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Separation of Ethanol during its Production by Fermentation using a Silicone Rubber Hollow Fiber Membrane Module

By

Sriram Kumarappan

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Abstract

Monosaccharides form the basis of more complex sugars. Sucrose consists of a fructose molecule linked to a glucose molecule. Both glucose and fructose are readily fermentable by brewers yeast. In this study, a mutant of *Saccharomyces cerevisiae* was used for the co-production of fructose and ethanol by selectively converting glucose to ethanol from a sucrose medium. To enhance the fermentation process, a pervaporation unit was coupled to the bioreactor to keep the ethanol concentration in the broth at a low level, thereby reducing the ethanol inhibition on the yeast. The membrane module consisted of silicone rubber hollow fibers assembled in an inside feed/outside vacuum module design. Various experiments changing the time at which pervaporation was started were carried out (after 6 hrs, 3hrs and 0 hrs), to examine its effects on the ethanol, fructose, biomass yields, ethanol productivity rates and the time required to complete the fermentation. Certain experiments were carried out by changing the flow rate through the membrane module. Two different membrane modules (different in area) were used for the co-production of fructose and ethanol.

Batch fermentation using a medium with 28 % (w/v) sucrose required about 30 h to decrease the glucose content to 1 % (w/v) (approx) with a biomass yield of 0.03 g/g, final ethanol of 5.9 %(w/v), and a specific growth rate of 0.091/h. Batch fermentation using the same medium, with pervaporation of ethanol required about 21 h or even less time depending on the time at which pervaporation was started for the glucose concentration to reach the same level as mentioned above. It required 21 h for glucose to be decreased to 1.6 % (w/v), with a fructose yield of 90.30 % and an ethanol yield of 73.5 %, if the membrane was initiated after 6 h of batch mode operation. It required 21 h for glucose to be decreased to 1.0 % (w/v), with a fructose yield of 86.2 % and an ethanol yield of 78.0 %, if the membrane was initiated after 3 h of batch mode operation. It required 21 h for glucose to be decreased to 0.5 % (w/v), with a fructose yield of 83 % and an ethanol yield of 80.1 %, if the membrane was initiated immediately at the start of the batch mode operation.

Changing the flow rate through the membrane module helped in acquiring higher ethanol yields with the large membrane module. Final ethanol produced with a flow rate of 5 mL/min through the membrane module was 5.23 %(w/v) , with an ethanol yield of 66.29 % after 22 h, compared to 6.05 %(w/v) , with an ethanol yield of 76.4 % after 19 h with a flow rate of 10 mL/min.

Finally, in the case of the small membrane module, higher glucose consumption rate and higher ethanol yields were observed with pervaporation/fermentation experiments initiated after 0 hours of batch fermentation. In the case of the large membrane module, higher ethanol yields and shorter fermentation times required to complete the fermentation process were observed with a flow rate of 10 mL/min through the membrane module.

Résumé

Les monosaccharides sont considérés comme étant l'unité élémentaire de sucres plus complexes. Le sucrose est à la base, constitué d'une molécule de fructose liée à une molécule de glucose et tout deux sont susceptible au processus de fermentation utilisant des levures variées. Dans l'étude présente, une levure mutante de l'espèce *Saccharomyces cerevisiae* a été utilisée pour la coproduction du fructose et de l'éthanol. Ceci dit, cette levure s'attaque uniquement au glucose dans un milieu de sucrose pour former l'éthanol. Afin d'auguementer l'efficacité de cette fermentation sélective, une unité de pervaporation ainsi qu'un bio-réacteur ont été lié en série afin de réduire la concentration d'éthanol à un stricte minimum de façon à diminuer l'effet inhibiteur de l'éthanol à des concentrations élevées. Le module composé de membranes perméables consistait de tubules de caoutchouc de silicone assemblés suivant un module concept «inside feed/outside vacuum». Plusieurs expériences ont été réalisées en variant le temps de pervaporation au cours de la fermentation (après 6 h, 3 h, 0h) pour examiner les effets de la concentration de l'éthanol, de la concentration du fructose, des rendements de biomasse, des taux de productivité d'éthanol et le temps requis pour compléter la fermentation. Certaines expériences furent réalisées en augmentant le débit à travers de la membrane du module. Deux différents modules à membrane (de surfaces différentes) ont été utilisés dans la production du fructose et de l'éthanol.

Le processus de fermentation dans un système fermé utilisant un médium consistant d'une solution de 28 % (poids/volume) de sucrose a requis 30 h pour diminuer la concentration approximative de glucose à 1 % (poids/volume) avec un rendement de biomasse de 0.03 g/g, une concentration d'éthanol de 5.9 % (poids/volume) et un taux de croissance de 0.091/h. Le processus de fermentation dans des conditions identiques avec la pervaporation de l'éthanol requiert un maximum de 21h dépendant de l'instant auquel le processus de pervaporation a été commencé pour que la concentration de glucose soit identique au niveau mentionné ci-haut. Un temps de 21 h est requis pour que la concentration de glucose diminue à 1.6 % (poids/volume) avec un rendement de fructose de 90.30 % et un rendement d'éthanol de 73.5 % si la membrane est activée de

après 6 h du début de l'essai. Un temps de 21 h est requis pour que la concentration de glucose diminue à 1.06 % (poids/volume) avec un rendement de fructose de 86.2 % et un rendement d'éthanol de 78.0 % si la membrane est activée après 3h du début de l'essai. Un temps de 18h a été requis pour que la concentration de glucose diminue à 0.5 % (poids/volume) avec un rendement de fructose de 83.0 % et un rendement pour l'éthanol de 80.1 % si la membrane fut activée immédiatement au début de l'opération.

L'augmentation du débit à travers le module ayant une membrane de plus grande superficie a permit au système d'acquérir un meilleur rendement d'éthanol. La concentration finale d'éthanol produit avec un débit de 5 mL/min du médium synthétique à travers de la membrane du module est de 5.23 % (poids/volume) avec un rendement d'éthanol de 66.29 % après 22 h comparer à un rendement de 6.05 % (poids/volume) avec un rendement d'éthanol de 76.4 % après 19 h avec un débit de 10 mL/min.

En conclusion, dans le cas du petit module de membrane, on a observé un taux plus élevé de consommation de glucose et de plus grands rendements d'éthanol avec pervaporation/expériences de fermentation lancées après 0 heures de fermentation en lots. Dans le cas du grand module de membrane, un éthanol plus supérieur rapporte et des temps plus courts defermentation requis pour compléter le procédé de fermentation ont été observés avec un débit de 10 mlminute par le module de membrane.

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Nomenclature

A	effective membrane area (m^2)
ADH	alcohol dehydrogenase
B_0	biomass concentration at the beginning of the fermentation (g/L)
B_1	biomass concentration at the beginning of the of the exponential growth phase(g/L)
B_2	biomass concentration at the end of the exponential growth phase (g/L)
B_t	biomass concentration at the end of the fermentation (g/L)
c_f	solute concentration in the feed
c_p	solute concentration in the permeate
DWG	distillers wet grains
DDGS	distilled dried grains with solubles
c_r	solute concentration in the retentate
E_t	normalized ethanol concentration at time t (% (w/v))
$(EtOH)_t$	experimental concentration of ethanol in the reactor at time t ((% (w/v))
F_t	normalized fructose concentration at time t (% (w/v))
G_t	normalized glucose concentration at time t (% (w/v))
h	hours
HCN	hydrogen cyanide
HFCS	high fructose syrup
HFS	high fructose syrup
HPLC	high performance liquid chromatography
J_{liquid}	flux for liquid phase ($kmol/m^2s^1$)
J_{vapor}	flux for vapor phase ($kmol/m^2s^1$)
J_i	flux of component i ($kmol/m^2s^1$)
J_j	flux of component j ($kmol/m^2s^1$)
l_{liquid}	length of the liquid-filled portion of the pore in the pore flow model (m)
l_M	thickness of the membrane (m)
l_{vapor}	length of the vapor-filled portion of the pore in the pore flow model (m)
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)
PTMSP	poly (1-trimethyl silyl-1-propyne)
p^{liquid}	total pressure of liquid phase (Pa)
p^{sat}	saturated pressure (Pa)
p^{vapor}	total pressure of vapor phase (Pa)
q_f	flow rate of the feed (m^3/s)
q_p	flow rate of the permeate (m^3/s)
q_r	flow rate of the retentate (m^3/s)
R	retention
S	recovery
S_0	initial experimental sucrose concentration ((% (w/v))
S_t	experimental sucrose concentration at time t ((% (w/v))
$(Sugar)_t$	concentration of sugar in the reactor at time t ((% (w/v))
t_1	time at which the exponential phase begins (hours)
t_2	time at which the exponential phase ends (hours)
t_p	pervaporation time (hours)
t_x	time at which the sucrose concentration becomes zero in the reactor (hours)
V_f	initial volume of the feed in batch operations
V_r	final volume of the retentate in batch operations
$V_{P,t}$	cumulative volume removed as permeate at time t (mL)
$V_{R,t}$	actual volume of the reactor at time t (mL)
VOC	volatile organic compounds
x_A, x_i	concentration of component A or i in the feed
x_B, x_j	concentration of component B or j in the feed
y_A, y_i	concentration of component A or i in the permeate
y_B, y_j	concentration of component B or j in the permeate
α	separation factor
μ	biomass specific growth rate (1/h)

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1. Introduction

1.1 Project Background and Context

Before the 1970's most of the sugar we ate came from sugar beets or sugar cane and was called sucrose. In the 70s, sugar from corn - corn syrup, fructose, dextrose, dextrine, and or high fructose corn syrup began to gain popularity as a sweetener because it was less expensive to produce. The story of high fructose corn syrup is one of the most "revolutionary" in food science in the last decade. Consumption has increased since its inception. It was produced by hydrolyzing corn starch. The corn starch is hydrolyzed and glucose, one of the products of hydrolysis is changed into fructose by an enzyme called isomerase. Fructose is a monosaccharide that is approximately 75% sweeter than sucrose (Inglett, 1981). High Fructose Corn Syrup (HFCS) is extremely soluble in water and mixes well in many foods. It is cheap to produce, sweet, and easy to store.

The use of HFCS in industries are mainly due to the following reasons: it retains moisture and/or prevents drying out; produces an osmotic pressure which is higher than sucrose or medium invert sugar and helps in controlling microbial growth; has high sweetness; costs less than liquid sucrose or corn syrup blends; provides a ready yeast-fermentable substrate; has low viscosity (Inglett, 1981; Coleman and Harbers, 1983; Volpe and Meres, 1976; Harris and Johnson, 1987).

The name "high fructose corn syrup" can be misleading; it is also made up of the two simple sugars glucose and fructose and differs only slightly from table sugar in the ratio of the two simple sugars. Most HFCS is manufactured as 42 HFCS or 55 HFCS, meaning it is 42 percent fructose or 55 percent fructose, with the remaining percentage being mainly glucose and oligosaccharides (Vuilleumier, 1993).

The major goal of this project was to develop and demonstrate a high-rate process for ethanol production at a lower cost, with the simultaneous production of fructose. The yeast *Saccharomyces cerevisiae*, better known as the Baker's yeast was used for this project. The yeast, *S. cerevisiae* ATCC 36858, studied in the laboratory (Koren and Duvnjak, 1990; 1993; Atiyeh and Duvnjak, 2001), was capable of hydrolyzing sucrose. Earlier studied microorganisms had the problems of production of unwanted by-products

and the consumption of fructose to a larger extent. Mutant strains of *S.cerevisiae* had zero fructose consumption in the presence of glucose, with the capability to produce high yields of ethanol (Koren and Duvnjak, 1989; 1990; 1993; Atiyeh and Duvnjak, 2001).

In many bio-reactions the fermentation product has an inhibitory effect on the productivity and viability of the microorganisms used (Gagne, 2001). Typical examples for such fermentations are the production of organic acids such as citric acid or lactic acid. An example for a very strong inhibition is the butanol-acetone fermentation. In organic acid production the toxic effect of the product is normally diminished by neutralization or precipitation (Nagashima, 1990). In case of ethanol fermentation these methods cannot be applied, but other methods like vacuum fermentation, flash fermentation, extractive fermentation and last but not the least, membrane separations will be effective (Mulder, 1991).

Pervaporation is one of the most promising membrane processes in a membrane bioreactor. There is a similar membrane process called membrane distillation. From an economical point of view membrane distillation is not to be considered seriously because its selectivity is too low. The selectivity cannot be improved in this process because it is determined by external factors (vapor-liquid equilibrium). Pervaporation, in its simplest form, is an energy efficient combination of membrane permeation and evaporation. In pervaporation, a separation of two or more components is accomplished across a non-porous or a porous membrane by the combination of two phenomena; differing rates of diffusion through the thin polymer membrane and an evaporative phase change analogous to a simple flash distillation step.

1.2 Objectives and Strategy

A mutant strain of *S. cerevisiae* ATCC 36858 was used in this project. It was used in the co-production of fructose and ethanol, for it did not have the ability to consume fructose in the presence of high concentrations of glucose. When sucrose (initial sucrose concentration of approximately 28 % w/v) starts to hydrolyze, glucose and fructose are accumulated in the reactor and glucose eventually is converted to ethanol. When the concentration of the ethanol in the reactor increases, it inhibits the yeast activity and thereby the fermentation time increases. Therefore, fermentation time should be

shortened while maintaining high yields in fructose and ethanol production. One way of doing this is to continuously remove the ethanol in the reactor. By maintaining a low level of ethanol in the reactor, the fermentation time would be lowered to a considerable extent.

The goal of the project was to enhance the co-production of fructose and ethanol from sucrose by *S. cerevisiae* ATCC 36858 by removing the ethanol as it was being produced. In this project, the bioreactor was coupled to a silicone rubber membrane module, which made it possible to remove ethanol continuously and maintain its concentration at a low level. Batch fermentation experiments with and without pervaporation were carried out to check the difference in fructose and ethanol yields. Two different types of hollow fiber membrane modules were used in this project, with the membrane area being the difference between the two modules. Pervaporation experiments with ethanol/water mixtures were also carried out with the two modules at various conditions to determine the membrane performance.

