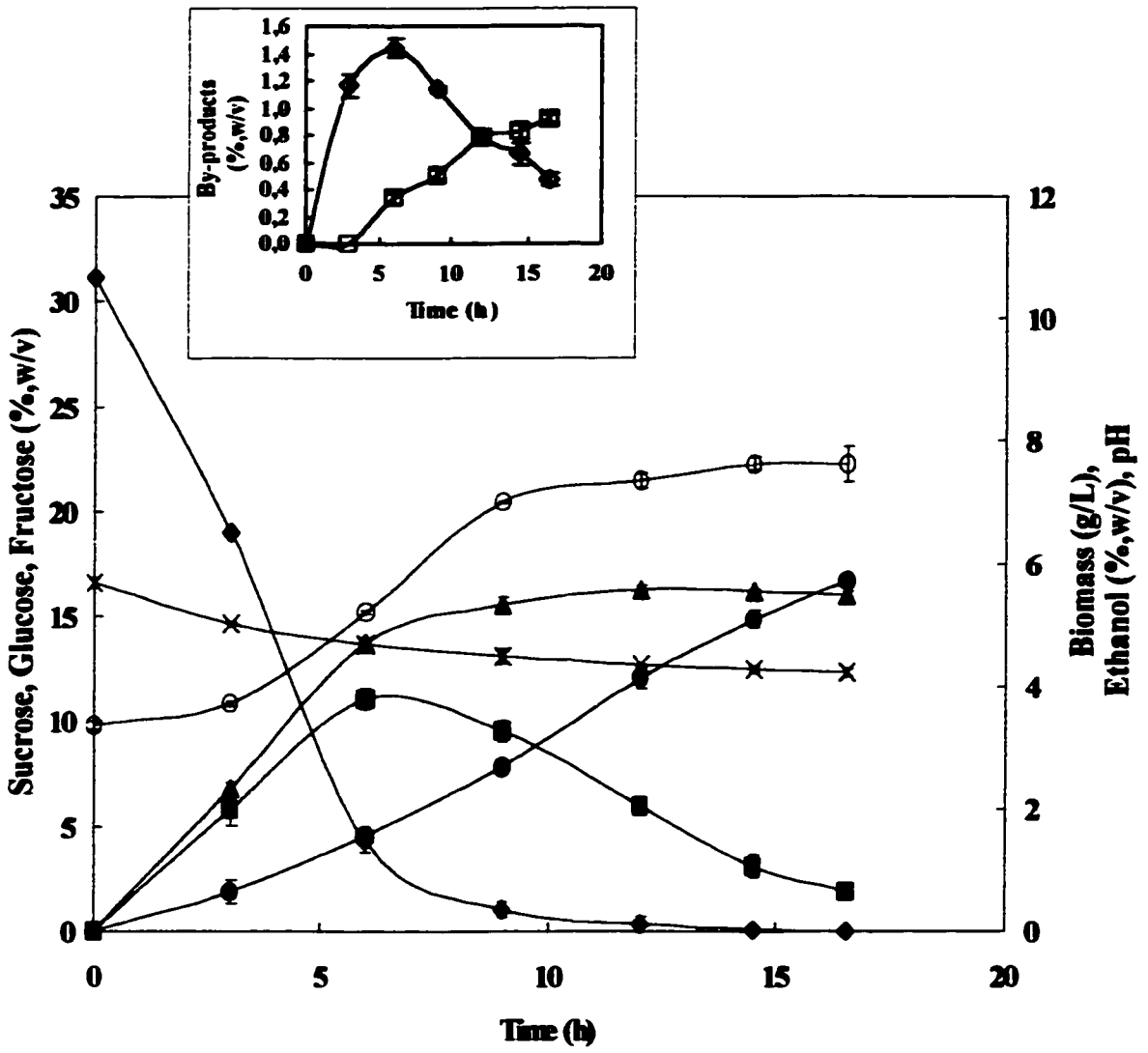


Figure 5.7 Normalized reactor data of batch fermentation/pervaporation using the laboratory pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 6 hours of batch fermentation; silicone rubber tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

with pervaporation is 16.5 hours instead of 27 hours (Figure 5.5) to reduce the glucose content to about the same 1.5% (w/v) level. Table 5.5 shows the fermentation parameters used for comparison with batch fermentation in the bioreactor presented in Table 5.4. All parameters are from the normalized data for accurate comparison. The pervaporation results themselves will be discussed later.

Table 5.5 Fermentation/Pervaporation I parameters.

	Fermentation/Pervaporation I
Initial Biomass Concentration (g/L)	3.37
Final Biomass Concentration (g/L)	9.22
Initial Sucrose Concentration (%,w/v)	31.1
Fermentation Time (h)	16.5
Final Glucose Concentration (%,w/v)	1.89
Final Fructose Concentration (%,w/v)	15.98
Final Ethanol Concentration (%,w/v)	5.72
μ (h^{-1})	0.07
Biomass Yield (g/g)	0.29
P (g/(L h))	3.47
Ethanol Yield (%)	75.3
Fructose Yield (%)	97.53
Fructose/Glucose Content (%)	89.4/10.6
Sucrose Hydrolysis Rate (g/(L h))	21.43

The system was operated as a batch reactor during the first six hours of fermentation to allow the ethanol concentration to reach a level of approximately 1.6% (w/v). A direct comparison of the coupled fermentation/pervaporation I with the batch fermentation in the bioreactor is possible, as stated previously. In both cases, initial conditions are identical, and final conditions (normalized concentrations) are approximately the same. The biomass yield and specific growth rate are the same as batch fermentation without membrane separation, i.e. 0.29 and 0.07h^{-1} respectively.

In both cases, the sucrose hydrolysis rate is identical with a value of 21 g/(L h). This was expected since most of the hydrolysis takes place in the first 6 hours of fermentation, at which point the membrane is not even in effect for the case of fermentation/pervaporation I.

The normalized final ethanol concentration in the broth is of 5.72% (w/v) compared to 5.83% (w/v) in batch fermentation without membrane. These correspond to ethanol yields of 75.3% and 78.3% respectively. This indicates that less ethanol is produced, but from the biomass yield data, it can be seen that the glucose did not go towards cell production. But the same amount of glucose was consumed and a small amount of fructose was even consumed. Therefore, a higher ethanol content would be expected, but this was not the case. This would indicate that somehow ethanol is lost somewhere in the process. When performing the experiments, a very strong ethanol smell was coming from the silicone rubber tubing used for broth circulation. It was therefore assumed that the ethanol was evaporating from the tubing, which accounted for the small loss in ethanol yield. To remedy this problem and to verify the assumption that ethanol is evaporating from the tubing, the next experiments were performed using Pharmed tubings for broth circulation. Going back to Table 5.5, the normalized fructose concentration at the end of the fermentation is lower (15.98% (w/v)) than in the batch system (16.11% (w/v)). These correspond to respective fructose yields of 97.5% and 99.9%. One factor leading to a smaller fructose yield is that the kestoses level is still at 0.4% (w/v), and since kestoses are made of two units of fructose and one unit of glucose, this would account for a lower fructose yield. Another factor, which is purely an

hypothesis to explain the decrease in fructose yield is that when ethanol inhibition is decreased, and the biomass is then more active, then the biomass may start consuming fructose. This would mean that by removing more ethanol, more fructose would be consumed. This possibility will be examined later on with other experimental results, and remains a hypothesis at this point. Also, this effect would probably only be seen at certain ranges of ethanol concentration, and only to a certain degree. The fructose/glucose content at the end of the process is 89.4%/10.6%, which is suitable for the production of crystalline fructose.

Figure 5.8 shows the membrane performance as a function of fermentation time. As seen in figure 5.6, the actual ethanol concentration in the reactor increases. This means that the membrane does not remove all the ethanol produced. In this case, the ethanol varies from 1.6% (w/v) to 3.2% (w/v) from the time the pervaporation is 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. Figure 5.8 shows that the flux varied from 27 g/(m^2 h) to 20 g/(m^2 h) compared to fluxes that vary from 32 g/(m^2 h) to 42 g/(m^2 h) with ethanol/water mixtures. One reason why the flux was lower in the case of fermentation/pervaporation is the presence of biomass, which decreased the flux by about 12% at high biomass concentrations as shown in Figure 5.3. As the reaction proceeds, the concentration of biomass in the reactor increases, and therefore more cells deposit on the membrane surface, which causes the flux to decrease with time. Another factor explaining why the flux was lower than expected by the pervaporation tests is the presence of sugars in the broth, which, as reported by Wood et al. (1994), has the effect of decreasing the total flux.