2. Literature Review

2.1 Introduction to Carbohydrate Chemistry

Carbohydrates are among the most abundant organic compounds found in both plants and animals. Carbohydrates are carbon compounds that contain large quantities of hydroxyl groups. The name *'carbohydrate'* arose from the mistaken belief that substances of this kind were hydrates of carbon, since the molecular formula of many could be expressed in the form $C_x(H_2O)_y$, for example glucose($C_6H_{12}O_6$), sucrose($C_{12}H_{22}O_{11}$) (Guthrie, 1974).

Usually carbohydrates appear at an early stage in the conversion of carbon dioxide and water by photosynthesis. Animals have no means of synthesizing carbohydrates from carbon dioxide and basically rely on plants for their supply. The carbohydrates are then converted into various other organic materials by a variety of biosynthetic pathways.

Carbohydrates serve as sources of energy (sugars) and stores of energy (starch and glycogen); they also form a major portion of the supporting tissue of plants (cellulose). Carbohydrates can be divided into broad groups: sugars and polysaccharides. Sugars are sweet, crystalline, and soluble in water. Their molecular weights are known exactly and are invariable for a given substance. The simplest sugars are the monosaccharides, which chemically are polyhydroxyaldehydes or polyhydroxyketones. Two of the most common monosaccharides are glucose and fructose. Glucose is the primary form of sugar stored in the human body for energy. Fructose is the main sugar found in most fruits. Both glucose and fructose have the same chemical formula ($C_6H_{12}O_6$), however they have different structures. A disaccharide is built up from two monosaccharide units with the formal elimination of one molecule of water. Common table sugar is sucrose, a disaccharide that consists of a glucose unit bonded to a fructose unit. The monosaccharides thus constitute the main building blocks of carbohydrates.

A disaccharide, trisaccharide, or a polysaccharide can be hydrolyzed by dilute aqueous acids or with the help of enzymes to the constituent monosaccharide.

2.1.1 Structures of Glucose and Fructose

Dumas in 1843 determined the empirical formula of the sugar to be CH_2O (Pigman and Goepp, 1948). Berthelot established the presence of a number of hydroxyl groups by the preparation of acetate (indicated by him to be a hexaacetate) and formulated glucose as a hexahydric alcohol; however as a result of additional studies, glucose was formulated as an aldehyde-alcohol with five carbon atoms (Pigman and Goepp, 1948). The six carbon nature and the various known properties of glucose were expressed by Fittig and Baeyer in 1868 to 1870 in the formula:

$(HO)H_2C\text{-}CH\ (OH)\text{-}CH\ (OH)\ -\ CH\ (OH)\ -\ CH\ (OH)\text{-}CHO$ (Fittig, Baeyer)

The Baeyer-Fittig formula was confirmed by molecular weight determinations by the formation of pentaacetates and other esters and by the exhibition of many aldehyde-type reactions (Pigman and Goepp, 1948). Thus, the reduction of the sugar produces a hexahydric alcohol (sorbitol), and oxidation with bromine or nitric acid produces a monobasic acid (gluconic acid). These reactions would be anticipated from the presence of an aldehyde group. By reduction (with hydrogen iodide) of the alcohol or acid obtained from glucose, sec-hexyl iodide or n-hexylic acid is obtained. The formation of the sec-hexyl iodide proves that the sugar has a straight chain. These and many other reactions support the Baeyer-Fittig formulation of glucose (Pigman and Goepp, 1948).

The presence of honey of a syrupy sugar different from glucose was recognized by many early workers, but the crystalline material was prepared first by Jungfleisch and Lefranc in 1881 (Pigman and Goepp, 1948). The name of levulose seems to have been applied first by Berthelot in 1860, whereas Emil Fischer in 1890 suggested the name fructose of this sugar (Pigman and Goepp, 1948).

Fructose must be constituted similarly to glucose, for it is reduced to hexahydric alcohols (mannitol and sorbitol). Mannitol has a straight chain structure as is shown by its conversion to sec-hexyl iodide by the action of hydrogen iodide. Oxidation of the sugar with nitric acid yields meso-tartaric acid (COOH-CHOH-CHOH-COOH), glycolic acid (CH_2OH-COOH) and oxalic acid (HOOC-COOH) and must take place by the cleavage of

the carbon chain. The formation of tartaric acid and glycolic acid would be expected if a ketone group is present at carbon 2. The existence of a ketone group is shown by the formation of a branched-chain acid when fructose is treated with HCN. The nature of the seven-carbon acid formed by the addition of HCN was shown by Kiliani who reduced it to 2-methylhexanoic acid (Pigman and Goepp, 1948). The original formula for fructose as proposed by Krusemann in 1876 and Kiliani in 1881 is given as follows (Pigman and Goepp, 1948):

(Fructose)

2.1.2 High Fructose Syrup

High Fructose (Corn) Syrup - HFS or HFCS - is a nutritive sweetener with high commercial potential. It is a product in which a large percentage of the glucose - derived from starch hydrolysis - has been converted into its sweeter-tasting isomer fructose, with the help of isomerase. HFCS is composed of either 42% or 55% fructose, with the remaining sugars being glucose and higher saccharides. HFCS is extremely similar to regular table sugar (sucrose), which is a 50/50 blend of fructose and glucose. It is usually found together with glucose. The crystal clear high fructose syrup has sweetness and calories approximately equal to that of a table sugar solution. It performs many of

the same functions as sugar, chiefly the "clean" sweetening of beverages, ketchup, dairy products, baked goods, and so on. HFS is usually sold at a price considerably below table sugar, hence it has gained popularity. High Fructose Corn Syrup is extremely soluble and hygroscopic in water. Generally, baked products made with HFCS will be softer than those made with sucrose.

From the perspective of the human body, there is very little difference between regular table sugar (sucrose) and HFCS. Sucrose and HFCS contain nearly the same ratio of glucose and fructose:

- Sucrose is 50% fructose and 50% glucose.
- HFCS is either 42% or 55% fructose and 42-52% glucose (with minor amounts of carbohydrate polymers).

Sucrose and HFCS differ by the bonding of their sugars:

- Sucrose is a disaccharide, in which fructose and glucose are linked by chemical bond.
- Fructose and glucose are free and unbonded sugars in HFCS.

Sucrose and HFCS have the same caloric density as many carbohydrates: both contribute 4 kilocalories per gram. HFCS-55 was designed to have identical sweetness to sucrose. The human body absorbs and metabolizes sucrose and HFCS identically, with one exception: the chemical bond linking glucose and fructose in sucrose must be hydrolyzed by invertase in the lining of the small intestine before absorption can occur. This enzymatic hydrolysis takes place very rapidly, so there is no practical difference in their subsequent absorption and metabolism. HFCS is far more common in the food supply now than in the early 1970s, when it was first developed, since there has been a largely concurrent decrease in sucrose consumption as HFCS gradually replaced sucrose in many foods and beverages.

2.1.3 Methods for the Production of High Fructose Syrup

Enzymes have played a drastic part in improving starch industries, which have brought about the development of entirely new products. Earlier, these processes used to be performed under high temperatures and pressures with acids. The invention of enzymes made these processes be performed under mild conditions.

The starch from corn is converted into glucose and other syrups by hydrolysis. The enzymes that include alpha-amylase, glucoamylase and glucose isomerase convert starch to high fructose corn syrup (HFCS). Alpha-amylase liquefies the starch slurry, so that the starch gets solubilized for the next step. It also splits the large amylose and amylopectin molecules into soluble dextrin fragments. Glucoamylase saccharifies (conversion of liquefied starch to sugars) the dextrins and hydrolyses the polymers into their individual dextrose units. Dextrose produced by the glucoamylase may be processed into finished syrup, dry dextrose, or converted to fructose. The next step performed by the glucose isomerase is an important part in the production of High Fructose Corn syrup (HFCS). Glucose isomerase rearranges dextrose to fructose, which is chemically similar, but twice as sweet as dextrose. The enzyme is immobilized onto a solid carrier held in a reactor with dextrose being passed through it. Repeated processing increases the concentration of fructose, with the possibility of making syrups of desired concentration. The HFCS production process is schematically represented by Figure 2-1.

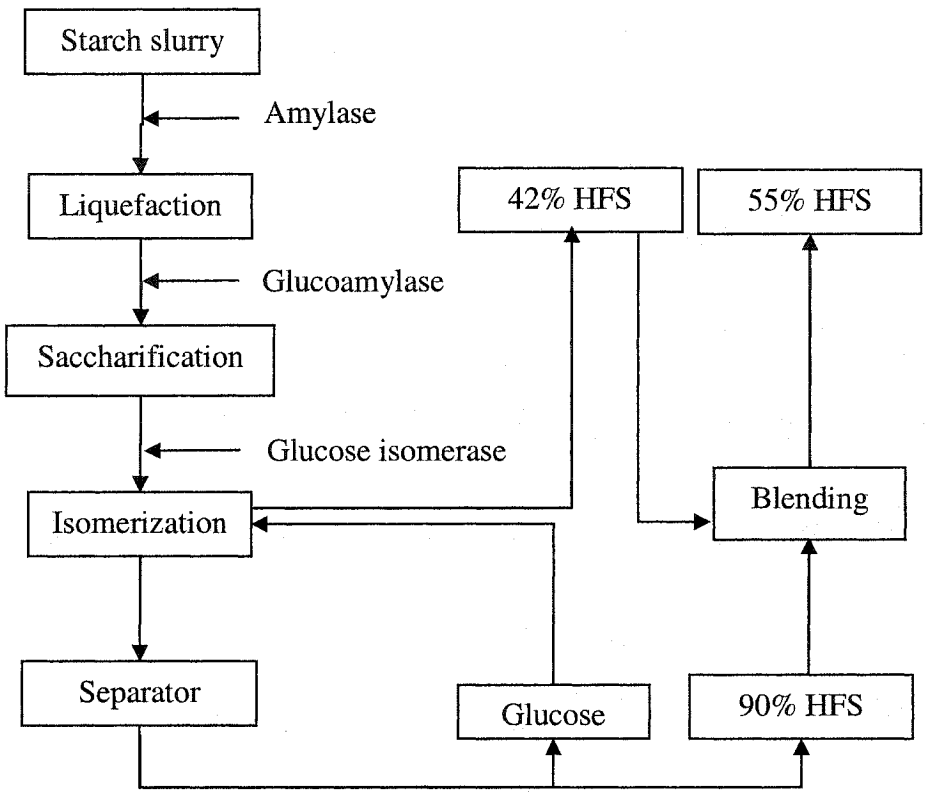

Figure 2.1: Schematic Representation of the Production of High Fructose Syrup (Chao Khun Agro Products, 1984).

2.1.4 Specifications of High Fructose Syrup

High Fructose Syrups are generally available as HFCS 42 and HFCS 55. Typical chemical and physical data of HFCS 42 and 55 are as follows:

Table 2.1: Specifications of High Fructose Syrup

Parameters	**HFCS 42**	**HFCS 55**
Percent Solids	70.5 - 71.5 % w/w	76.5 - 77.5 % w/w
Carbohydrate component		
a)Fructose	NLT 42.0 % w/w	NLT 55.0 % w/w
b)Dextrose	NLT 51.0 % w/w	NLT 40.0 % w/w
c)Higher Saccharides	NMT 7.0 % w/w	NMT 5.0 % w/w
Conductivity Ash	NMT 0.05 % w/w sulfated	NMT 0.05 % w/w sulfated
Taste	Free from foreign tastes	Free from foreign tastes
Odor	Free from Objectionable odor	Free from Objectionable odor
Temperature	NMT 30°C	NMT 30°C
Chlorides	NMT 50 mg/kg	NMT 50 mg/kg
pH (Undiluted)	3.5 - 4.5	3.5 - 4.5
Sulfur Dioxide	NMT 3.0 mg/kg	NMT 3.0 mg/kg
Color (RBU)	25 Max	25 Max
Turbidity	Free	Free
Brix@20°C	69.1 - 70.1	74.8 - 75.8

NLT = Not less than; NMT = Not more than; Max = Maximum;

RBU = Reference based Units.

(Chao Khun Agro Products, 1984)

2.1.5 Applications of High Fructose Syrup

High Fructose Syrup has been used for a variety of applications. Some of them are as follows:

Table 2.2: Applications of High Fructose Syrup

1.Direct Human Consumption	
2. Pharmaceuticals	
3. Confectionery	Prevents sugar crystallization.
4. Winery and Brewery	High fermentability.
5. Bakery Non-Fermented and Fermented Products	Caramelization during baking. Hold the freshness of cream.
6. Dairy Products	Increases moisture content.
7. Preserved Foods	Oxygen barrier around food surface.
8. Carbonated and Non-carbonated Soft-drinks	Water soluble. Mixes easily and pumpable in manufacturing.
9. Tobacco Industry	Good color.
10. Honey Bee Feeding	Carbohydrate source.
11. Frozen Live-Stock Coating	Lower freezing temperature.
12. Concentrated Fruit Juices and Flavor Syrups	Lower development of bacteria, yeast and mold. Lower crystallization.

(Chao Khun Agro Products, 1984)

2.2 Traditional Methods for the Production of Ethanol

Ethanol, otherwise known as ethyl alcohol is a clear, colorless, flammable oxygenated fuel. Currently fuel ethanol is primarily made from corn in the United States. Traditional corn to ethanol production is accomplished in two ways:

1) Wet Milling
2) Dry Milling (Mann and Bryan, 2001)

Both processes essentially include the same steps:

1) Preparing the feedstock
2) Fermenting simple sugars
3) Recovery of the alcohol and residual non-alcohol materials.

The preparation of material for fermentation is the essential difference between these two processes.

Wet Milling

The corn kernel is presoaked and then milled to produce streams of germ, fiber, and starch. Steeping conditions the grain for milling. In spite of softening the kernel for milling, steeping also breaks down the protein holding the starch particles and helps in removing the soluble constituents (Buchheit, 2002).

The germ is extracted then to produce corn oil, the most important byproduct obtained in the wet milling process. The starch fraction then undergoes centrifugation and saccharification to produce gluten wet cake, which is the second important byproduct. The ethanol is then distilled leaving thin stillage, which when dewatered leaves condensed distiller's solubles. The wet milling process is a very cost intensive process.

Dry Milling

Dry milling involves breaking and cleaning down the kernel corn into very fine particles using a hammer mill. The starchy material produced from grinding contains germ, fiber, gluten and other impurities. In order to produce ethanol this starch should be separated or converted to low molecular fermentable sugars (Mann and Bryan, 2001), which is achieved via hydrolysis. The hydrolysis process is stimulated by the action of enzymes or a dilute acid.

The glucose-laden mash is then transferred to fermenters, where yeast is added for the final conversion to alcohol. The product obtained is then transferred to a distillation unit where the alcohol is separated from the solids and water. The resulting solid is dewatered in a centrifuge and then sent to the dryer as distiller's wet grains (DWG). The liquid from the centrifuge is then concentrated in evaporators and the resulting syrup is blended with the dried distillers grains creating distilled dried grains with solubles (DDGS), a high value feed stuff. DDGS is the byproduct obtained after removing ethanol via distillation from the yeast fermentation. The germ, gluten and the fiber should be removed in the dry milling process. The germ is usually removed by sieving and prepress-solvent extraction is used to remove the oil from the germ. The actual processes of production of ethanol, whether it is from wet or dry milling, requires only the carbohydrate part of the grain, while the other materials including protein, fiber, oil, ash, are superfluous to the process.

Lower capital costs make dry milling process a better option for the production of ethanol. Lower energy requirements, more sophisticated automation, lower cost of enzymes, advanced yeast strains, are some of the advantages in this process, with the main one being better ethanol yields.

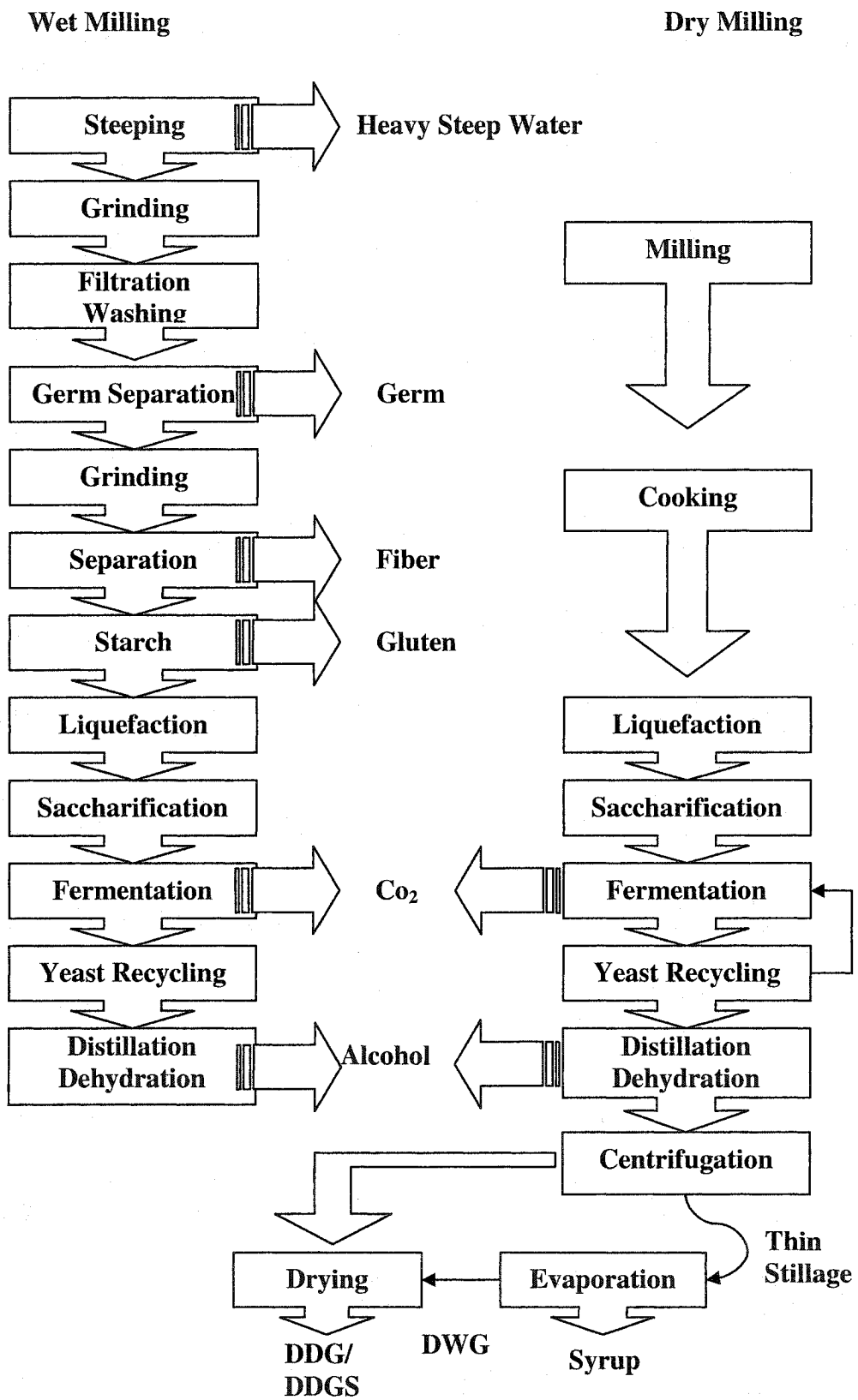

Fig 2.2: Production of Ethanol using Dry and Wet Milling (Buchheit, 2002)

2.3 Ethanol and Fructose Production with Yeasts

Fermentation describes the anaerobic degradation of sugars into various products to obtain energy for biosynthesis and maintenance of the yeast cells. Glucose is converted to pyruvate in a sequence of 10 enzyme-catalyzed reactions in a pathway that is called either glycolysis or the Embden-Meyerhof-Parnas pathway (Figure 2.3). Glucose and fructose enter glycolysis directly, while galactose is converted to glucose 1-phosphate, then to glucose 6-phosphate.

The glycolysis reactions can be arranged into three major parts. The first part does not involve oxidation-reduction and results in the production of glyceraldehydes 3-phosphate. The second part involves oxidation-reduction and results in the formation of pyruvate. In the third part, the pyruvate formed can proceed by two routes in the presence or absence of oxygen. One path way leads to the production of ethanol and CO_2. The pyruvate loses its carboxyl group by the action of pyruvate decarboxylase to form acetaldehyde. The acetaldehyde is then reduced to ethanol through the action of alcohol dehydrogenase.

The development of large scale fermentation contributes itself to the conventional ethanol fermentation techniques with respect to both basic and applied research. Most alcoholic fermentations are batch systems, which are characterized by low productivity, sensitivity to contamination due to low cell density under anaerobic conditions (Nagashima, 1990). Increase in cell density improves ethanol productivity, as do continuous ethanol fermentation systems (Nagashima, 1990). Physical separation of cells by centrifugation, membrane technology, and gravity separation of cells using very strongly flocculant microorganisms, all followed by cell re-circulation are new techniques developed to achieve these goals (Nagashima, 1990).

With *Saccharomyces* yeasts in alcoholic fermentations, Lactobacillus is a typical contaminant which may even form flocs with the yeast. When this happens, it would be very difficult to remove the bacteria from the yeast. Another important observation is the decline in the fermentation rate as the ethanol concentration increases, which thereby limits the final concentration and time to complete substrate conversion. Ethanol

tolerance and ability to accumulate high ethanol concentrations are strain-dependent characteristics (Nagashima, 1990). *S.cerevisiae* is one of the better ethanol-tolerant

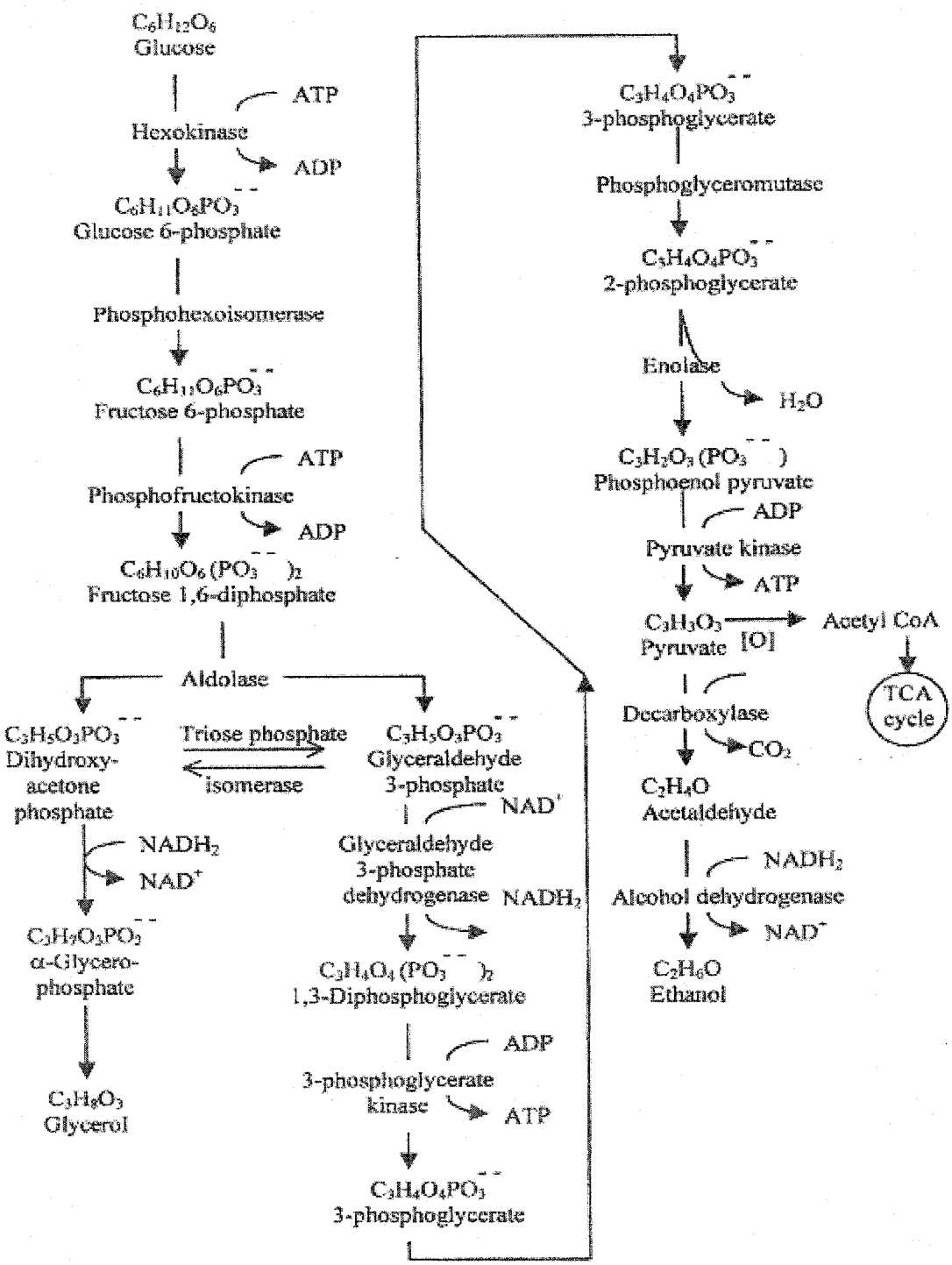

Figure 2.3: Embden-Meyerhof-Parnas pathway for the fermentation of glucose by *S.cerevisiae* (Agarwal, 1990)

organisms used in industrial processes (Nagashima, 1990). Environmental parameter such as pH, temperature, osmotic pressure, carbon dioxide levels may directly affect yeast growth and ethanol productivity.

All living organisms are constantly subjected to a variety of physical, chemical, or biological damages usually referred to as stress. Among environmental stresses that yeast cells may undergo, ethanol constitutes the main stress factor during fermentation processes. The toxic effects of ethanol on *S.cerevisiae* cells involve the modification of the membrane lipid composition and a reduction of metabolic activity, inhibition of glucose up-take, suppression of growth rate and product formation.

S.cerevisiae and related species are alcohol-tolerant, which is believed to be due to a polygenic involvement as shown by Ismail and Ali (Nagashima, 1990). Earlier work indicated that key glycolytic enzymes such as hexokinase and also alcohol dehydrogenase were involved. More recent studies of phosphoglycerate kinase, phosphoglyceromutase, and pyruvate decarboxylase have shown these enzymes to be sensitive towards ethanol. Ethanol accumulation in the cells leads to membrane destruction (Nagashima, 1990). Ethanol tolerance is improved by the structure of the cell membrane through adequate yeast growth and fermentation conditions. Andreasen and Stier reported that to survive under anaerobic conditions yeast cells required sterols and unsaturated fatty acids (Nagashima, 1990). The absence of both sterols and unsaturated fatty acids alters the membrane structure. Such effects are responsible for the slow start of fermentation in beer brewing when yeasts at the end of previous fermentations are reused (Nagashima, 1990). Cell viability and ethanol production seem to be optimal when cells are in growing conditions (Nagashima, 1990). To achieve this, nitrogen should be available in adequate form. Oxygenation at regular intervals is better than supplying the media with sterols and unsaturated fatty acids.

Kinetic analysis for the batch ethanol fermentation of *S.cerevisiae* revealed that lower culture temperatures caused slower growth and slower ethanol production; however the final cell mass and ethanol concentrations reached levels which were higher than those for higher culture temperatures. A kinetic model based on the integrated ethanol concentration was proposed which could simulate temperature dependent ethanol

fermentation and hence suggesting that the model could be therefore be useful for the optimization of ethanol batch fermentation by controlling the culture temperature as an operating variable (Nanba et al., 1987).

S.cerevisiae ATCC 36858 was used in batch processes to produce fructose syrups and ethanol from synthetic media with high sucrose concentrations. With sucrose concentrations between 180 g d/m^3 and 726 g d/m^3, a yield of 92% fructose was obtained. Ethanol yield was about 82 % in media with sucrose concentrations up to 451 g d/m^3 (Atiyeh and Duvnjak, 2001).