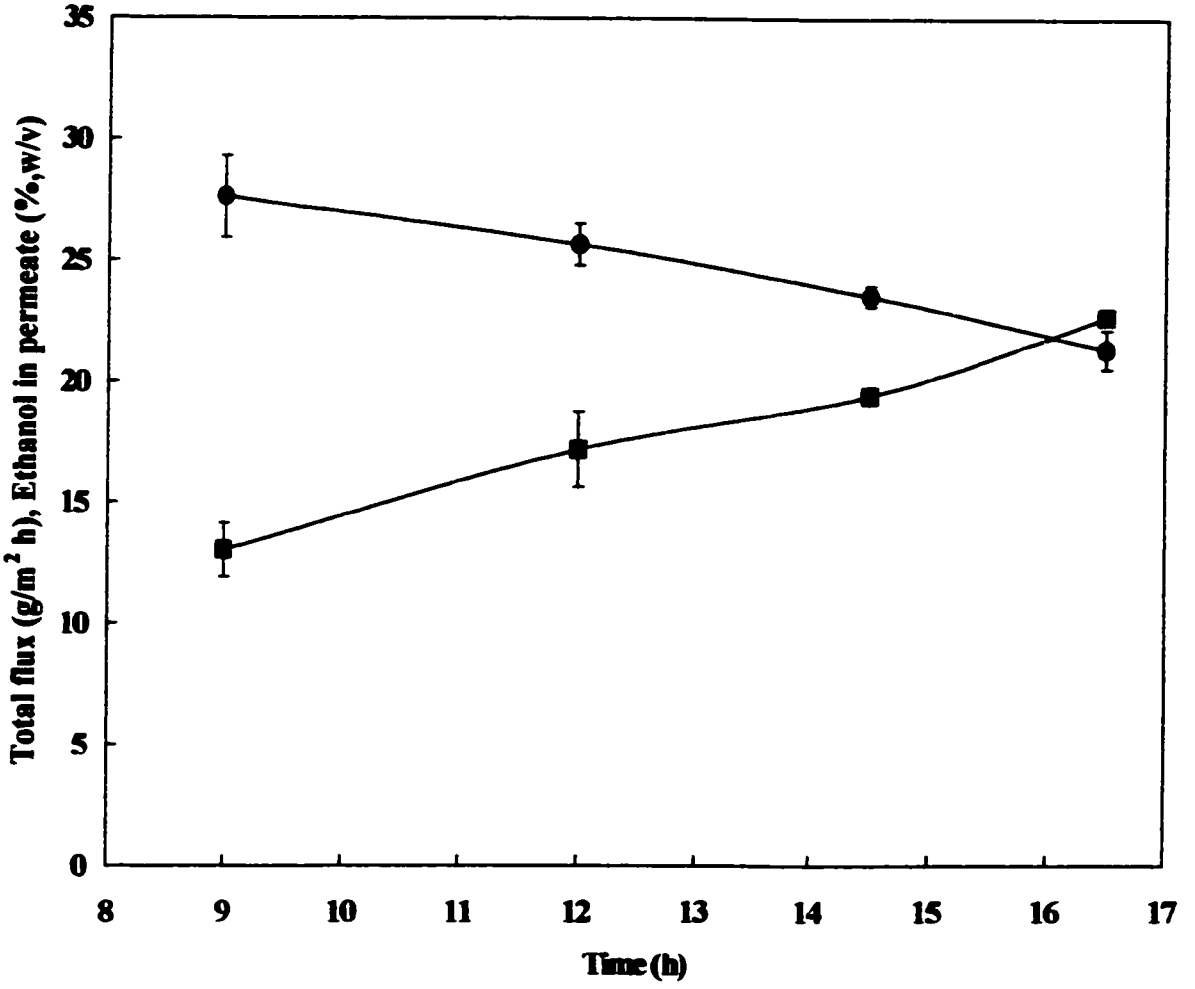


Figure 5.8 Membrane performance of the laboratory pervaporation module coupled to a bioreactor. Silicone rubber tubing was used for broth circulation, and membrane was initiated after 6 hours of batch fermentation. (●) Total Flux; (■) [EtOH] permeate.

The concentration of ethanol in the permeate shown in Figure 5.8 increased from 13% (w/v) to 22% (w/v). This trend is the same as observed during the pervaporation tests looking at the effect of ethanol in the feed (Figure 5.1). Since ethanol concentration in the reactor increases with time, it can therefore be expect that ethanol in the permeate will also increase with time. From the tests of ethanol/water mixtures, when the feed ethanol concentration was increased from 1.6% (w/v) to 3.5% (w/v), the permeate ethanol concentration increased from 12% (w/v) to 25% (w/v). In the case of fermentation/pervaporation I, the feed range was approximately 0.6% (w/v) to 3.2%(w/v). Therefore, the results from fermentation/pervaporation are comparable to pervaporation tests performed with ethanol/water mixtures. The small difference is in the selectivity and the ethanol permeate concentration, which are higher in fermentation conditions. This is because of the presence of biomass and sugars, which have been shown to provoke a similar effect in Figure 5.3. Wood et al. (1994) also observed that effect.

When looking at fermentation/pervaporation literature, most studies have been done using continuous fermentation systems, as opposed to the batch system used in the present study. And, most of those studies do not report results from the point of view of fermentation; they only report pervaporation results. One prior study will be summarized shortly here for the purpose of comparison in trends observed. Nakao et al. (1987) performed continuous fermentation/pervaporation of glucose into ethanol by *S. cerevisiae*. Their membrane module was made of PTFE. When coupled to a pervaporation unit, the fermentation performance was improved: the ethanol productivity increased from 1.6 g/(L h) to 4.9 g/(L h), the ethanol concentration in the broth and in the permeate were respectively 2.4% (w/w), and 18% (w/w) when fermentation was coupled to pervaporation compared to 6.5% (w/w) in the broth during continuous fermentation without ethanol separation. As was presented with the results of this study, Nakao et al. observed an increase in the ethanol concentration in the permeate with time before the system reached steady state. They also observed an approximate constant flux with time.

In conclusion, the advantage of using the coupled fermentation/pervaporation I system is that less time is required to reduce the glucose content to a 1.8% (w/v) concentration. A gain of 10.5 hours was observed without compromising the ethanol and fructose yields, which have respective values of 75.3% and 97.5%. No blocking of the membrane fibres was observed (the flow rate was never zero), but a small decrease in flux was observed with time (fouling to the deposit of biomass cells on the membrane surface). The biomass yields and specific growth rate remained unchanged in the coupled system compared to the batch system. The membrane behaved in the same trends as observed with ethanol/water mixtures, only with slightly different values.

5.4.2 Batch Fermentation/Pervaporation II

The second set of experiments was conducted using the laboratory membrane module under the following operating conditions: an initial reactor volume of 150 mL, an initial sucrose concentration of 31% (w/v), an initial biomass concentration of 3.1g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation, and Pharmed tubing for broth circulation in the membrane module. The system was initially kept in batch operation for 6 hours, and then pervaporation was started. Figure 5.9 shows the actual reactor data as a function of time, and Figure 5.10 shows the normalized data as a function of time. Table 5.6 shows the fermentation parameters used for comparison.

Table 5.6 Fermentation/Pervaporation II parameters.

	Fermentation/Pervaporation II
Initial Biomass Concentration (g/L)	3.10
Final Biomass Concentration (g/L)	9.86
Initial Sucrose Concentration (%,w/v)	31.10
Fermentation Time (h)	16.5
Final Glucose Concentration (%,w/v)	2.10
Final Fructose Concentration (%,w/v)	15.71
Final Ethanol Concentration (%,w/v)	5.92
μ (h^{-1})	0.08
Biomass Yield (g/g)	0.35
P (g/(L h))	3.59
Ethanol Yield (%)	78.6
Fructose Yield (%)	96.4
Fructose/Glucose Content (%)	88.2/11.8
Sucrose Hydrolysis Rate (g/(L h))	21.99

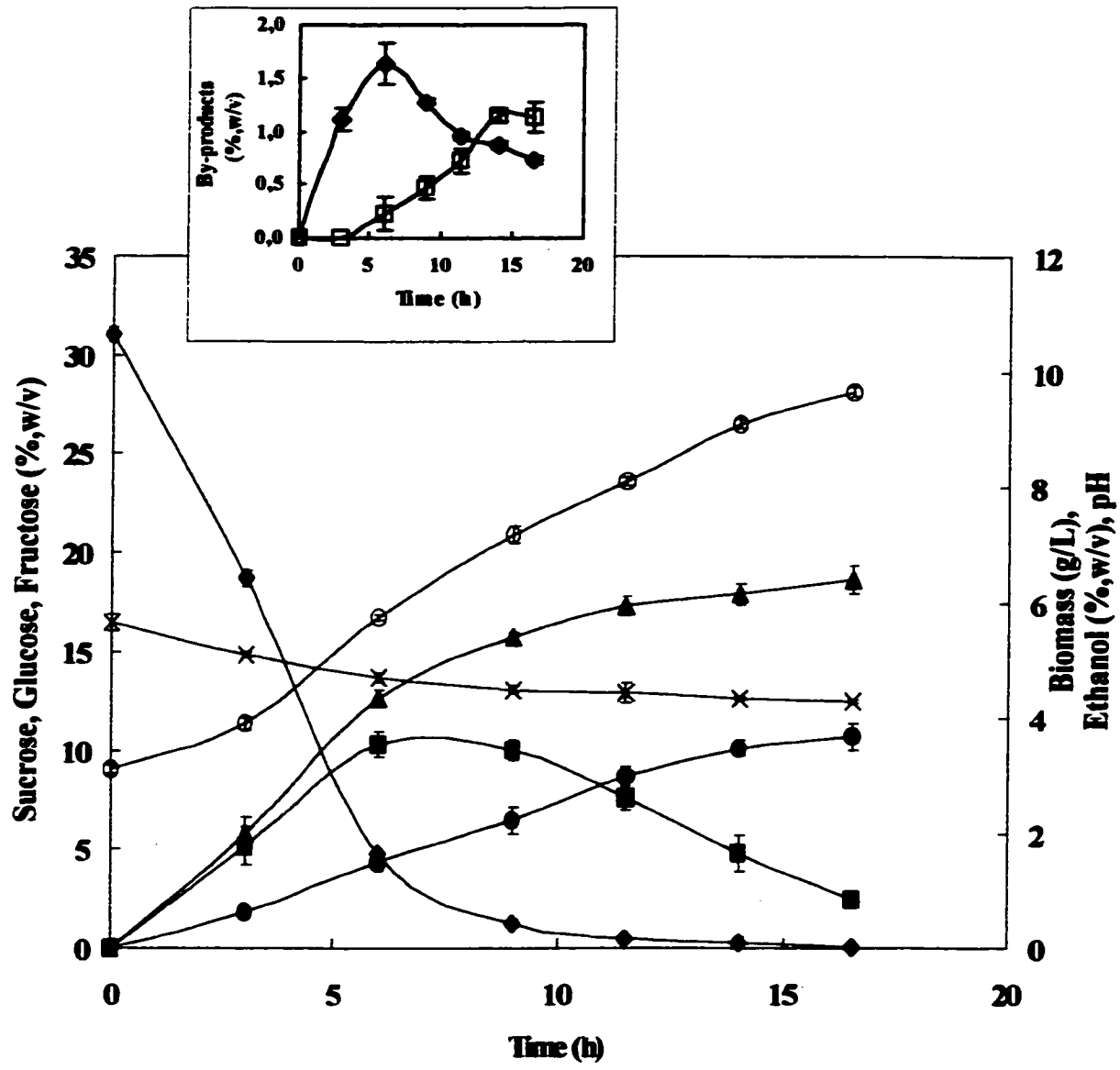


Figure 5.9 Actual reactor data of batch fermentation/pervaporation using the laboratory pervaporation module. Initial sucrose concentration, 31%w/v; initial biomass concentration, 3.1 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 6 hours of batch fermentation; Pharmed tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