S.cerevisiae ATCC 36859 was immobilized and used for the continuous production of high fructose syrup. Continuous fermentation was performed in an immobilized cell reactor, where the cells were immobilized in Ca-alginate beads. No fructose consumption and no formation of byproducts were observed (Lamarche, 1988). Synthetic and complex media containing high fructose corn syrup supplemented with Jerusalem artichoke juice was used for the production of pure fructose syrup by the conversion of glucose into ethanol. A fructose yield of 99% was obtained with a feed of 10.1 % w/v glucose and 9.8 % w/v fructose mixture at a dilution rate of 0.106/h. Purification of this product with activated carbon and ion-exchange resins produced high fructose syrup which was suitable for human consumption (Koren and Duvnjak, 1989).

S.cerevisiae ATCC 39859 immobilized into small cubes of wood produced ethanol and very enriched fructose syrup from glucose/fructose mixtures through the selective fermentation of glucose. Immobilization maintained the cells in a viable state and did not create any barrier between the cells and the solution in the system. A maximum ethanol productivity of 21.9 g/ L h was obtained from a feed containing approximately 10 % w/v of both fructose and glucose, with a fructose yield of 99%. The ethanol productivity and its concentration increased by 13% at a dilution rate of 0.74/h with the addition of 30 mg/L oleic acid (Guenette and Duvnjak, 1995; 1996).

In-situ recovery of ethanol from fermentation by different hydrophobic adsorbents was studied by Einicke et al., (1991). It was shown that in a low concentration range, pentasil zeolites have a high selectivity for ethanol and the product inhibition was stopped when the zeolite NaZSM-5 was brought into contact with the fermentation broth. It also

changed the ethanol production rate dramatically, depending on the glucose-to-adsorbent ratio.

Economic analyses of the production of ethanol using different fermentation processes were examined. Experiments using a mutant strain of *S.cerevisiae* ATCC 36859 were performed using batch, semi-batch and semi-batch coupled to pervaporation and vacuum membrane distillation processes. The fermentation medium contained glucose, fructose, yeast extract and peptone. The installation of a new plant and the adaptation of an existing ethanol plant to the selective fermentation of glucose from sugarcane hydrolysate were considered. The use of pervaporation was to minimise the inhibition of the micro organisms and facilitate product recovery. The results show that the selective fermentation coupled to a membrane process to remove ethanol is an attractive process to increase ethanol production economics, although membrane performance and costs should be still improved (Luccio et al., 2002).

Conventional yeast fermentation coupled to a flat-plate membrane pervaporation unit to recover continuously an enriched ethanol stream from the fermentation broth was performed. The pervaporation module contained 0.1 m^2 commercially available polydimethyl-siloxane membrane and consistently produced a permeate of 20-25% (w/w) ethanol while maintaining a level of 4%-6% ethanol in a stirred tank reactor (O'Brien and Craig, 1996).

A hydrophobic porous membrane made of polypropylene was used to study the effect of ethanol removal by pervaporation when glucose was fermented by immobilized baker's yeast. It was seen that the rate of ethanol production was 2 times higher than that in fermentation without pervaporation. In fed-batch fermentations, the total ethanol without pervaporation produced was 381 g compared to 780 g of ethanol produced with pervaporation. The conversion rate of glucose to ethanol was also shown to be 96.3 % of the theoretical yield (Kaseno et al., 1998).

The effects of the introduction of cell recycling on ethanol productivity were examined for a continuous membrane fermentor-separator (CMFS) with continuous removal of ethanol by pervaporation. The effects of pervaporation for systems with and without a cell separator were compared in terms of yeast cell density, substrate utilization, ethanol concentration in the fermentation broth and productivity. The results

of cell wash-out allows the CMFS with cell separator unit to operate at a very high value of dilution rate and increases the productivity of ethanol at the same value of the pervaporation factor (PF) compared with a system without cell separator. Pervaporation factor (h^{-1}) is proportional to the rate of permeation and inversely proportional to the volume of the fermentor. An increase in the value of PF always results in an increase in the ethanol productivity (Kargupta et al., 1998).

A coupled fermentation/pervaporation process using ethanol perm selective silicate membranes coated with silicone rubber was used for the production of highly concentrated ethanol solutions. The process exhibited about a 20 % increase in the average glucose consumption rate as compared with that without pervaporation. In the case of silicate membrane without coating, the membrane flux and the ethanol concentration in the permeate decreased with the fermentation time. The completely recovered ethanol solution was 30 %(w/w) in the permeate. Coating a silicate membrane with silicone rubber produced 70 %(w/w) ethanol in the permeate. In the case of fermentation/pervaporation with silicone rubber coated membrane too, the flux greatly decreased. It was suggested that the glycerol produced in the fermentation, which is not adsorbed by the membrane, might have affected the pervaporation fluxes (Ikegami et al., 2002).

Pervaporation techniques have also been used to remove butanol from fermentation broths. Butanol, a superior liquid fuel, can be produced anaerobically by *Clostridium beijerinckii or C. acetobutylicum* from agricultural biomass. However, the cultures are strongly inhibited by the butanol. In bioreactors, butanol concentration higher than 25 g/L is rarely reached. Low butanol concentration hampers economic recovery of this fuel. The performance of these membranes varied significantly depending upon chemical composition of the membrane and means of removal of butanol vapors from the membrane surface. Butanol vapors were removed from membrane surface either by sweep gas or vacuum. When applied, vacuum offered high flux as compared to sweep gas (Qureshi et al., 2002).

Continuous ethanol fermentation with membrane separator of pervaporation was performed using hydrophobic micro porous hollow fibres of polypropylene. An ethanol-water mixture of high ethanol concentration was recovered continuously and directly

from the fermentation broth. Intermittent back flushing by compressed nitrogen gas prevented degradation of the permeation rate. The selectivity of the membrane for ethanol was a little bit smaller than that of distillation at normal pressure. The permeation rate of ethanol was almost linearly proportional to the ethanol concentration of the feed broth. The carbon dioxide generated by fermentation helped the permeation rate (Matsumoto et al., 1986).

Pervaporation can be utilized for recovery of ethanol from fermentation broths, which suggests a continuous fermentation process for fuel ethanol production. Based upon previously established laboratory performance data for pervaporation, a flux of 0.15 kg/ m^2 h and a selectivity of 10.3, process equipment was designed, sized and costs estimated for the pervaporation, fermentation, distillation and dehydration sections of a commercial-scale fuel ethanol plant. It can be compared to a typical dry milling ethanol facility plant. For a baseline membrane cost of \$200 m^2, costs for the fermentation-pervaporation process were slightly higher. Sensitivity analyses on the pervaporation performance parameters indicated that with minor improvements in either flux or selectivity, the entire pervaporation process for ethanol recovery from fermentation broths could be cost-effective (O'Brien et al., 2000).

2.4 Introduction to Membrane Processes

Membrane processes are new methods of separation. Today membrane processes are used in a wide range of applications with the number still growing. The different types of membrane processes are as follows: microfiltration (MF), ultrafiltration (UF), reverse osmosis (RO), electrodialysis (ED), pervaporation (PV), membrane distillation (MD), and separation by liquid membranes (LM).

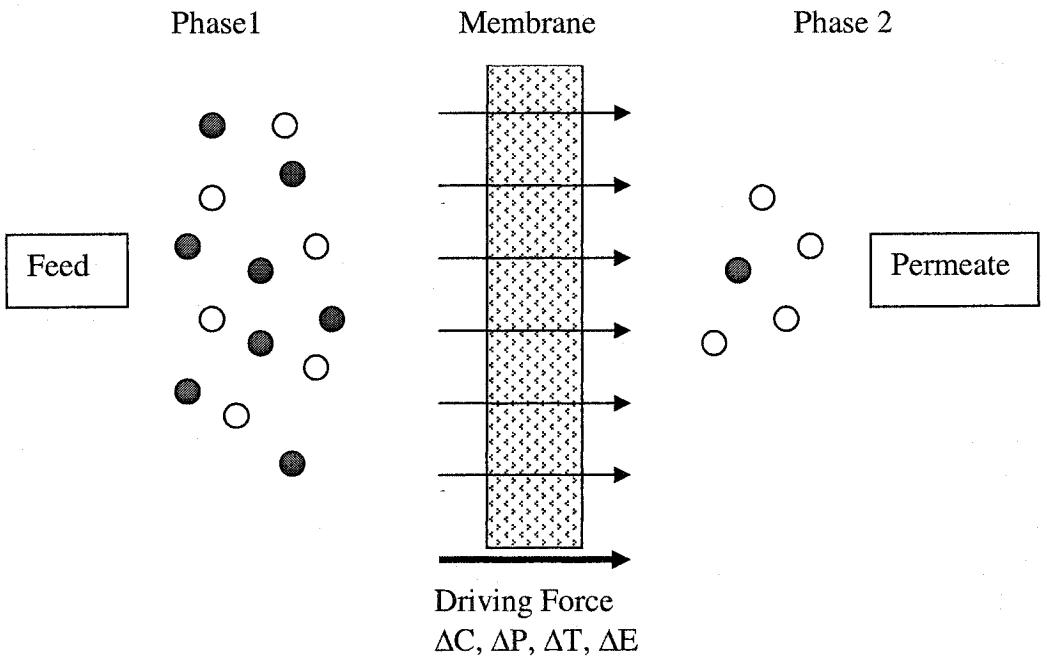

Figure 2.4: Schematic representation of a two-phase system separated by a membrane (Mulder, 1991).

Membranes are classified based on the different separation principles or mechanisms and specific problems can cover the broad size range from particles to molecules. In spite of these various differences, all membrane processes have one thing in common, i.e., membrane. The membrane is the heart of every membrane process and is a permselective barrier between two phases.

Phase 1 is usually considered as the feed or upstream side phase while phase 2 is considered the permeate or downstream side. Separation can be achieved because of the tendency of the membrane to transport one component from the feed mixture more readily than any other components (Mulder, 1991).

The performance or efficiency of a membrane is determined by two parameters.

a) Selectivity

b) Flow through the membrane(Flux)

The flux is defined as the volume (or) weight (or) mole flowing through the membrane per unit area and time.

The selectivity of a membrane is generally expressed as the retention (R) or the separation factor (α). For mixtures, consisting of mostly water and a solute, it is more convenient to express the selectivity in terms of the retention R towards the solute. The solute is partly or completely retained while the solvent (water) molecules pass through the membrane. The retention R is given by,

$$R = \frac{C_p - C_f}{C_p} = 1 - \frac{C_f}{C_p} \qquad (1)$$

Where,

C_f is the solute concentration of the feed

C_p is the solute concentration in the permeate

R, as a dimensionless parameter, does not depend on the units in which the concentration is expressed. The value of R varies between 100% (complete retention of the solute) and 0% solute (solute and solvent pass through the membrane freely).

Membrane selectivity towards gas mixtures and mixtures of organic liquids is usually expressed in terms of a separation factor α.

For a mixture consisting of components A and B, the separation factor $\alpha_{A/B}$ is given by:

$$\alpha_{A/B} = \frac{y_A/y_B}{x_A/x_B} \quad (2)$$

Where,

y_A and y_B are the concentrations of components A and B in the permeate

x_A and x_B are the concentrations of the components A and B in the feed

The definition of selectivity α is chosen usually in such a way that its value is greater than unity. If the permeation rate of component A through the membrane is larger than that of component B, the separation factor is denoted as $\alpha_{A/B}$; likewise if component B permeates preferentially, then the separation factor is given by $\alpha_{B/A}$. If $\alpha_{A/B} = \alpha_{B/A} = 1$, it means that no separation has been achieved.

2.5 Pervaporation

2.5.1 What is Pervaporation?

Pervaporation is one of the few membrane processes which can separate a mixture of two or more miscible liquids into products in which some of the constituents are concentrated. The analogous non membrane based process is distillation although the mechanism of separation is quite different. The term "pervaporation" is used to describe a separation process, where a liquid mixture is in direct contact with one side of a non-porous membrane, and where the permeated product, enriched in at least one of the components of the liquid mixture, is removed from the other side of the membrane as vapor.

In a pervaporation process, one side of a non-porous membrane is in direct contact with a liquid mixture, whereas the permeated product is removed from the other side of the membrane as vapor. This is effected by keeping the partial vapor pressure at the permeate side below that of the feed liquid mixture. Composition of the permeate is determined by the permeation rate of the components in the feed and may be totally

different from liquid-vapor equilibrium curves. Pervaporation is a dynamic process, operating far away from thermo-dynamic equilibrium conditions.

2.5.2 Two Dominant Causes of Separation in Pervaporation

Pervaporation involves separation of a feed liquid. This is a unique technique among various other membrane separation techniques because of the fact that a phase change occurs across the membrane. The partial pressure differences created between the feed and permeate side of the membrane is the driving force for the mass transport. The separation is a function of relative permeation rates of the components through the membrane.

The selectivity in pervaporation is caused by the following two component steps,

a) An evaporation process in the feed liquid is elevated to a vapor that is in the thermodynamic equilibrium with the feed liquid. Mathematically speaking, this process is defined as,

$$\alpha_{vap} = \frac{(Y_i / Y_j)_{eq.vapor}}{(X_i / X_j)_{feed}} \quad (3)$$

b) A Membrane transfer process in which the vapor diffuses from the feed side to the permeate side. Mathematically speaking, the second process can be defined as the,

$$\alpha_{memb} = \frac{(Y_i / Y_j)_{perm.\ vapor}}{(X_i / X_j)_{eq.vapor}} \quad (4)$$

Mass flux across the membrane is due to the partial pressure difference between the feed saturated vapor pressure and the permeate vapor pressure. Experimental data for water flux across a silicone rubber pervaporation membrane with a thickness of 20 μm shows that as the permeate pressure increases and approaches the feed pressure, the flux decreases to zero in a linear matter. Temperature is also a factor in this flux. As the

temperature of the feed is increased, the system is more able to handle a higher flux of water. This is the reason why most feed streams pass though a heater before entering the membrane housing. Heating is also to supply the heat of vaporization. The final separation of the feed liquid is the product of the separation achieved by the evaporation of the liquid, α_{vap}, and the separation achieved by the permeation through the membrane, α_{memb}. Since the separation factor of a contaminated feed stream is based upon the product of the evaporation factor and the membrane factor, the separation factor may be due more to one factor than another.

The separation factor also plays an important role in pervaporation. For example, the 200- to 500-fold separation achieved by pervaporation membranes in ethanol dehydration is almost entirely attributed to the separation factor of the membrane which is more permeable to water than ethanol. On the other hand, when attempting to separate volatile organic compounds (VOC's) from a dilute aqueous solution, the evaporation factor is greater than the membrane factor. The separation factor for a VOC will increase the more hydrophobic the membrane becomes, but the contribution to the total separation due to membrane will increase. As material continues to pass through a membrane, "swelling" effect makes the membrane more permeable but less selective, until a point of unacceptable selectivity is reached and the membrane must be regenerated.

Advantages of Pervaporation over other separation techniques:

1) Effective and economic separation of mixtures of substances with small difference in boiling point and azeotropic mixtures.
2) Modular membrane design.
3) No entrainers for separation of azeotropic mixtures.
4) Reduced capital costs compared to conventional systems.

2.6 Selection of Polymers for Pervaporation Membranes

The efficiency of a pervaporation process is controlled mainly by the intrinsic properties of the polymers used for membrane preparation. Hence the selection of polymer material is usually a major factor in the development of pervaporation membranes.

Pervaporation is controlled by the mass transport mechanism as in the solution-diffusion model. In the case of PDMS (Polydimethyl Siloxane) membrane, being a rubbery polymer, the permeability increases with the increasing mass of permeants (Bell et al., 1988). Molecules with larger solubilities will more readily permeate through the membrane (Bell et al., 1988). In the case of membranes prepared from glassy polymers, permeability decreases with increasing molecular mass because decrease in diffusivity is more significant than increase in solubility when molecular mass is increased. The difference between rubbery and glassy polymers is due to their structural properties.

The solubility plays a major role in determining the permeability to a large extent in rubbery polymer membranes. In rubbery polymers, such as PDMS, the macromolecules are in a coiled state, in which the polymer segments are in a continuous vibrational and rotational movement.

The permeability is determined by the following two steps.

1) Ability of the permeating molecule to dissolve intermolecular attractions between the polymer segments
2) By increasing the number and the dimensions of the moving polymer segments

Both can be achieved when permeant molecules are dissolved in a rubbery polymer. In a glassy polymer, there is very little movement of the polymer segments. The distance between the two segments is fixed, with no direct free path in the direction of macroscopic flow. The distance is not uniform throughout the entire polymer bulk and minimally influenced by the permeants (Bell et al., 1988). Thus, with the diffusion path not longer than the membrane thickness, the selectivity is determined by the size of the permeating molecules, which must pass through the existing spaces between the rigid polymer segments of a glassy polymer.

2.7 Pervaporation of Ethanol/Water Mixtures

The separation of alcohol-water mixtures by pervaporation has been preferentially carried out through hydrophobic membranes because it permeates more alcohol through the membrane. Most polymeric membranes are water perm-selective as the molecular size of water is smaller than that of alcohol. Ethanol perm-selective membranes have been restricted to silicon and fluorine containing polymers, for example, polydimethylsiloxane (PDMS), poly (1-trimethylsilyl-1-propyne) (PTMSP), and poly (tetrafluoroethylene) derivatives (Kang et al., 1994; Wang et al., 1999; Ishira et al., 1986; Chen et al., 1998).

Polyvinyl alcohol/cyclodextrin (PVA/CD) membrane was utilized in the pervaporation of ethanol/water mixtures, with the content of CD up to 33 %. The addition of CD increased the water selectivity especially at lower (<40%) and higher (>90%) ethanol concentrations in the feed. The water selectivity through the sorption equilibrium, on the contrary, decreased by addition of CD; this result indicated that the effect of CD on the diffusion coefficient was predominant on pervaporation performance. At lower ethanol concentrations, the addition of CD increased the permeation rate of water because of large increase in the diffusion coefficient of water. The permeation rate of ethanol decreased due to the large decrease in the diffusion coefficient of ethanol. At higher ethanol concentrations, the permeation rate of water slightly decreased; since even though the diffusion coefficient of water increased the water content in the membrane decreased. The permeation rate of ethanol largely decreased because the solubility as well as the diffusion coefficient decreased (Yamasaki et al., 1994).

PTMSP (poly (1-trimethylsilyl-1-propyne)) membrane shows preferential permeation of ethanol in the pervaporation of ethanol/water mixtures. The selectivity is due to the existence of the large free volume and the hydrophobicity of the membrane surface. The separation factor reaches 10.7 and after a certain period of time decreased to an average value of 8. The initial value is similar to that of PDMS, a well known ethanol selective membrane. Although PTMSP shows good characteristics for the ethanol-water mixtures, the separation factor and the specific permeation rate decrease with the operating time, because of the swelling (Gonzalez-Velasco et al., 2002).

A variety of copolymers composed of polysiloxane and phosphate esters were coated on the porous PVDF substrate and cured to form a composite membrane, which was used for the pervaporation of ethanol-water mixtures. An interfacial layer was deposited on the PVDF substrate prior to the composite membrane to enhance the adhesion of the silicone and PVDF layers. It was shown that the inherently hydrophobic membranes were highly ethanol selective, especially when the ethanol concentration in the feed was low (Chang and Chang, 2002).

PDMS (polydimethylsiloxane) membranes were used in this project because it was the simplest silicone rubber material. It also had a high rate of diffusion of the small penetrants. Its industrial interest and its use as a selective membrane in various biomedical applications also prompted the use of PDMS. With PDMS membranes, the flux increases with the increase in feed ethanol concentration (Kimura and Nomura, 1998). Also the total flux decreases with an increase in the downstream pressure and the selectivity remains constant with increase in feed ethanol concentration. Usually, PDMS membranes exhibit low flux and low selectivity.

Separation factors of PDMS (polydimethylsiloxane), either blended or copolymerized with acrylate or methacrylate polymers, are not only a function of the composition of the membrane polymers but are also decisively determined by membrane morphology. Diffusion coefficients for ethanol and water in polymer blends are smaller than in PDMS without blending. The flux decreases consistently in comparison to PDMS. The selectivity of PDMS can be attributed to its preferential sorption, which overrides the difference in diffusion coefficients. However it is not the case in polymer blends. The sorption coefficients of polymer blends are higher than PDMS but do not lead to higher fluxes or increased selectivities necessarily (Jopski et al., 1989).

Pervaporation selectivity of silicone rubber membranes can be drastically improved by the addition of zeolites. When these membranes were tested with ethanol/water mixtures, it influenced both the sorption and diffusion characteristics, which resulted in a change in the properties due to zeolite sorption. The addition of silicate enhanced the ethanol flux, thereby increasing both total flux and selectivity (Hennepe et al., 1987).

3. Data Analysis and Modeling

3.1 Pervaporation Modeling

Modeling the mass transfer in pervaporation is necessary to understand and improve the performance of the process. The model chosen for this work is based on the pore model introduced by Okada and Matsuura (1988). It is a semi-empirical model which combines the features of theoretical with empirical approaches. Semi-empirical models cover the key parameters and give a basic understanding of how different parameters influence the process.

Basic Assumptions:

a) A bundle of straight cylindrical pores, distributed over the surface area of the membrane, penetrate the selective layer of the membrane.
b) Length of the pores l_M is equal to the thickness of the selective layer.
c) Membranes are in isothermal condition.

From the pore inlet at the feed side, a distance of the pore l_{liquid} is filled with liquid and the remaining distance l_{vapour} is filled with vapor. Evaporation takes place at the liquid-vapor boundary. The driving force of the process is the pressure difference between the liquid feed p^{liquid} and the vapor permeate p^{vapour}. At the boundary between the liquid and the vapor, the pressure is the saturated vapor pressure p^{sat} of the feed component. In the length of the pore filled with liquid, l_{liquid}, the permeating components are driven by liquid transport and similarly in the length of the pore filled with vapor, l_{vapour}, the permeating components are driven by vapor transport. It should also be noted that there should be a discontinuous change at the liquid/vapor boundary with respect to the density and the composition.

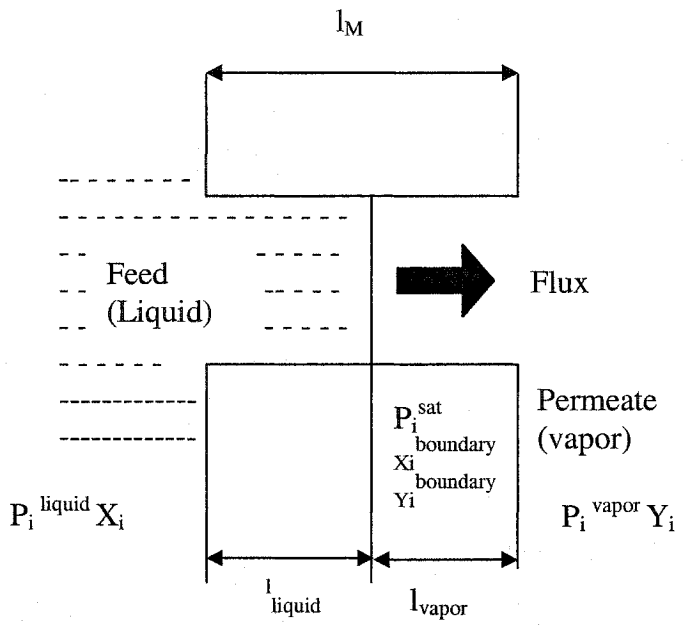

Figure 3.1: Schematic representation of a Pore Flow Model (Lipinizki and Tragardh, 2001).

For single component permeation, the flux in the liquid part, J_{liquid}, of the membrane can be written as:

$$J_{liquid} = \frac{A^{Pore}}{l_{liquid}(p^{liquid} - p^{sat})} \tag{5}$$

The flux in the vapor part, J_{vapour}, of the membrane can be expressed as:

$$J_{vapour} = \frac{B^{Pore}}{l_{vapor}((p^{sat})^2 - (p^{vapour})^2)} \tag{6}$$

where, $\mathbf{A^{Pore}}$ and $\mathbf{B^{Pore}}$ are constants.

$\mathbf{l_{liquid}}$ is the length of the liquid-filled portion of the pore in the pore flow model in metres.

$\mathbf{l_{vapor}}$ is the length of the vapor-filled portion of the pore in the pore flow model in metres.

$\mathbf{p^{liquid}}$ is the total pressure of the liquid phase in Pascals.

$\mathbf{p^{sat}}$ is the saturated pressure in Pascals.

At steady state, both fluxes are equal.

$$J = J_{liquid} = J_{vapour}$$

Hence the overall flux of a component can be written as,

$$J = \frac{A^{Pore}}{l_M (p^{liquid} - p^{sat})} + \frac{B^{Pore}}{l_M ((p^{sat})^2 - (p^{vapour})^2)} \quad (7)$$

These constants (A^{Pore}, B^{Pore}) are treated purely as empirical parameters accounting for the pressure effects on the liquid and vapour transport on the pores. l_M is the thickness of the membrane. This single-component model could be used to describe the flux of pure organic compounds and water through cellulose acetate and PDMS membranes at various permeate pressures (Lipinizki and Tragardh, 2001).

When it comes to binary mixtures l_{liquid} is neglected, considering that the pore diameter is sufficiently small and the liquid/vapor boundary comes near the pore inlet. Hence the flux of the components in the binary mixture of the i^{th} and j^{th} species can be written for a binary mixture as,

$$J_i = \frac{B_i^{Pore}}{l_M ((p^{sat})^2 - (p_i^{vapour})^2)} \quad (8)$$

$$J_j = \frac{B_j^{Pore}}{l_M ((p^{sat})^2 - (p_j^{vapour})^2)} \quad (9)$$

where, J_i is the flux of component i and J_j is the flux of component j.

Applying this model to the ethanol-water system, the flux and permeate concentrations were calculated and found to be in good agreement with the experimental

results. It should also be noted that the constants for the single component pervaporation could not be used for the binary mixture pervaporation.

The driving force for the pore model is different from that of the solution-diffusion model. The solution-diffusion model uses the concentration difference between the feed and permeate side, whereas the pore model for the single component is based on the pressure difference between the feed pressure and permeate pressure. The key feature of the pore flow model is the liquid-vapour boundary inside the membrane. While the solution-diffusion model assumes that the evaporation takes place on the permeate side of the membrane, the pore model assumes that evaporation of the permeating components takes place at the liquid/vapor boundary inside the membrane. The limitations of the pore flow model are similar to that of the solution-diffusion model. The complexity of the model increases, when the coupling effects are taken into consideration.

3.2 Fermentation/Pervaporation Data Analysis

Fermentation/Pervaporation data analysis should be performed in order to determine the total volume of the liquid in the reactor which has been removed .The volume of the liquid in the reactor keeps decreasing due to the following two reasons:

a) Ethanol and water are being constantly removed by pervaporation.
b) Samples are taken every 3 hours to analyze the sucrose, glucose, fructose, and ethanol content.

Due to constant volume removal, the performance of the membrane was depicted in two different ways:

a) Actual reactor data: These are the actual values of concentration inside the reactor and permeate, respectively, at each point in time.
b) Volume corrected reactor data: These are normalized values determined as though the permeate would have been put back in the reactor at each point in time.

The feed solution in the reactor becomes more concentrated due to the constant removal of ethanol and water. The volume concentrated data correspond to the point where no concentration effect has taken place. The volume removed for sample analysis is not considered in the calculation of volume concentrated 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) (Gagne, 2001).

The volume concentrated concentration of sugars can be calculated for any point in time during the fermentation using the following equation:

$$\text{Sugars} = \frac{(\text{Sugar})_t * V_{R,t}}{V_{R,t} + V_{P,t}} * 100 \qquad (10)$$

where, $(\text{Sugar})_t$ = 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 volume concentrated concentration of ethanol can be calculated for any point in time during the fermentation using the following equation:

$$E_t = \frac{([\text{EtOH}]_t * V_{R,t})/100 + M_{P,t}}{V_{R,t} + V_{P,t}} * 100 \qquad (11)$$

where, $[\text{EtOH}]_t$ = 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).

Error bars on figures represent the standard deviation for analytical samples.