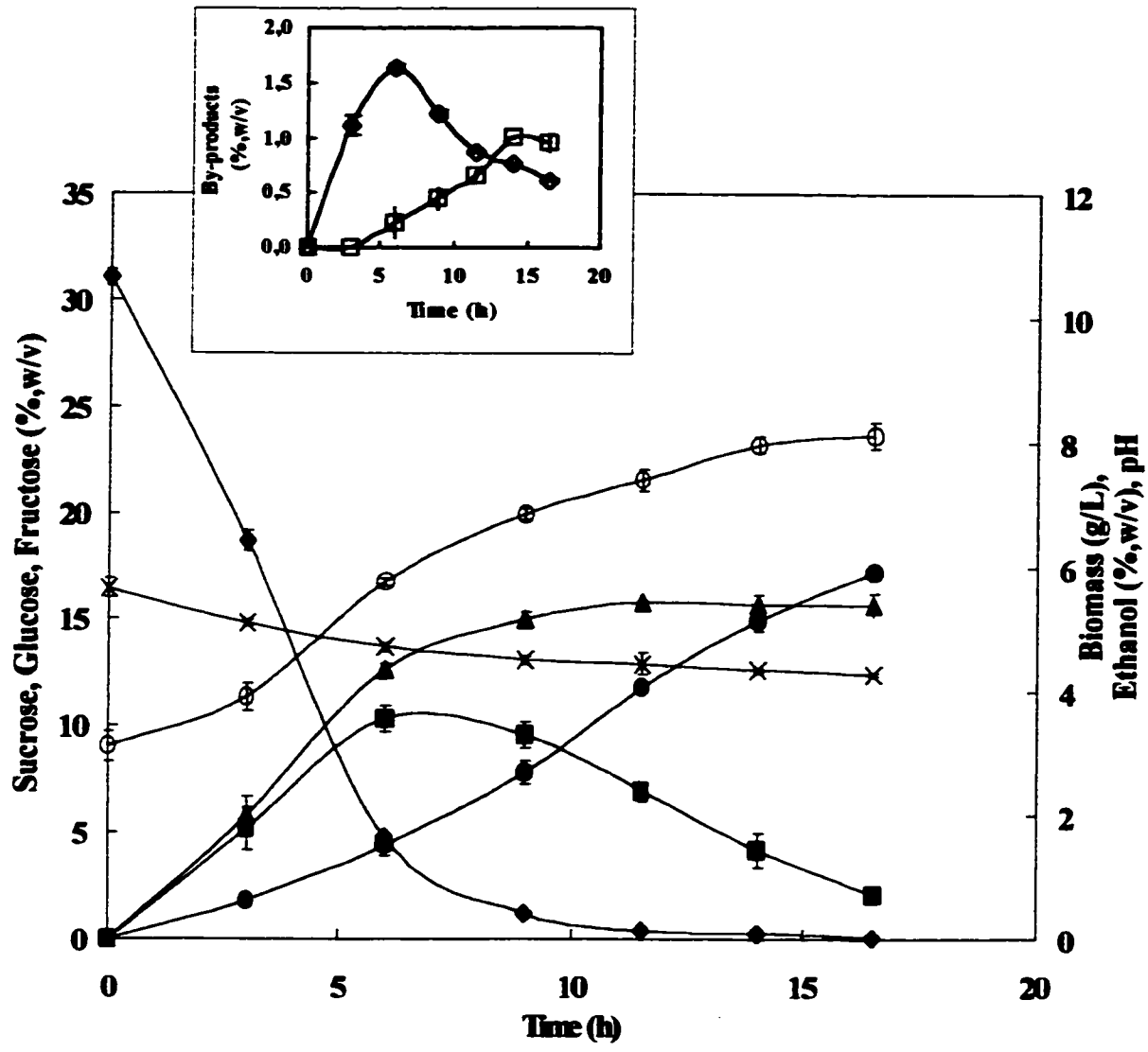


Figure 5.10 Normalized reactor data of batch fermentation/pervaporation II using the laboratory pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.1 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 6 hours of batch fermentation; Pharmed tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

Since the only difference in batch fermentation/pervaporation I and II was the tubing used for broth circulation, all parameters are expected to be approximately the same as in fermentation/pervaporation I, except the parameters related to ethanol and the final glucose concentration. The actual ethanol concentration in the reactor is 3.6% (w/v) at the end of the fermentation, compared to 3.2% (w/v) when silicone rubber tubing was used. The normalized ethanol concentration was 5.92% (w/v) with Pharmed tubing compared to 5.72% (w/v) when silicone rubber tubing was used and the ethanol yield increased to 79% compared to the previously observed 75%. Since more ethanol was produced in the same amount of time, the ethanol productivity increased from 3.47g/(L h) to 3.59g/(L h) by changing the tubing. The final normalized glucose concentration in the reactor is 2.1% (w/v) compared to 1.9% (w/v). The glucose is higher in the case of Pharmed tubing because more ethanol was present in the reactor and therefore the inhibition on the yeast was slightly stronger, and a bit less glucose was consumed. The sucrose hydrolysis rate and the biomass related parameters are almost identical in both cases, as well as the fructose/glucose content, which is near the 90%/10% range at the end of fermentation. Since only the change in tubing made it possible to increase ethanol yield, the assumption that ethanol was diffusing through the silicone rubber tubing was verified, and from then on, only Pharmed tubing was used for further experiments.

As for the pervaporation results shown in Figure 5.11, the same trends as previously described (Figure 5.8) were observed, and approximately the same amount of ethanol was removed in each set of experiments, but as already seen, more ethanol was produced using Pharmed tubing by consuming less glucose. The flux still decreased with time from 24g/(m^2 h) to 18g/(m^2 h), and the ethanol concentration in the permeate still increased from 12% (w/v) to 23% (w/v) for the same reasons as described previously. The flux range in this case is somewhat lower compared to the 27-20g/m^2 h range observed when the set-up was with silicone rubber tubing. This has nothing to do with the type of tubing used, it is rather caused by the fouling of the membrane at this point. Insufficient cleaning of the membrane after using it for a long time may have caused this decrease in flux. Between each run, the membrane was cleaned with 80% (v/v) ethanol solution to ensure no more biomass was left active to reproduce inside the hollow fibres.

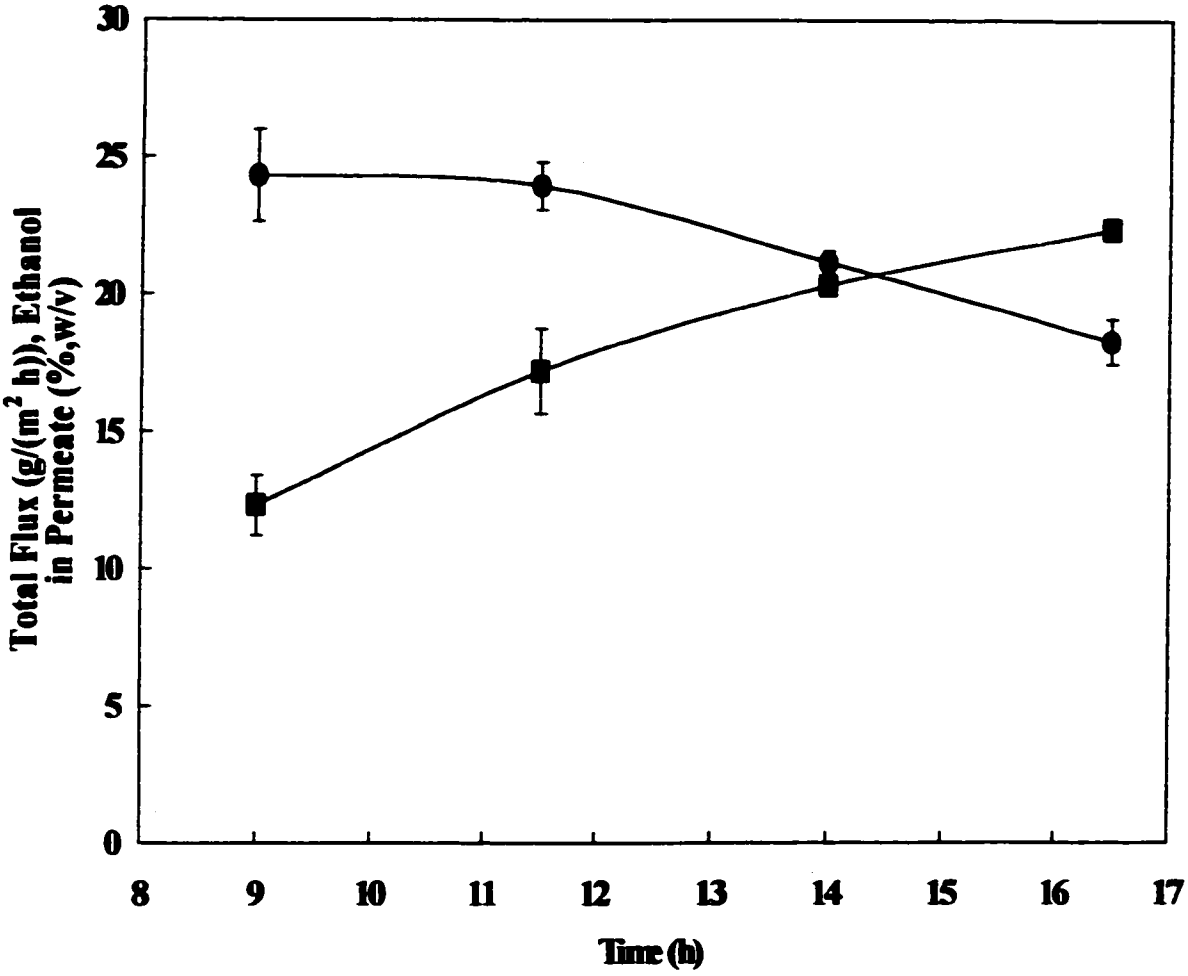


Figure 5.11 Membrane performance of the laboratory pervaporation module coupled to a bioreactor. Pharmed tubing was used for broth circulation, and membrane was initiated after 6 hours of batch fermentation. (●) Total Flux; (■) [EtOH] permeate.

If the membrane was not flushed thoroughly, there may have been biomass left on the membrane surface (not necessarily active), therefore decreasing the flux. This type of fouling was observed after about 10 runs, but if a more efficient method of cleaning the membrane would be used, this might not be observed at all.

In conclusion, these experiments have shown that if silicone rubber tubing is used for broth circulation, some ethanol will permeate through this tubing, and the ethanol yield will be lower than expected. Pharmed tubing, on the other hand, is known to be less permeable to ethanol and other volatile organics (VWR Canlab Catalog), and when this tubing is used, the ethanol yield will be improved.

As mentioned in section 5.1, after about 10 runs, the laboratory module was breaking apart and it became impossible to repair. In order to continue the study, a commercial module was purchased and an identical set of runs as described in fermentation/pervaporation II was done to compare the performance of the two modules.

5.4.3 Batch Fermentation/Pervaporation III

The third set of experiments was conducted using the commercial membrane module under the following conditions: an initial reactor volume of 150 mL, an initial sucrose concentration of 31% (w/v), an initial biomass concentration of 3.28 g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation, and Pharmed tubing for broth circulation in the membrane module. The system was initially kept in batch operation for 6 hours, and then pervaporation was started. Figure 5.12 shows the actual reactor data as a function of time, and Figure 5.13 shows the normalized data as a function of time. Table 5.7 shows the fermentation parameters used for comparison with the runs done using the laboratory module.