3.3 Data Analysis

Fermentation Data Analysis

i. Fructose Yield

Theoretically 1g of sucrose hydrolyses to 0.526g of glucose and 0.526g of fructose. The fructose yield can be calculated as:

$$\text{Fructose Yield (\%)} = \frac{F_t}{(S_o - S_t) * 0.526} \qquad (12)$$

where, F_t = fructose concentration at time t in (%(w/v))

S_o = initial sucrose concentration at time (%(w/v))

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

The kestoses, which are made up of a glucose unit and two fructose units are not taken into account, because their levels at the end of batch fermentation are almost zero and in all the fermentation/pervaporation experiments their levels are below 0.7 %(w/v).

ii. Ethanol Yield

Theoretically 1g of glucose or 1 g of fructose yield 0.51g of ethanol. The ethanol yield should be done purely on the basis of sugars consumed. The glycerol which is a by-product in this process is not accounted because the levels of glycerol rarely go beyond 1% (w/v). The biomass used for maintenance and reproduction is not taken into account too. The ethanol yield can be calculated as follows:

$$\text{Ethanol Yield (\%)} = \frac{E_t}{[((S_o-S_t)* 0.526)-G_t]*0.51+ [((S_o-S_t)* 0.526)-F_t]*0.51} * 100 \quad (13)$$

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

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

iii. Ethanol Productivity

The ethanol productivity at any point t is calculated as follows:

$$P \text{ (g/ (L h))} = \frac{E_t}{t} \quad (14)$$

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

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

iv. Biomass Yield

The biomass yield was calculated based on total carbohydrate consumption:

$$\text{Biomass Yield (g/g)} = \frac{B_t - B_o}{((S_o-S_f)* 0.526)-G_f) + ((S_o-S_f)* 0.526)-F_f)} \quad (15)$$

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

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

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

v. Average Sucrose Hydrolysis Rate

The sucrose hydrolysis rate was calculated when the sucrose level in the reactor was zero.

$$\text{Sucrose Hydrolysis Rate: (g/ L h)} = \frac{S_o}{t_x} \quad (16)$$

where, S_o = initial sucrose concentration (g/L)

t_x = time at which the sucrose becomes zero in the reactor (hours)

vi. Maximum Specific Growth Rate

The maximum specific growth rate was calculated during the beginning of the exponential phase and at the end of the exponential phase.

$$\mu\ (h^{-1}) = \ln \frac{B_2}{B_1} * \frac{1}{t_2 - t_1} \quad (17)$$

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)

vii. The total flux was calculated according to the following equation:

$$\text{Total Flux } (g/m^2h) = \frac{m_p}{t_p * A} \quad (18)$$

where, m_p = Mass of permeate (g)

t_p = pervaporation time (h)

A = effective membrane area (m^2)

4. Materials and Methods

4.1 Yeast Culture Maintenance

The yeast *Saccharomyces cerevisiae* ***ATCC 36858*** was kept on malt agar slants *medium 1*. The medium was sterilized at 115°C for 15 min, inoculated after cooling and incubated at 30°C for 4-5 days. The slants were stored in a refrigerator at 4°C until needed. The yeasts were transferred to fresh slants every two months to maintain high metabolic activity. Medium 1 contained 45 g of Bacto Malt Agar and 1 L of deionized water.

4.2 Inoculum Preparation

Inoculum was prepared by aseptically transferring a loopful of cells from the test tube slants into a sterilized liquid *medium 2*(100mL), in 500 mL Erlenmeyer flasks. The flasks were then placed in a rotary shaker (Lab-Line Instrument Inc, Illinois, USA) at 200 rpm and 33°C for 24-30 hours. Then the flasks were placed in a refrigerator at 4°C to allow the biomass to settle down for 5-7 hours. The supernatant was decanted out and the concentrated biomass was used to inoculate reactors for the simultaneous production of fructose and ethanol. Table 4.1 shows the medium used for the inoculum preparation.

Table 4.1: Medium 2

10.0 g	glucose
30.0 g	yeast extract
3.5 g	peptone
2.0 g	KH_2PO_4
1.0 g	$MgSO_4.7H_2O$
1.0 g	$(NH_4)_2.SO_4$
Up to 1 L	distilled water

4.3 Fructose and Ethanol Production by Batch Fermentation

Batch fermentations were carried out in a bioreactor with an initial sucrose concentration of approximately 28% (w/v) after inoculation in *medium 3*. It was done in 500 mL Erlenmeyer flasks containing 100 mL of sterile medium 3. The medium was asceptically inoculated with biomass previously prepared as described in section 4.2 and the flasks were placed in a rotary shaker at 200 rpm and 33°C. The initial volume of the broth after inoculation varied depending on the type of membrane used. For the smaller membrane module, the initial volume of the broth was 150 mL, whereas with respect to the larger membrane module, it was 275 mL. The amount of biomass added also varied depending on the type of membrane module used. For the smaller module, 3.5 g/L of biomass was used, whereas with respect to the larger one, 5.5 g/L of biomass was used. The broth was constantly mixed using a Fisher Scientific magnetic stirrer. The temperature in the bioreactor was maintained at 33°C using a Thermo mix 2, which controlled the temperature of the water bath. The temperature inside the bioreactor was read using a digital thermometer. Samples of 5 mL were taken from the bioreactor every 3 hours using a sterilized pipette, and then centrifuged and analyzed to determine biomass, sugars, and ethanol concentrations. All tests mentioned above were done in duplicates too. Figure 4.1 depicts the batch fermentation performed in a bioreactor (Gagne, 2001). Table 4.2 shows the medium used for batch fermentation.

Table 4.2: Medium 3 (batch fermentation medium)

Approx. 28% (w/v)	sucrose
30.0 g	yeast extract
3.5 g	peptone
2.0 g	KH_2PO_4
1.0 g	$MgSO_4.7H_2O$
1.0 g	$(NH_4)_2.SO_4$
Up to 1 L	distilled water

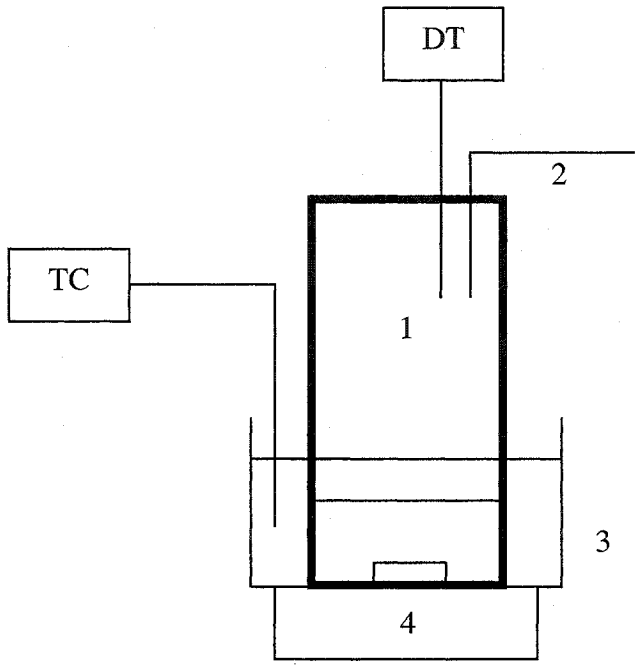

Figure 4.1: Batch fermentation in a small capacity bioreactor. (1) Bioreactor; (2) CO_2 vent; (3) Water bath; (4) Magnetic stirrer; (TC) Temperature control unit; (DT) Digital thermometer.

4.4 Description of the Hollow Fiber Membranes

As mentioned earlier, two different hollow fiber membrane modules were used for this project. Both of them were silicone rubber (Cross Linked PDMS) based membrane modules. The main difference between the two types of membrane modules was the effective membrane area and the total number of fibers in the membrane module. The specifications of the two membrane modules, M60-75S and M60-4000, are given in Table 4.3.

Table 4.3 Specifications of the two membrane modules used

Description	*M60 – 75S*	*M60 – 4000*
Membrane Area	0.09 m^2	0.457 m^2
Length of Fiber	140 mm	140 mm
Number of Fibers	750	4000
Outer Diameter	320μm	320μm
Inner Diameter	200μm	200μm
Thickness	60μm	60μm
Max. Allowable Pressure	1.5 kg/cm^2	1.5 kg/cm^2

4.5 Analysis

4.5.1 Ethanol

Ethanol concentrations were determined enzymatically using alcohol dehydrogenase (Bernt and Gutmann, 1974), which involves the oxidation of ethanol to acetaldehyde in the presence of nicotinamide adenine dinucleotide (NAD) by alcohol dehydrogenase according to the following equation:

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

The concentration of NADH, which is proportional to the amount of ethanol present, is measured spectrophotometrically at 340nm, since the equilibrium is shifted completely to the right at pH equal to 8.7. The reagents required for this analysis are buffer solution, NAD solution and ADH solution. The buffer solution was prepared by dissolving 10 g $Na_4P_2O_7.10H_2O$, 2.5 g semicarbazide hydrochloride, 0.5 g glycine in 250 mL of deionized water. The pH of the solution was adjusted to 8.7 by adding 4N KOH and the entire solution is diluted to 300mL. The NAD solution was prepared by adding 50mg NAD into 3 mL of deionized water. The ADH solution was prepared by adding 5mg ADH in 1 mL of deionized water.

A calibration curve was made using ethanol solutions containing between 0.020 % (w/v) and 0.0115 %(w/v) ethanol. Hence, the samples for analysis were diluted to bring the ethanol concentration below 0.0115 % (w/v). Then, 3 mL of buffer solution, 0.10 mL of NAD solution, 0.20 mL sample and 0.02 mL of ADH solution were added to a test tube. The tube contents were thoroughly mixed by shaking and incubated at 37°C for 25 min. The absorbance of the solution was measured against a reference at 340 nm and 25°C, using a Beckman DU 640 spectrophotometer equipped with a temperature controller (Beckman, California, USA). The reference was prepared by replacing the 0.20 mL sample with 0.2 mL deionized water. Ethanol yields were calculated on the basis of total sugars that were consumed at the maximum ethanol concentration.

4.5.2 pH Measurement

The pH measurements were conducted using an Accumel Research AR50 dual channel pH/ion/conductivity meter. The pH meter was calibrated with pH reference buffer solution of 4.00, 7.00, and 10.00 prior to each measurement.

4.5.3 Biomass

Biomass concentration was determined by the dry weight method. In this method, a known volume of the sample was centrifuged at 17,000 g for 15 min; the supernatant was collected and analyzed for sugar and ethanol content while the biomass was washed with deionized water and re-centrifuged. The biomass was then transferred to a pre-weighed aluminum dish and dried at 105°C overnight. The dish and the dried biomass were then reweighed and the biomass concentration was expressed in g/L.

4.5.4 Sugars

Sucrose, glucose, fructose, kestoses and glycerol were measured using a 600E system controller Water's high performance liquid chromatograph (HPLC) with Water's 410 differential refractometer as the detector and a Sugar-Pak 1 column (Waters, Massachusetts, USA) operated at 75°C. The flow rate of the mobile phase, deionized water containing approximately 50 mg/L EDTA-disodium-calcium salt was maintained at 0.5mL/min. The column was packed with a micro particulate cation exchange gel in calcium form, and was effective in separating sugars and alcohols according to their molecular weight. About 20 μL of solutions, with known concentrations from 1.0 and 4.0 g/L of sugars, glycerol and kestoses were injected into the HPLC to prepare standard curves. About 20 μL of samples, diluted to sugar levels below 4.0 g/L, was injected to the column for analysis. The acquired chromatographic data were analyzed using the Millennium Chromatography Manager System version 2.1.

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 50mg/L calcium EDTA solution.

4.6 Pervaporation Test of Ethanol/Water Mixtures

To determine the performance of the membrane, preliminary tests were carried out using the hollow fiber silicone membrane module. The schematic diagram of the pervaporation system is shown in Figure 4.2, in which the bioreactor contained aqueous ethanol solution. The feed solution was pumped through the module with a peristaltic pump. The feed solution here was a mixture of ethanol and water. Pharmed tubing was used for circulating the feed solution through the module. The feed entered the inside of the fibers, and vacuum was applied on the outside of the fibers with a Welch Duo Seal vacuum pump model 1400. Downstream pressure was measured with an MKS type 122A pressure transducer and controlled by an MKS type 651 pressure controller. The temperature was constantly monitored using a thermometer and maintained at 33°C. The permeation rate was determined by collecting the sample for a predetermined period. The amount of collected sample was determined by weighing the condenser before and after sample collection. The sample in the condenser was subjected to analysis for ethanol concentration.

Both modules were tested under the following conditions:

- Downstream pressure, 1 torr; flow rate through the membrane module, 0.5 mL/min, 2.5 mL/min ; turnover time through the reactor, 110 min, 300 min; temperature 33°C; total permeation time, 6 hours; ethanol concentrations in the feed, 2 %(w/v), 3 % (w/v), 4 % (w/v), 5 %(w/v).