Table 5.7 Fermentation/Pervaporation III parameters.

	Fermentation/Pervaporation III
Initial Biomass Concentration (g/L)	3.28
Final Biomass Concentration (g/L)	9.66
Initial Sucrose Concentration (%,w/v)	31.10
Fermentation Time (h)	16.5
Final Glucose Concentration (%,w/v)	1.40
Final Fructose Concentration (%,w/v)	15.76
Final Ethanol Concentration (%,w/v)	6.28
μ (h^{-1})	0.08
Biomass Yield (g/g)	0.32
P (g/(L h))	3.81
Ethanol Yield (%)	79.5
Fructose Yield (%)	96.5
Fructose/Glucose Content (%)	91.8/8.2
Sucrose Hydrolysis Rate (g/(L h))	21.97

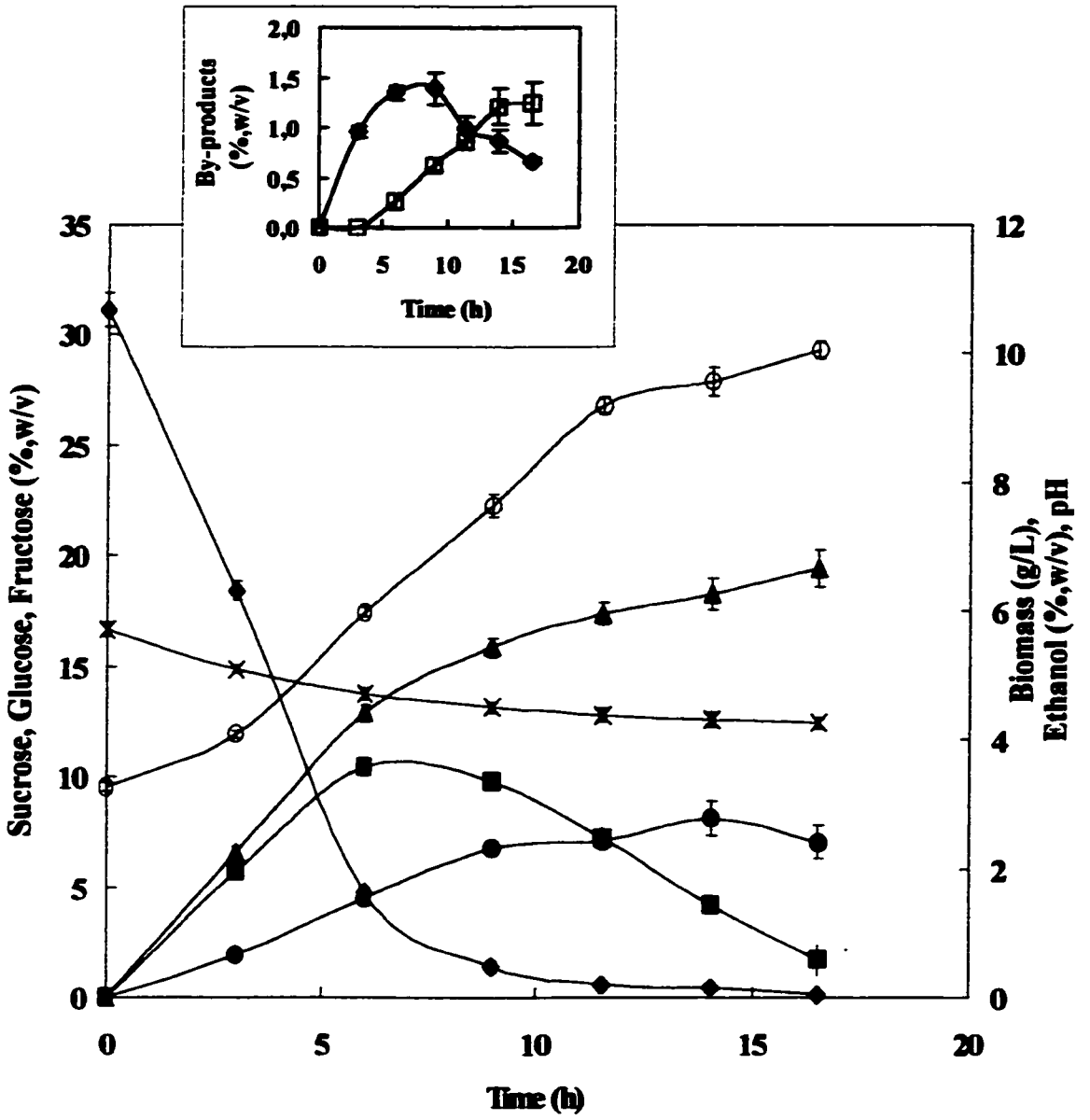


Figure 5.12 Actual reactor data of batch fermentation/pervaporation using the commercial pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.1 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 6 hours of batch fermentation; Pharmed tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

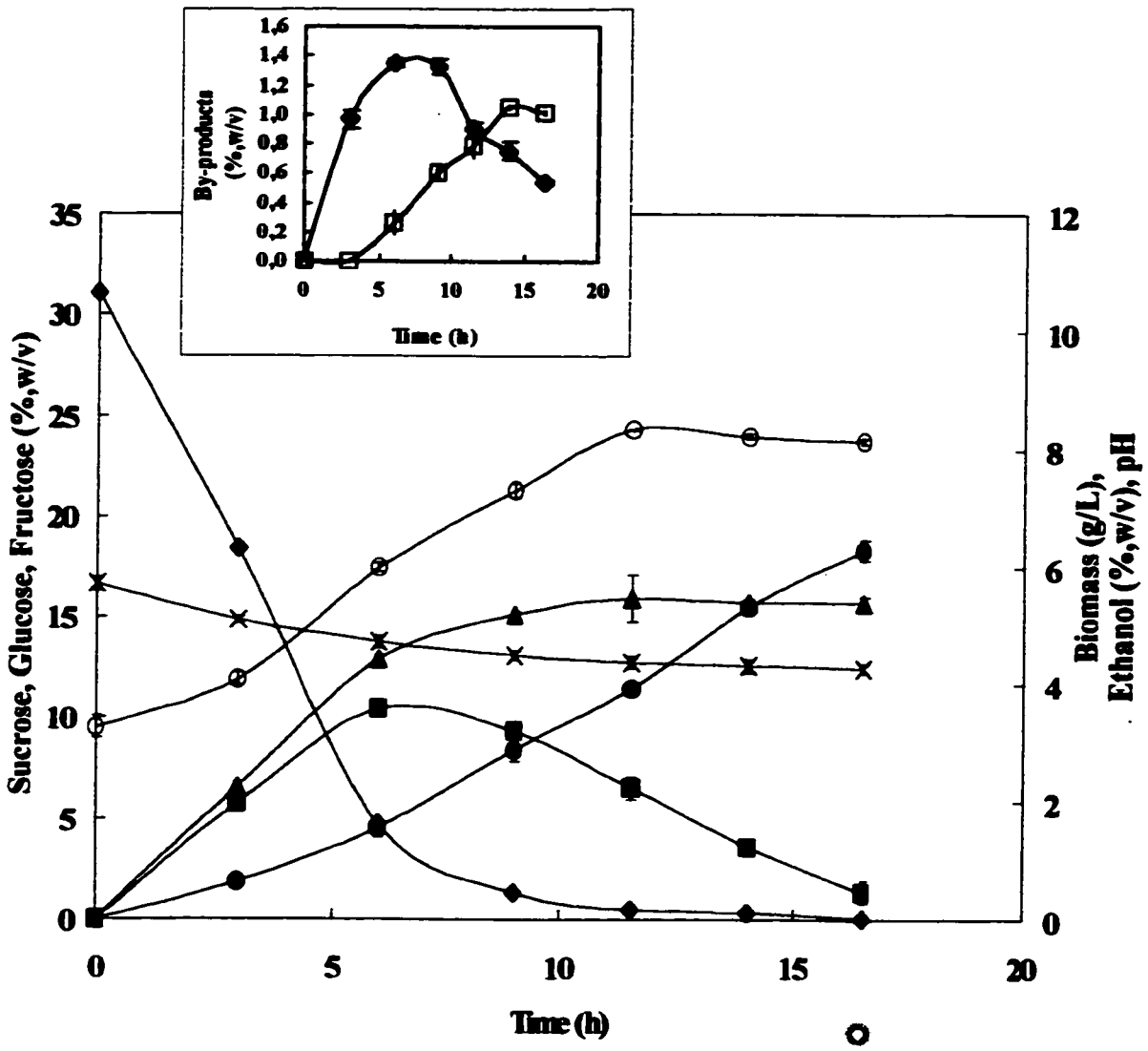


Figure 5.13 Normalized reactor data of batch fermentation/pervaporation using the commercial pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.1 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 6 hours of batch fermentation; Pharmed tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

This set of experiments will be compared to fermentation/pervaporation II since the only difference between these two experiments is the module used for pervaporation. The first thing to point out is that after the same amount of time (16.5 hours), the normalized final glucose concentration is lower when the commercial module is used with a value of 1.4% (w/v) compared to a 2.1% (w/v) concentration with the laboratory module. 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 is shown by the actual ethanol concentration in the reactor, which is 2.4% (w/v) instead of 3.6% (w/v) with the laboratory module. Therefore, the commercial module removed more ethanol during the same period; and since ethanol concentration in the reactor was kept at a lower level, less inhibition on the yeast occurred. Since the commercial module removed a larger quantity of ethanol, the actual reactor ethanol concentration remained lower, which allowed the yeast to use more glucose for the production of ethanol. Therefore, more ethanol was produced, and ethanol yield and productivity were higher with respective values of 79.5% and 3.81g/L h compared to 78.6% yield and 3.59g/L h productivity in fermentation/pervaporation II where the laboratory module was used. The biggest advantage is that since more ethanol is removed, more glucose is consumed, and therefore the final fructose/glucose content reached 92%/8%, which is sufficient for crystallization. Biomass parameters (biomass specific growth rate and biomass yield) are the same in both cases as well as sucrose hydrolysis rate. And by-products levels are still very low at values of 1 % (w/v) for glycerol and 0.5% (w/v) for kestoses.

As for the pervaporation results shown in Figure 5.14, the same trends as previously described are observed. More ethanol was removed with the commercial module. This was shown by the actual ethanol concentration in the reactor, which was kept lower during the experiments. 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 with the laboratory module. The flux still decreased with time, but from 28g/(m^2 h) to 24g/(m^2 h), and the ethanol concentration in the permeate was still increasing, but from

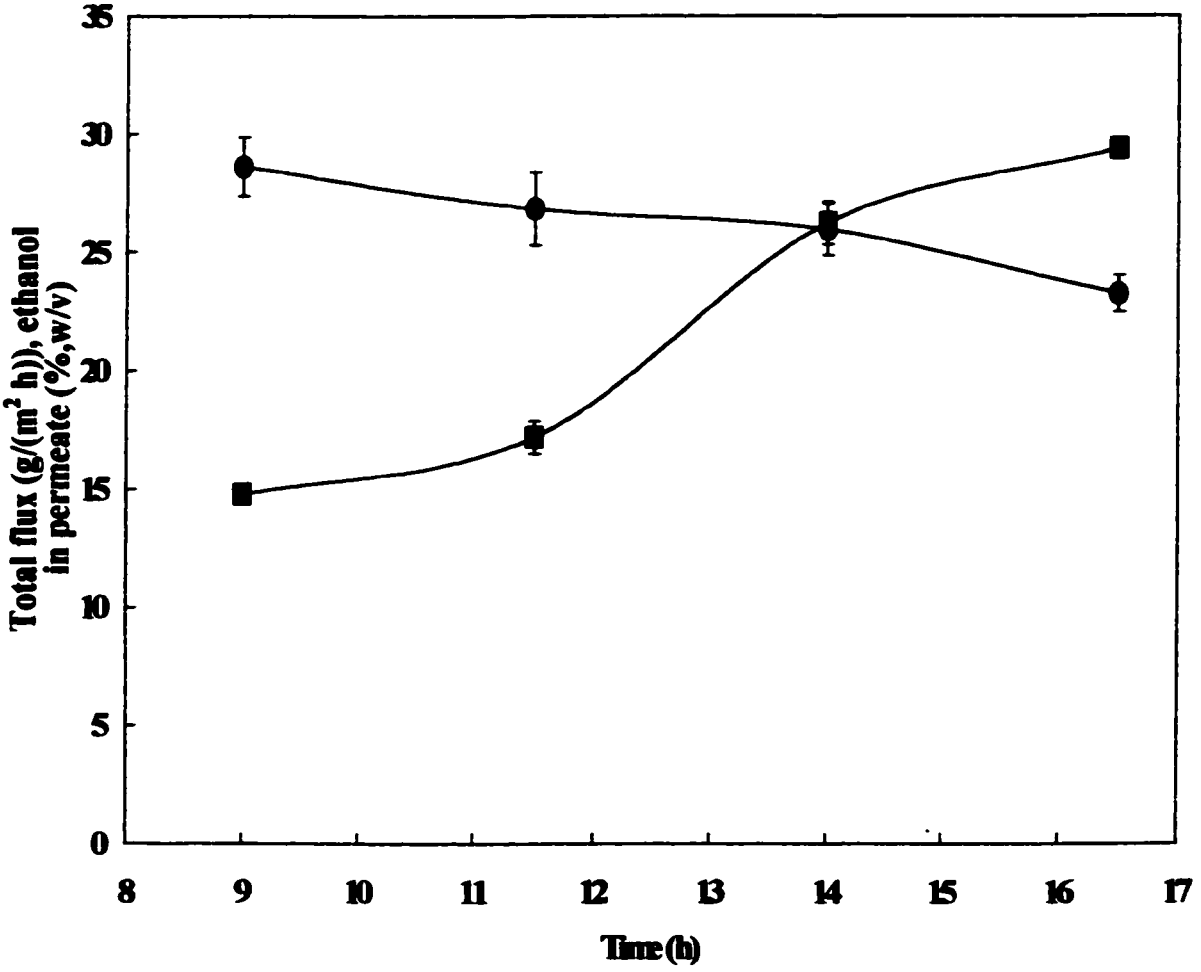


Figure 5.14 Membrane performance of the commercial pervaporation module coupled to a bioreactor. Pharmed tubing was used for broth circulation, and membrane was initiated after 6 hours of batch fermentation. (●) Total Flux; (■) [EtOH] permeate.

15% (w/v) to 30% (w/v) for the same reasons as described previously. It is possible that the actual flux from 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. The commercial module was used for the remainder of the study (about 10 runs) and no leak was ever observed, and the glue withstood the vacuum very well.

In conclusion, the commercial module removes a larger amount of ethanol in the same reaction period, leading to a further decrease in ethanol inhibition. As a result, the final glucose concentration was lowered, producing syrups of higher fructose content. The set of experiments which will be presented next were performed with exactly the same conditions as just described here, with fermentation/pervaporation III, except that the membrane was started after three hours of batch fermentation instead of six hours.

5.4.4 Batch Fermentation/Pervaporation IV

The fourth set of experiments was conducted using the commercial membrane module under the following operating conditions: an initial reactor volume of 150 mL, an initial sucrose concentration of 31% (w/v), an initial biomass concentration of 3.3 g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation, and Pharmed tubing for broth circulation in the membrane module. The system was initially kept in batch mode operation for 3 hours, and then pervaporation was started. Figure 5.15 shows the actual reactor data as a function of time, and figure 5.16 shows the normalized data as a function of time. Table 5.8 shows the fermentation parameters used for comparison.

Table 5.8 Fermentation/Pervaporation IV parameters.

	Fermentation/Pervaporation IV
Initial Biomass Concentration (g/L)	3.32
Final Biomass Concentration (g/L)	10.90
Initial Sucrose Concentration (%,w/v)	31.10
Fermentation Time (h)	16.5
Final Glucose Concentration (%,w/v)	1.15
Final Fructose Concentration (%,w/v)	14.48
Final Ethanol Concentration (%,w/v)	7.23
μ (h^{-1})	0.08
Biomass Yield (g/g)	0.36
P (g/(L h))	4.43
Ethanol Yield (%)	82.84
Fructose Yield (%)	88.45
Fructose/Glucose Content (%)	92.8/7.2
Sucrose Hydrolysis Rate (g/(L h))	24.69

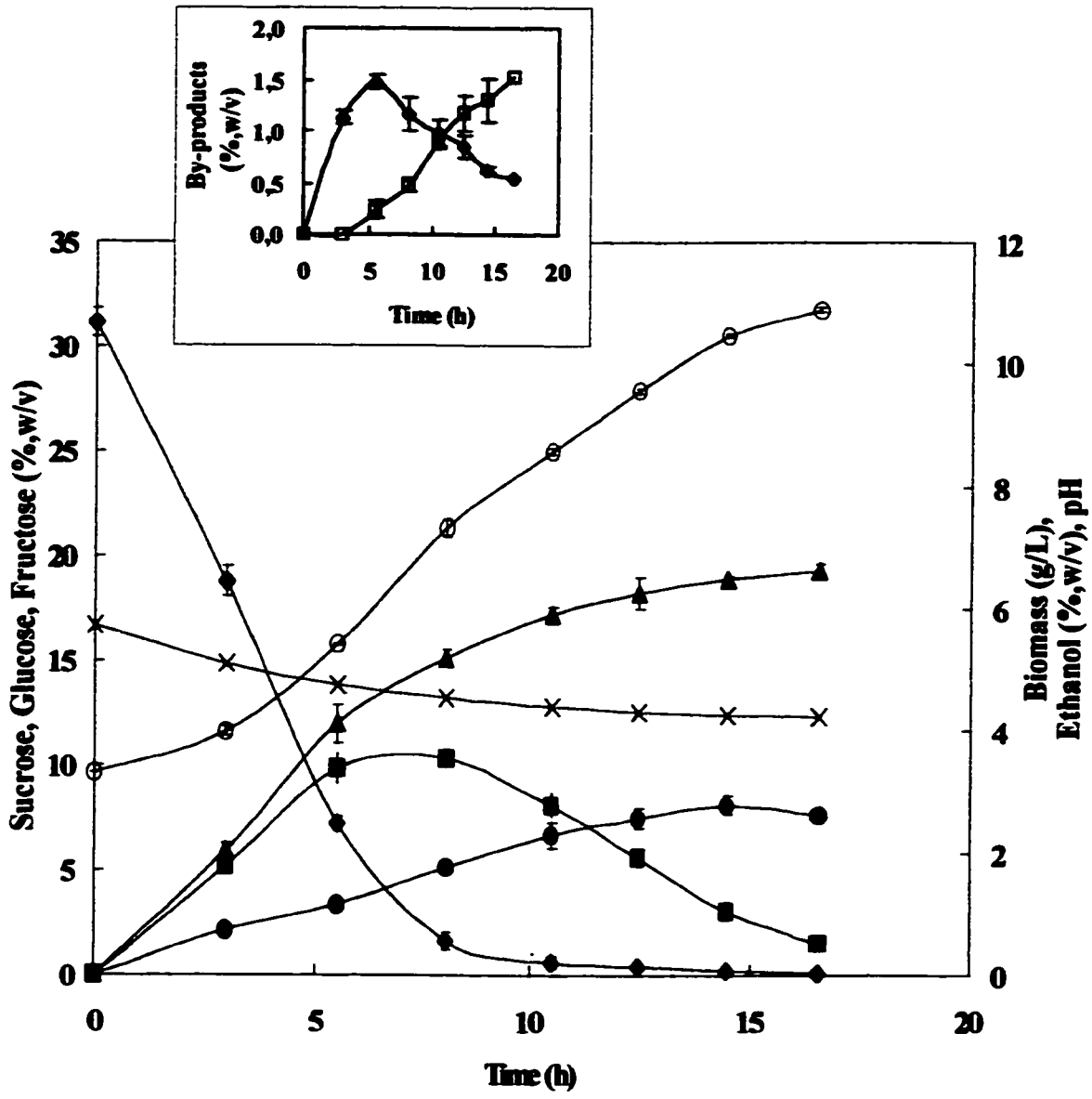


Figure 5.15 Actual reactor data of batch fermentation/pervaporation using the commercial pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L, temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 3 hours of batch fermentation; Pharmed tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (O) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