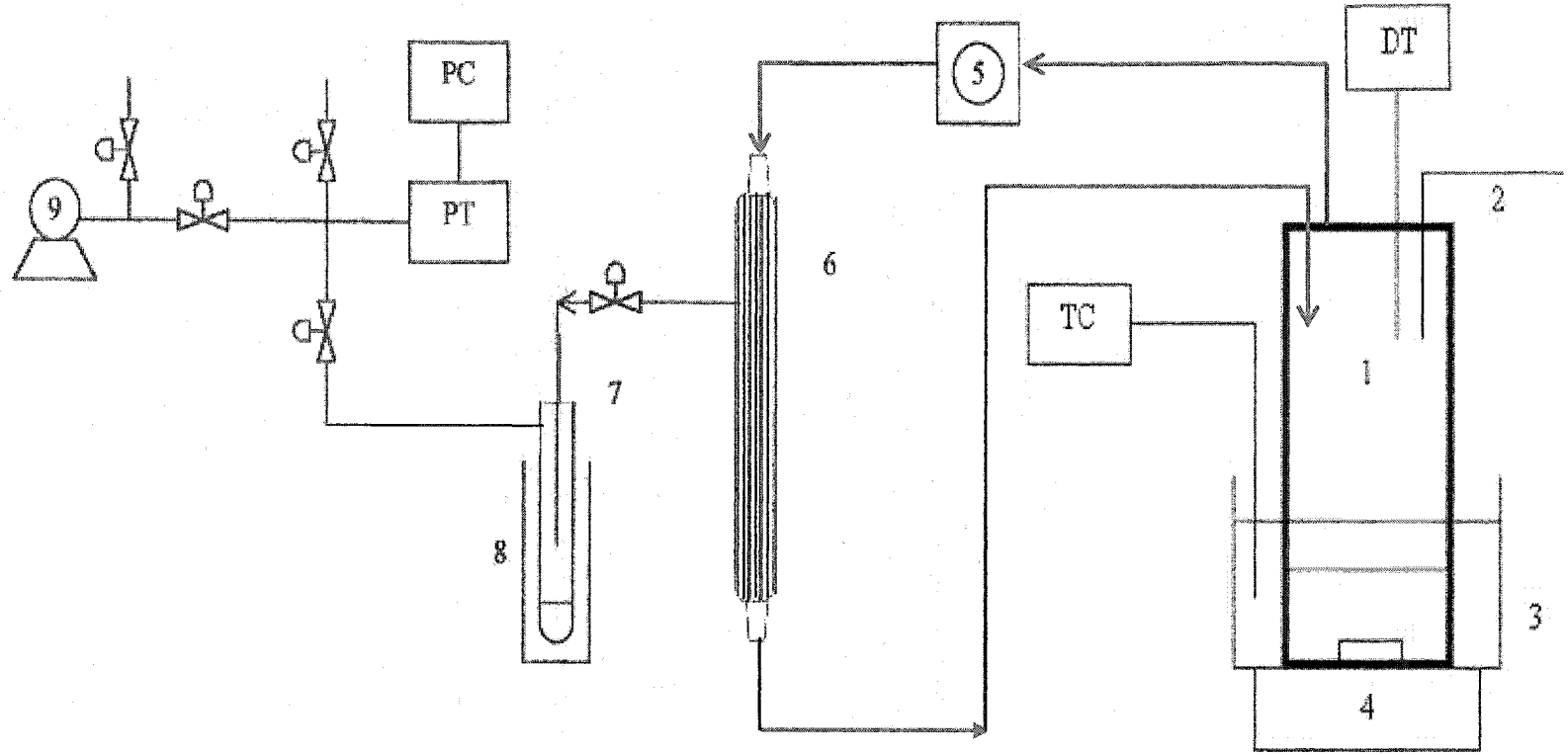

Figure 4.2 Schematic diagram of the pervaporation experimental setup (1) Bioreactor; (2) CO_2 vent for bioreactor; (3) Water bath; (4) Magnetic stirrer; (5) Peristaltic pump; (6) Membrane module; (7) Condenser; (8) Liquid N_2 bath; (9) Vacuum pump; PC – Pressure Control unit; TC – Temperature Control unit; DT – Digital Thermometer; PT – Pressure Transducer.

4.7 Batch Fermentation Coupled with Pervaporation

A bioreactor was used to carry out all the fermentation experiments. The initial sucrose was close to 28 % (w/v) for all the experiments. The concentration of initial biomass varied with the type of membrane module used. The medium contained 3.5 g/L of biomass when the small membrane module was used compared to a 5.5 g/L when the larger membrane module was used. The volume of the sucrose medium in the reactor was different depending on the type of the membrane module used; i.e. 150 mL after inoculation for the small membrane module and 275 mL for the large membrane module. The sucrose medium, after being prepared, was sterilized in an autoclave for 20 minutes at 110°C. After the medium was cooled down, it was inoculated with biomass of desired concentration. The system was left in batch mode for a predetermined period to allow the biomass to produce some ethanol, before the circulation of fermentation broth through the hollow fiber module was initiated. The flow rate for the fermentation broth through the membrane module was maintained at 5 mL/min and 10 mL/min for the small and large hollow fiber module, respectively. Simultaneously, the vacuum pump on the permeate side was started. Pharmed tubing was used for the circulation of the fermentation broth. Certain experiments were carried out with the membrane module initiated immediately after the start of the fermentation or 3 hours after the start of the fermentation. In all the experiments, the downstream pressure and the bioreactor temperature were maintained at 1 torr and 33°C, respectively. The permeate vapor was collected 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. When the condenser was changed, a sample of 5 ml was taken from the reactor using a sterilized pipette and was analyzed to determine biomass, sugars, and ethanol concentrations.

Table 4.4 shows the various experiments performed under various conditions.

Table 4.4 Different types of experiments performed under the following conditions

S. No.	Description	Type	Initial Sucrose Conc.	Flow rate	Pervaporation Initiated Time
A	Hollow fiber module	small	Approx. 28% (w/v)	5 mL/ min	After 6 hrs of batch fermentation
B	Hollow fiber module	small	Approx. 28% (w/v)	5 mL/ min	After 3 hrs of batch fermentation
C	Hollow fiber module	small	Approx. 28% (w/v)	5 mL/ min	After 0 hrs of batch fermentation
D	Hollow fiber module	large	Approx. 28% (w/v)	5 mL/ min	After 3 hrs of batch fermentation
E	Hollow fiber module	large	Approx. 28% (w/v)	10 mL/ min	After 3 hrs of batch fermentation

5. Results and Discussions

5.1 Batch Fermentation without Pervaporation

Batch fermentation was performed in a bioreactor to compare with fermentation/pervaporation experiments. The feed medium for batch fermentation was sucrose with a concentration of 28.3 % (w/v), and an initial biomass of approximately 3.6 g/L. The results are shown in Table 5.1 and Figure 5.1.

Table 5.1 Batch Fermentation in a Bioreactor

Description	Bioreactor
Initial Biomass Concentration (g/L)	3.6
Final Biomass Concentration (g/L)	8.0
Initial Sucrose Concentration (%w/v)	28.3
Fermentation Time (h)	30
Final Glucose Concentration (%w/v)	1.0
Final Fructose Concentration (%w/v)	14.1
Final Ethanol Concentration (%w/v)	5.9
Maximum Specific Growth Rate μ (1/h)	0.091
Biomass Yield (g/g)	0.03
Ethanol Productivity P (g/ (L h))	2.0
Ethanol Yield (%w/v)	78.9
Fructose Yield (%w/v)	94.7
Sucrose Hydrolysis Rate (g/ (L h))	30.7

As the sucrose in the reactor starts to hydrolyze, concentrations of glucose and fructose increase. However, since the yeast begins to consume glucose, glucose concentration reaches a maximum value and then starts to decrease. Fructose concentration on the other hand, reaches its maximum value, and remains at the same

level for the remaining period of the fermentation. As the glucose is being consumed, ethanol concentration increases in the reactor. As the concentration of ethanol in the reactor increases, the reaction becomes slower, because of the inhibition of ethanol by the yeast.

The fermentation was stopped after 30 hours, at which time the final glucose concentration in the bioreactor was 1.0 %(w/v). Since the theoretically achievable glucose concentration is 14.9 %(w/v), this means that 13.9 %(w/v) was consumed. The final fructose concentration in the reactor was 14.1 %(w/v), out of a possible 14.9 %(w/v), which gives a fructose yield of 94.7 %. It also shows that when glucose is present in the reactor, most of the fructose in the reactor remains unconverted. The small amount of fructose lost may have been used towards maintenance purposes. The fructose produced can be used for the production of crystalline fructose. A maximum of 7.60 %(w/v) of ethanol could be produced from the 14.9 %(w/v) of available glucose. With 1.0 %(w/v) glucose still remaining in the reactor, the maximum achievable ethanol would be 7.08 %(w/v). It should also be noted that the yeast must have utilized both glucose and fructose for growth purposes. Since only 5.9 %(w/v) ethanol was found in the final stage of the reaction, the ethanol yield is 78.9 %. The ethanol productivity was 2 g/ (L h). Both the fructose and ethanol yields were good, but the point of concern was the time taken to reach that stage.

The biomass, initially at 3.6 g/L, started to increase significantly and finally ended up at a concentration of 8.0 g/L, which gave a biomass yield of 0.03 g/g. The specific growth rate was 0.091/h. The sucrose hydrolysis rate was 30.7 g/ (L h). All the sucrose in the reactor was consumed during the first 9 hours, which was the reason for such a high sucrose hydrolysis rate.

It should be noted that the kestoses formed initially disappeared at the end of the experiment (Figure 5.1). Kestoses, which consists of two units of fructose and one unit of glucose, seem to be consumed eventually. Glycerol is another by-product, which was produced by *S.cerevisiae* ATCC 36858. The formation of glycerol depends on the yeast strain and the composition of the fermentation media.

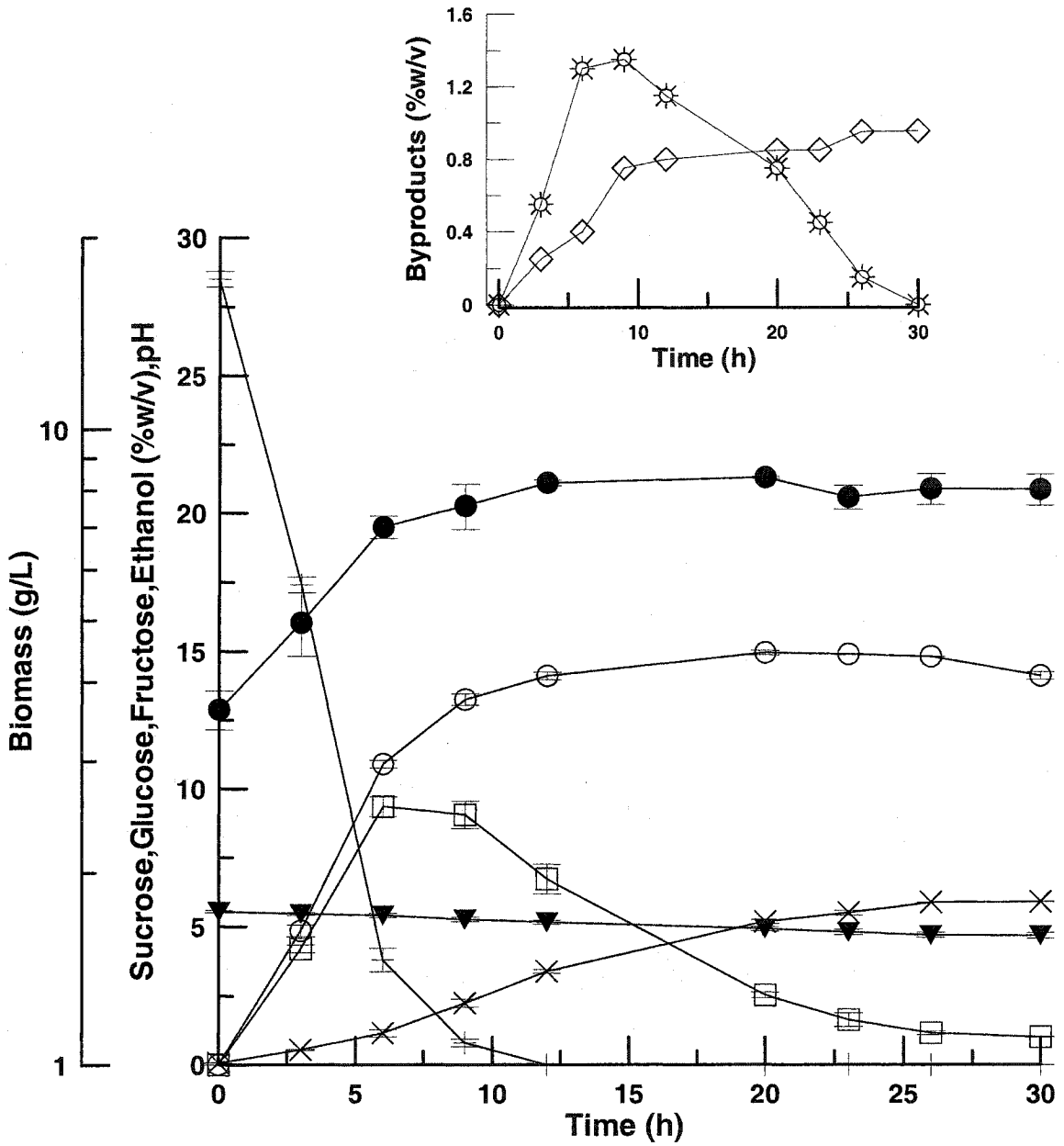

Figure 5.1 Results of batch fermentation performed in a small bioreactor without pervaporation. Initial sucrose concentration, 28.3 % (w/v); initial biomass concentration, 3.62 g/L, temperature 33°C; initial volume, 150 mL; (†) Sucrose, (□) Glucose, (○) Fructose,(●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

5.2 Pervaporation of Ethanol/Water Mixtures using the Small Commercial Module (M60-75S)

In order to check the performance of the membrane module coupled to a bioreactor, pervaporation tests of ethanol/water mixtures at various concentrations were carried out.

The performance test was carried out with a smaller hollow fiber membrane module at different ethanol concentrations in the feed. The operating conditions were as follows: initial volume of the ethanol/water mixture in the reactor 150 mL, downstream pressure of 1 torr, feed temperature of 33°C, flow rate of 0.5 mL/min through the membrane module and a turnover time of 300 min through the reactor. The experimental results are given in Table 5.2 and Figure 5.2. From the figure, it is seen that the total flux, including water and ethanol along with the ethanol concentration increased with an increase in ethanol concentration, while the separation factor decreased. The total flux increased from 22.2 g/ (m^2h) to 33.3 g/ (m^2h) and the ethanol concentration in the permeate increased from 18 % (w/v) to 32 % (w/v) as the ethanol concentration in the feed increased from 2 % (w/v) to 5 % (w/v). When it came to the separation factor, it decreased from 10.8 to 8.9 as the feed ethanol concentration increased from 2 % (w/v) to 5 % (w/v). These values were in good correspondence to the values reported for the pervaporation of ethanol/water mixtures through silicone rubber membranes (Kimura et al., 1982; Nakao et al., 1987). The values reported by Kimura and Nakao also showed a slight decrease in the separation factor and an increase in the total flux and permeate ethanol concentration with an increase in the feed ethanol concentration. When the ethanol and water flux are given separately as in Table 5.2, it can be seen that both fluxes increase but the ethanol flux increases much faster than the water flux, with an increase in the feed ethanol concentration. The higher water flux at higher feed ethanol concentrations can be explained by the swelling of the membrane. Swelling results in the better permeability of water through the membrane than ethanol and eventually makes it less selective to ethanol.