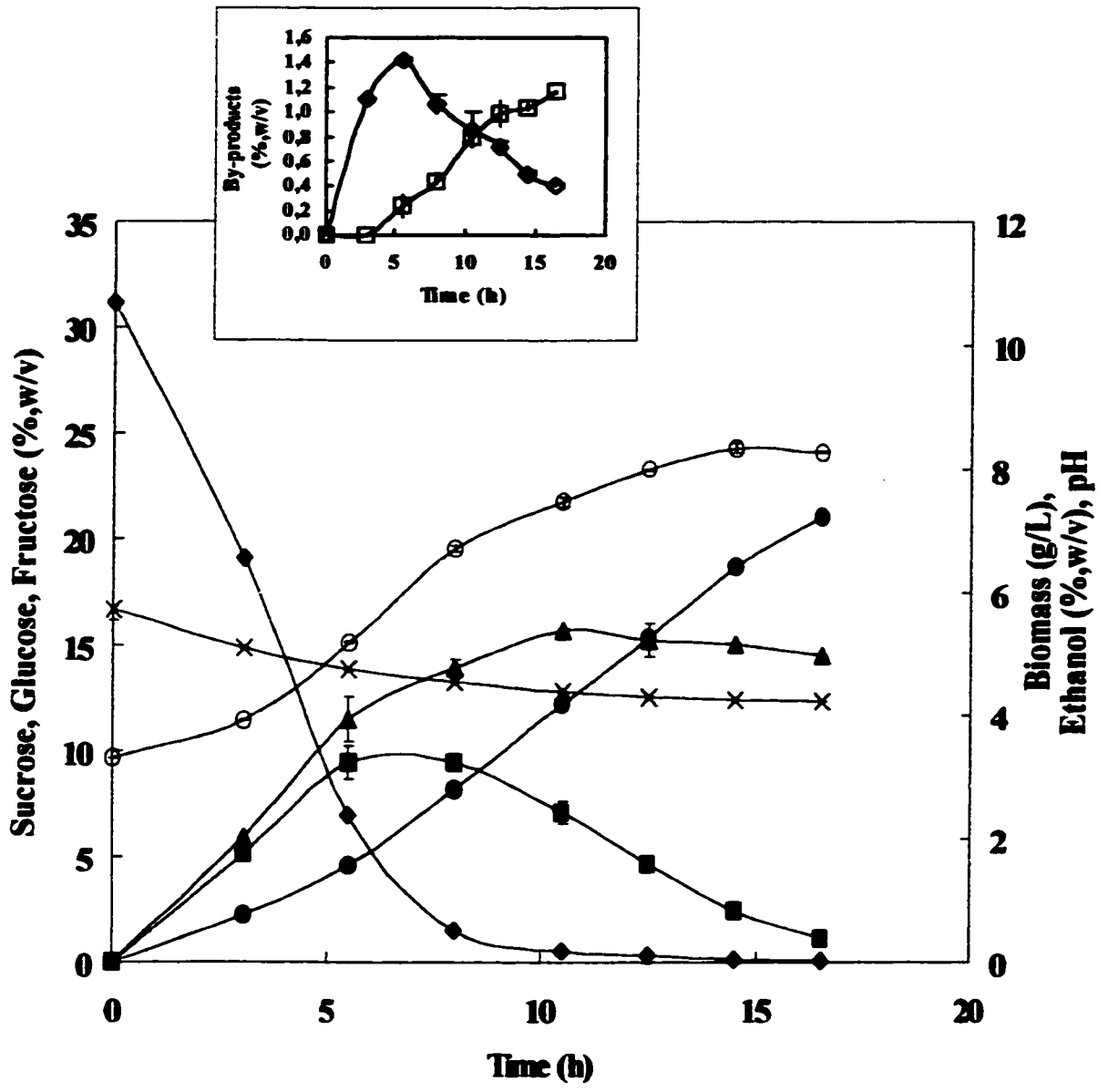


Figure 5.16 Normalized reactor data of batch fermentation/pervaporation using the commercial pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 3 hours of batch fermentation; Pharmed tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol.

This set of experiments will be compared to fermentation/pervaporation III since the only difference between fermentation/pervaporation III and IV is the length of the batch mode operation after which pervaporation was started. Comparing Tables 5.7 and 5.8 a large decrease in fructose yield, which is now 88% compared to 96%, is immediately noticed. Therefore, more fructose has been converted to ethanol, and this can be reflected by the final normalized ethanol concentration, which is 7.2% (w/v) compared to a 6.3% (w/v) concentration. Even though more ethanol has been produced, the final actual ethanol concentration is about the same in both cases at a 2.6% (w/v) concentration. When pervaporation was initiated after 3 hours of batch fermentation, the ethanol was being removed earlier in the process. Since the fermentation was allowed to proceed for the same amount of time, which was 16.5 hours, this means that the yeast was subjected to pervaporation for a longer period. In fermentation/pervaporation III and IV, approximately the same amount of glucose was consumed; i.e. when pervaporation was initiated after 3 hours, the final glucose concentration was 1.1% (w/v) compared to 1.4% (w/v) when pervaporation was initiated after 6 hours of batch fermentation. The actual ethanol concentrations at the time when pervaporation was initiated were 0.6% (w/v) after 3 hours of batch, and 1.6% (w/v) after 6 hours of batch. It was lower in fermentation/pervaporation IV, which was expected since after 3 hours, the yeast produced less ethanol during the batch phase. Then, the actual ethanol concentration in the broth reached a point where it was the same in both cases (2.6 %(w/v)), and this was the point when the fructose yield started to decrease drastically. Therefore, if the yeast is subjected to pervaporation for a long period of time at low ethanol inhibition level, fructose will be consumed according to the previously described hypothesis, which remains just that. This hypothesis would need to be verified with specific experiments. If the fermentation had been stopped after 14.5 hours, then the fructose yield would have been 92%, and the ethanol yield 82% (calculated on sugar consumption at this time point of the process). If the above hypothesis is correct, fermentation should not be coupled with pervaporation for a too long period unless the ethanol concentration in the reactor is at a beneficial level for the non-consumption of fructose. As for all the other parameters of comparison, they are all the same in both cases, which demonstrates that only fructose becomes affected by a long exposure to pervaporation process.

As for the pervaporation results shown in figure 5.17, the same trends as previously described are observed once again. More ethanol was removed when the membrane was initiated earlier in the process, but since more was also produced because of fructose conversion, the final actual ethanol concentration in both cases was the same. The flux decreased with time from 26g/(m^2 h) to 23g/(m^2 h), and the ethanol concentration in the permeate increased from 11% (w/v) to 29% (w/v) for the same reasons as described previously. Here the range of ethanol in the permeate is larger because when the membrane was initially started, the actual ethanol concentration in the reactor was only 0.6%w/v instead of 1.6%w/v after 6 hours. As seen previously (figure 5.1), the ethanol permeate concentration decreases with a decrease in feed ethanol concentration.

In conclusion, beginning the pervaporation after 3 hours instead of 6 hours would be beneficial time-wise because the process could be stopped earlier with only a small decrease in fructose yield. The process should not be allowed to continue any longer than 14.5 hours, because the loss in fructose yield is too great. An economical analysis would be needed to determine which sets of conditions are better for the overall process, but this is beyond the scope of the present study.

The following set of experiments was performed with exactly the same conditions as just described except that the membrane was stopped after 14.5 hours of batch fermentation instead of 16.5 hours, as suggested by the results just described. The advantage of decreasing ethanol inhibition is that more sugar can theoretically be processed in shorter time periods. To verify this, fed-batch experiments were performed, and this will be presented next.

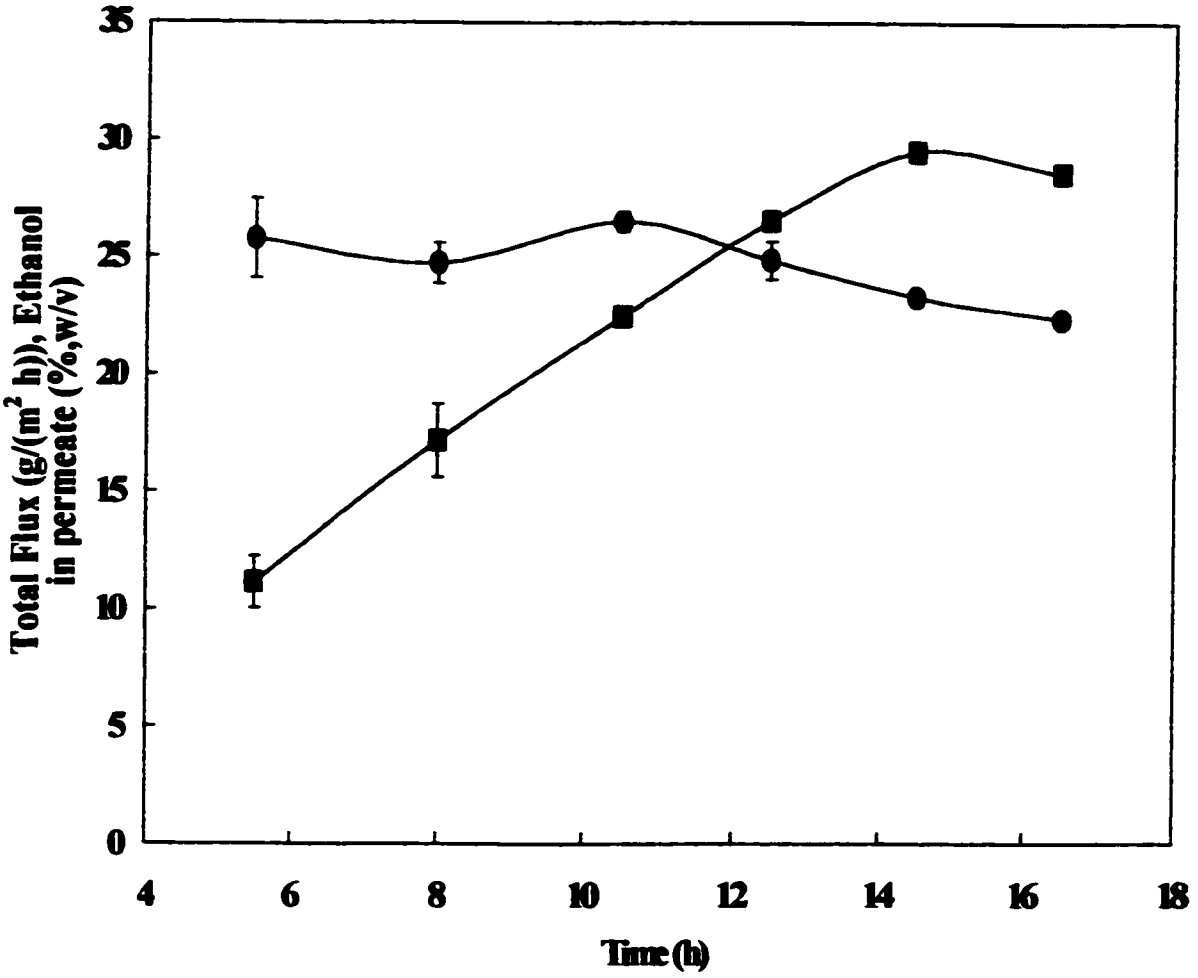


Figure 5.17 Membrane performance of the commercial pervaporation module coupled to a bioreactor. Pharmed tubing was used for broth circulation, and membrane was initiated after 3 hours of batch fermentation. (●) Total Flux; (■) [EtOH] permeate.

5.4.5 Fed-Batch Fermentation/Pervaporation

The fifth set of experiments was conducted using the commercial membrane module under the following conditions: an initial reactor volume of 150 mL, an initial sucrose concentration of 31% (w/v), an initial biomass concentration of 3.3 g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation, and Pharmed tubing for broth circulation in the membrane module. The system was initially kept in batch mode operation for 3 hours, and then pervaporation was started. After a total of 14.5 hours after inoculation, the pervaporation was stopped, and fresh concentrated sucrose solution was added to the reactor. The system was then left in batch mode for an additional 10 hours without membrane separation. The added sucrose plus the initial sucrose at time zero combined together correspond to a total sucrose concentration of 40% (w/v) to be processed by the yeast. Therefore, the comparison parameters presented were calculated based on a 40% (w/v) sucrose solution, even though the figures indicate a 31%w/v initial sucrose concentration. Therefore, the comparison of this fed-batch could be made with batch fermentation with a 40% (w/v) initial sucrose concentration. Figure 5.18 shows the actual reactor data as a function of time, and Figure 5.19 shows the normalized data as a function of time. Table 5.9 shows the fermentation parameters used for comparison.

Before looking at the parameters in Table 5.9, which were determined at the 24.5 hour point, it is worthwhile to look at the fructose yield and ethanol yield at the time when the pervaporation was stopped, before the sucrose was added. At this point, the yields were 79% ethanol and 91% fructose (calculated based on sugar consumption at the point 14.5 hours before fresh sucrose was added), as expected from the results of fermentation/pervaporation IV.

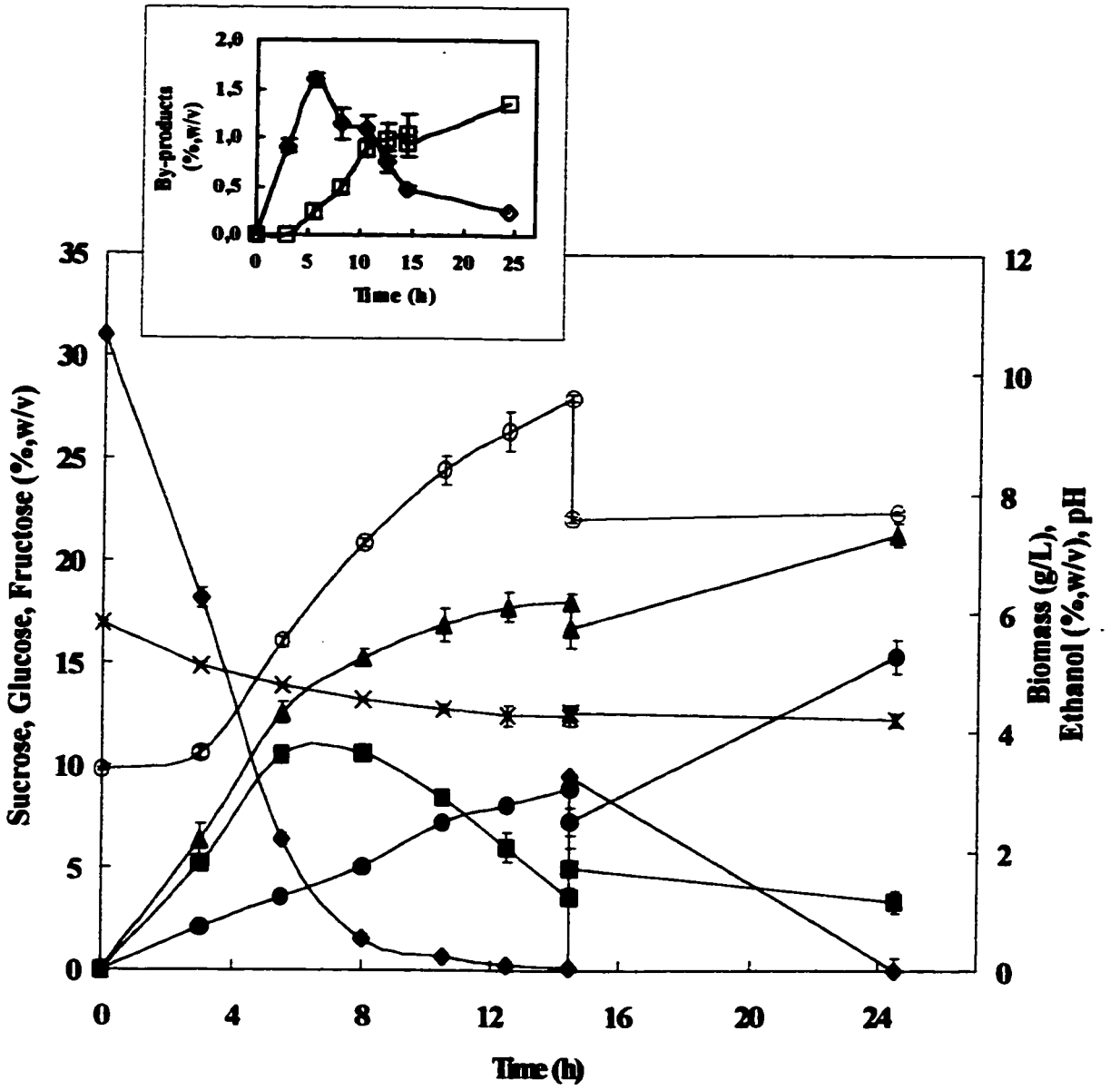


Figure 5.18 Actual reactor data of fed-batch fermentation/pervaporation using the commercial pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 3 hours of batch fermentation; Pharmed tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol

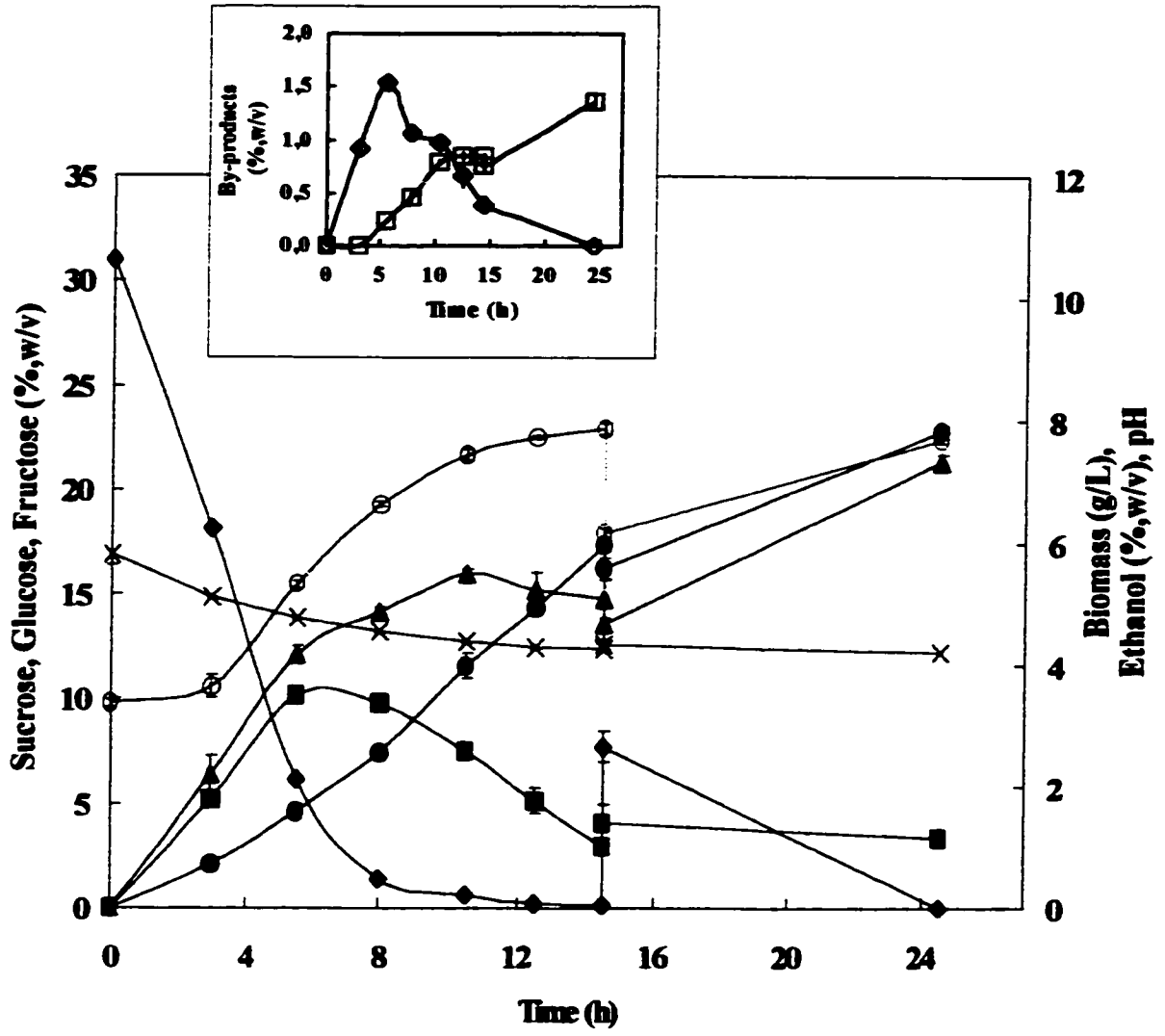


Figure 5.19 Normalized reactor data of fed-batch fermentation/pervaporation using the commercial pervaporation module. Initial sucrose concentration, 31% (w/v); initial biomass concentration, 3.3 g/L; temperature, 33°C; initial volume, 150 mL; pervaporation initiated after 3 hours of batch fermentation; Pharmed tubing used for broth circulation. (◆) Sucrose; (■) Glucose; (▲) Fructose; (●) Ethanol; (○) Biomass; (×) pH; (◇) Kestoses; (□) Glycerol

Table 5.9 Fed-Batch Fermentation/Pervaporation parameters.

	Fed-Batch Fermentation/Pervaporation
Initial Biomass Concentration (g/L)	3.36
Final Biomass Concentration (g/L)	7.69
Initial Sucrose Concentration (%,w/v)	31.00
Fermentation Time (h)	24.5
Final Glucose Concentration (%,w/v)	3.38
Final Fructose Concentration (%,w/v)	21.38
Final Ethanol Concentration (%,w/v)	7.84
μ (h^{-1})	0.06
Biomass Yield (g/g)	0.34
P (g/(L h))	3.20
Ethanol Yield (%)	82.75
Fructose Yield (%)	98.67
Fructose/Glucose Content (%)	86.4/13.6
Sucrose Hydrolysis Rate (g/(L h))	21.30

It can be seen from Figures 5.18, 5.19, and from Table 5.9, that in 24.5 hours, the yeast was able to process the equivalent of a 40% sucrose solution. Without attaching the hollow fibre module, this process would require well over 40 hours, so a gain of about 15.5 hours and over could be obtained. This shows that when the ethanol inhibition is decreased, the advantage of processing more sugars in the same time period than without hollow fibre module is gained. In this fed-batch fermentation, the normalized final fructose and glucose concentrations are 21% (w/v) and 3.4% (w/v), which gives a fructose/glucose content of 86%/14%. If the fermentation would have 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 hours, the actual ethanol

concentration in the reactor is up to 5% (w/v), a concentration at which ethanol inhibition is high. To further decrease the glucose content would take too much time for the advantage gained. Therefore, it would be best to restart pervaporation after 24.5 hours, or it would have been even better never to stop it after 14.5 hours, after the addition of sucrose. All other parameters are in the same ranges as observed with other experiments. It can also be noticed in Figure 5.19 that the normalized concentration of kestoses is 0.05 % (w/v) and that of glycerol is 1.4 % (w/v). Thus, the concentration of by-products is slightly higher than other modes of operation.

As for the pervaporation results shown in Figure 5.20, the same trends as previously described are observed. The flux still decreased with time and was in the range of 20-23g/(m^2 h), and the ethanol concentration in the permeate still increased from 12% (w/v) to 28% (w/v) for the same reasons as described previously. The flux range, in this case, was somewhat lower compared to the 28-24g/(m^2 h) range observed before, when the commercial module was used for the first time. Insufficient cleaning of the membrane, after a long time period of time, may have caused this flux decline, as explained earlier.

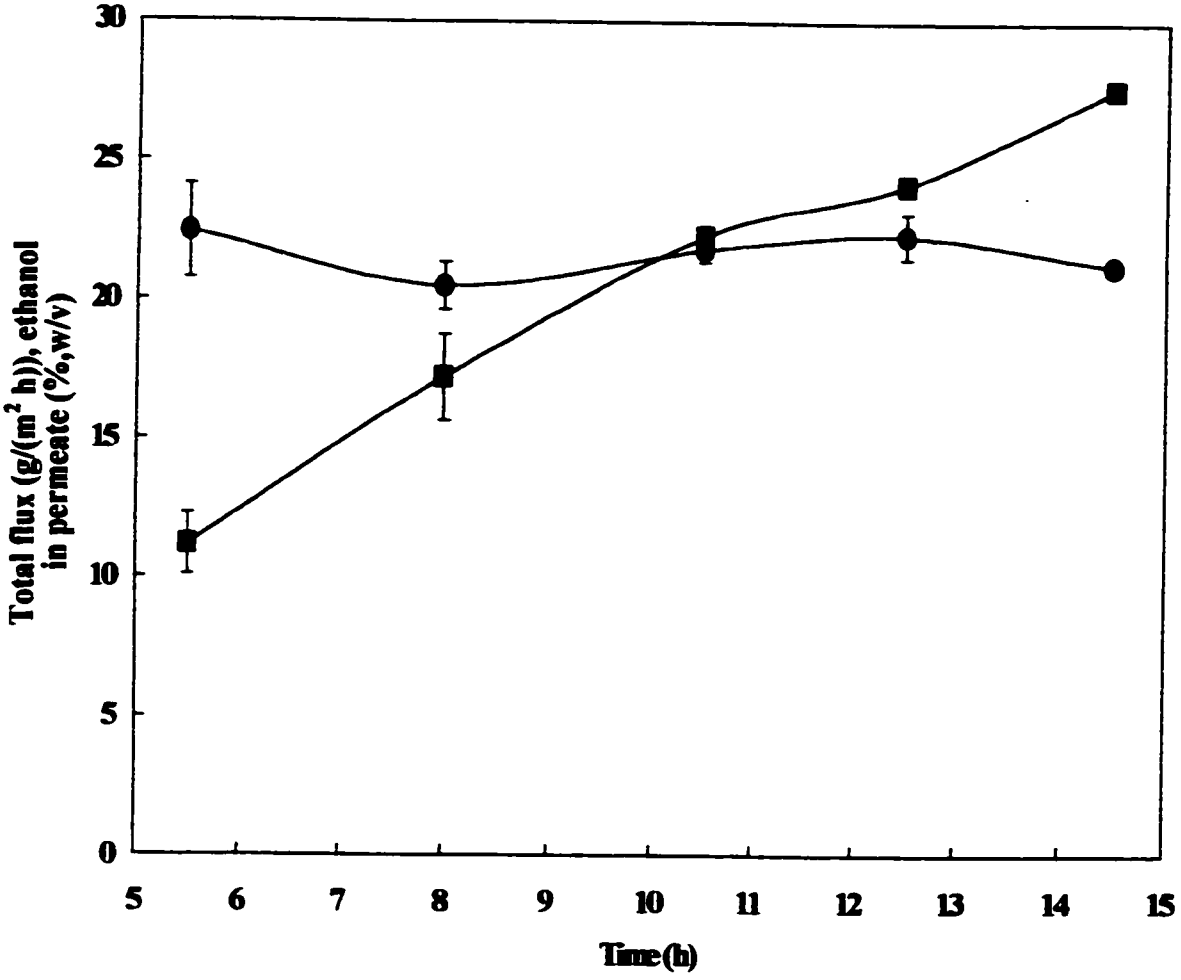


Figure 5.20 Membrane performance of the commercial pervaporation module coupled to a bioreactor in fed-batch mode. Pharmed tubing was used for broth circulation, and membrane was initiated after 3 hours of batch fermentation. (●) Total Flux; (■) [EtOH] permeate.

6. Conclusions

- Pervaporation tests performed with ethanol/water mixtures showed that, as the ethanol concentration in the feed increased, the total flux and the ethanol concentration in the permeate increased, but the selectivity decreased because of the swelling effect on the membrane.

- Pervaporation tests performed with ethanol/water mixtures showed that as the downstream pressure was increased (less vacuum is applied), the total flux decreased and the ethanol concentration in the permeate and the selectivity remained about constant, with a very slight decrease. This was also due to swelling of the membrane.

- Pervaporation tests performed with ethanol/water/biomass mixtures showed that in the presence of biomass, the total flux decreased, while the ethanol concentration in the permeate and the selectivity increased slightly. This was due to the biomass cells depositing on the membrane surface.

- Batch fermentation performed in a bioreactor produced a final broth with a fructose/glucose content of 91%/9% after 27 hours of fermentation. The fructose and ethanol yields were 78% and 99% respectively. The final ethanol concentration was 5.8% (w/v) and the ethanol productivity was 2.16g/L h.

- Batch fermentation/pervaporation performed with the laboratory module initiated after 6 hours of fermentation, using silicone rubber tubing for broth circulation (fermentation/pervaporation I) required 16.5 hours instead of 27 in batch fermentation, which is a gain of 10.5 hours. The biomass behaved in the same way as in batch alone, the major difference, besides the time requirement, was that the ethanol yield was lower even though the same amount of glucose had been consumed. This indicated that ethanol was lost somewhere in the process.

- Batch fermentation/pervaporation performed with the laboratory module initiated after 6 hours of fermentation, using Pharmed tubing for broth circulation (fermentation/pervaporation II) also required 16.5 hours instead of 27. The only difference observed was that with the Pharmed tubing, the ethanol yield was higher, indicating that the ethanol was evaporating through the silicone rubber tubing in the fermentation/pervaporation I experiment.

- Batch fermentation/pervaporation performed with the commercial module initiated after 6 hours of fermentation, using Pharmed tubing for broth circulation (fermentation/pervaporation III) also required 16.5 hours instead of 27 in batch fermentation. The major difference was that the commercial module removed more ethanol, and therefore more glucose was consumed to produce a final broth with a fructose glucose content of 92%/8%.

- Batch fermentation/pervaporation performed with the commercial module initiated after 3 hours of fermentation, using Pharmed tubing for broth circulation (fermentation/pervaporation IV) also required 16.5 hours instead of 27, but would ideally have been stopped after 14.5 hours in order to obtain high fructose yields. Otherwise, fructose is consumed leading to a yield of 88%. Therefore, it can be concluded that there is a further gain in time if the pervaporation is initiated earlier in the fermentation, but the experiment has to be stopped before fructose starts to be consumed.

- Fed-Batch fermentation/pervaporation performed with the commercial module initiated after 3 hours of fermentation, using Pharmed tubing for broth circulation showed that a large amount of sucrose can be processed in less time than without membrane separation. The fed-batch was the equivalent of a 40% sucrose batch, and it was done in 24.5 hours instead of well over 40 hours. The yields of ethanol as well as fructose remained high.

- The pervaporation results in each of these set of experiments showed the same trends: the flux decreased with time, and the permeate concentration and selectivity decreased with time because the concentration of biomass increased with time and more and more cells deposited on the membrane surface.

- The commercial module had a higher flux and selectivity than the laboratory module. The commercial module removed more ethanol, and therefore improved the process slightly more because its surface area was larger.

- In general, this study has demonstrated that the simultaneous production of fructose and ethanol by the fermentation of sucrose feed by *Saccharomyces cerevisiae* 58 can be enhanced time wise by coupling the bioreactor to a pervaporation unit. The ethanol being removed, the inhibition was also removed, and the process required less time to accomplish without compromising the fructose and ethanol yields.

7. Recommendations

The process was shown to be successful, but it is not perfect. Many future studies may be performed to further enhance the process or simply to improve it. Some recommendations for future studies would be:

- When building the membrane module, test different glues, such as LTV silicone, and improve the method of assembling the fibre bundle;

- To verify the hypothesis that when more ethanol is removed, more fructose is consumed, perform batch experiments of fermentation in fructose mediums containing various amounts of ethanol;

- Explore other methods of cleaning the membrane module in order to have more efficient means of flushing the biomass from the hollow fibres after experiments are conducted.

- When performing fed-batch experiments, leave the pervaporation unit running after sugar addition. More sugar would be processed within a shorter time period. To be able to do this, the whole system would need to be automated for liquid nitrogen addition in the cold trap, for autosampling, and for condenser changes, or larger condensers would be needed;

- Test different types of membrane materials for their suitability to be coupled with a bioreactor. Perhaps membranes with larger flux to use less membrane area, or membranes with higher selectivities;

- Perform extensive fouling studies.

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