Table 5.2 Experimental total, water, and ethanol flux as a function of ethanol feed concentration

Ethanol in Feed % (w/v)	Total Flux g/ (m^2h)	Ethanol Flux g/ (m^2h)	Water Flux g/ (m^2h)	Separation factor
2	22.2	4	18.2	10.75
3	26.0	6	20	9.65
4	29.6	8.3	21.3	9.33
5	33.3	10.7	22.6	8.94

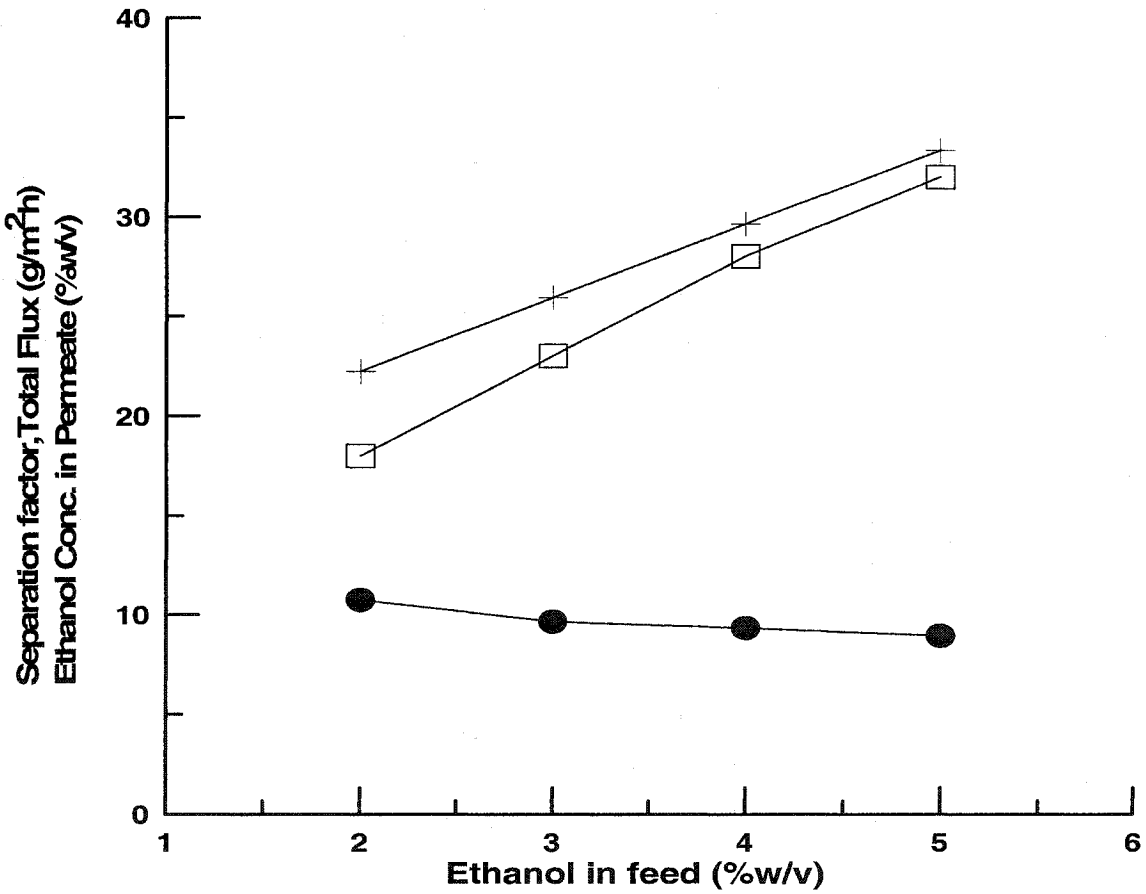

Figure 5.2 Effect of feed ethanol/water concentration on the membrane performance for pervaporation through silicone rubber hollow fibers. Downstream pressure of 1 torr; temperature, 33°C; circulation rate, 0.5 mL/min. (●) Separation factor, (□) Total Flux, (+) Ethanol concentration in the permeate

5.3 Batch Fermentation Coupled with Membrane Separation

The batch fermentation was performed with pervaporation to compare the results with batch fermentation without pervaporation. The bioreactor used for the batch fermentation experiment was the same in both cases. The main parameter that would be taken into consideration would be the data corresponding to volume corrected glucose concentration between 1- 2 % (w/v) that are usually obtained at the end of the experiment.

5.3.1 Batch Fermentation coupled with Pervaporation A:

The first set of experiments was conducted using the commercial membrane module (M60 - 75S) under the following conditions: initial volume of the fermentation broth in the reactor 150 mL (sucrose medium along with the inoculum), an initial sucrose concentration of 27.1 %(w/v), a biomass concentration of 3.5 g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation and a flow rate of 5 mL/min through the membrane module. The system initially underwent batch fermentation for 6 hours before pervaporation was started. Experimental results shown in Table 5.3 are volume corrected concentrations obtained at the end of the experiment. Figure 5.3 shows the actual reactor data as a function of time, and Figure 5.4 shows the volume corrected data as a function of time. The velocity, which was calculated using the flow rate through the membrane module and the cross sectional area of the membrane module was found to be $1.049 * 10^{-4}$ m/s.

Table 5.3 Fermentation/Pervaporation A Parameters

Description	Fermentation/Pervaporation A
Initial Biomass Concentration (g/L)	3.5
Final Biomass Concentration (g/L)	7.1
Initial Sucrose Concentration %(w/v)	27.1
Fermentation Time (h)	21
Final Glucose Concentration %(w/v)	1.6
Final Fructose Concentration %(w/v)	12.9
Final Ethanol Concentration %(w/v)	5.3
Maximum Specific Growth Rate μ (1/h)	0.099
Biomass Yield (g/g)	0.03
Ethanol Productivity P (g/ (L h))	2.5
Ethanol Yield %(w/v)	73.5
Fructose Yield %(w/v)	90.3
Sucrose Hydrolysis Rate (g/ (L h))	28.8

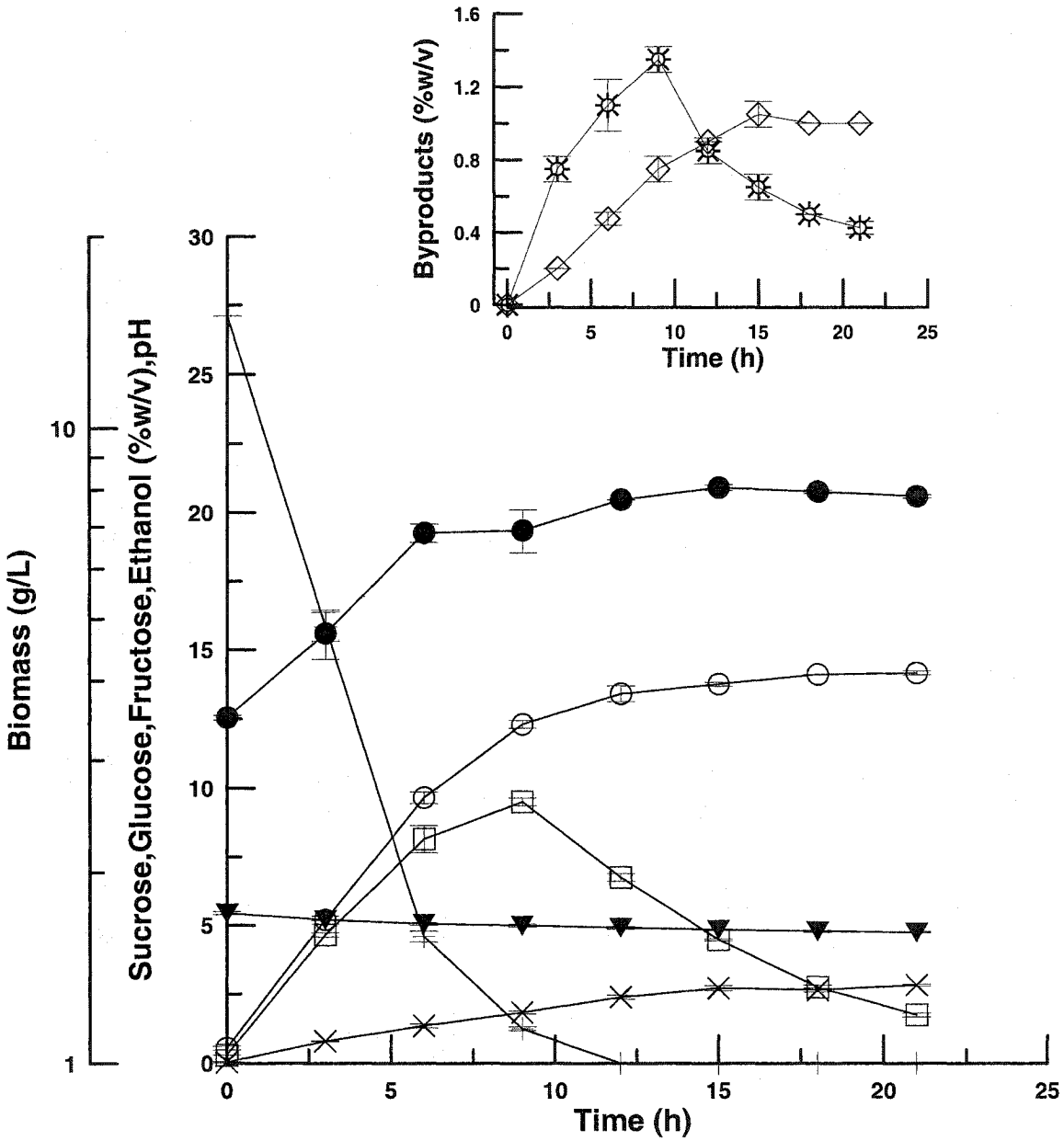

Figure 5.3 Actual reactor data of batch fermentation/pervaporation using the commercial small pervaporation module: Initial sucrose concentration, 27.1 % (w/v); initial biomass concentration, 3.5 g/L, temperature 33°C; initial volume, 150 mL; pervaporation initiated after 6 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

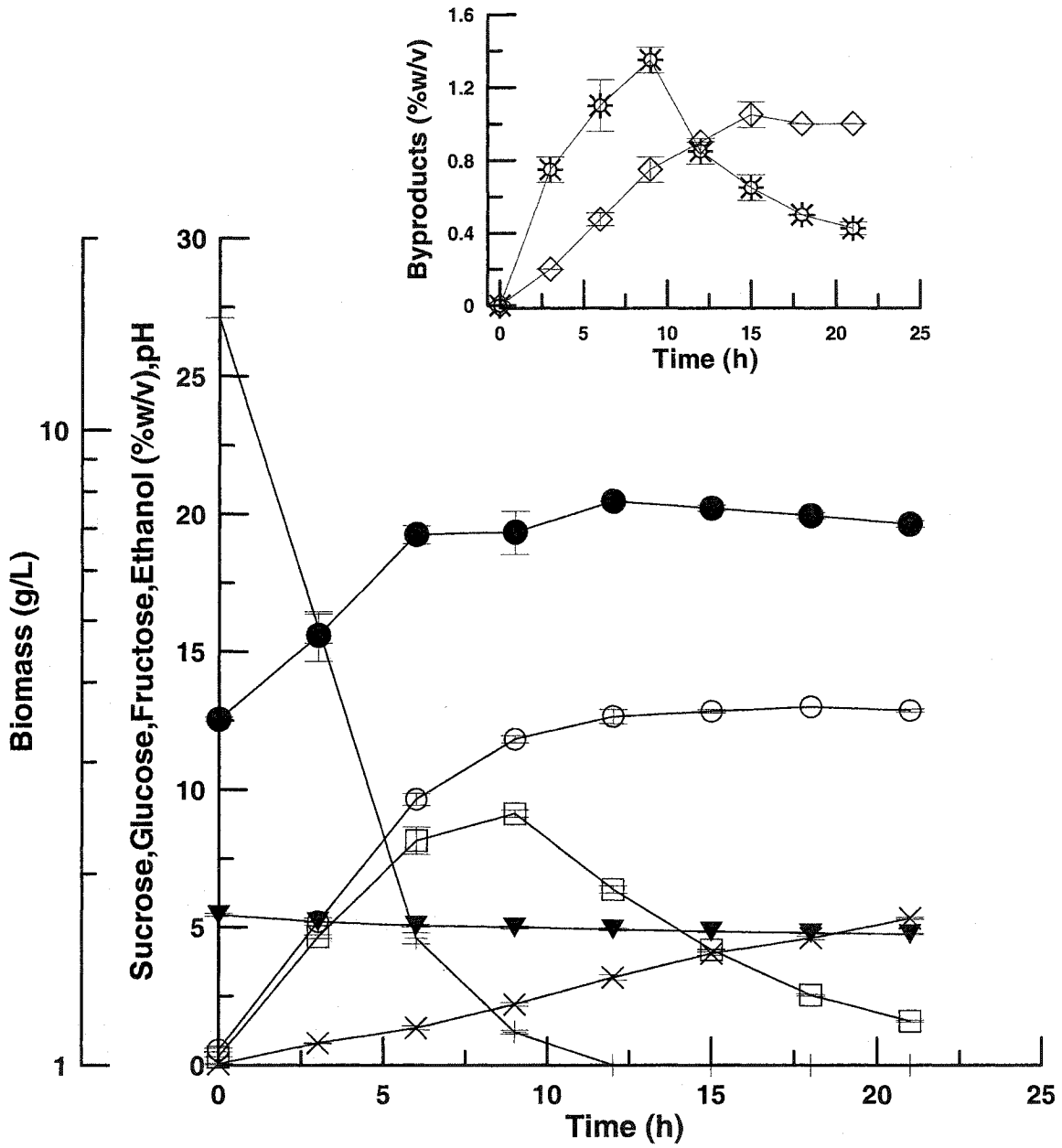

Figure 5.4 Volume corrected reactor data of batch fermentation/pervaporation using the commercial small pervaporation module: Initial sucrose concentration, 27.1 % (w/v); initial biomass concentration, 3.5 g/L, temperature 33°C; initial volume, 150 mL; pervaporation initiated after 6 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

The system was successful in removing the ethanol from the fermentation broth where the ethanol concentration without volume correction is 2.8 %(w/v) at the end of the fermentation instead of 5.9 %(w/v) in batch without membrane separation. The advantage of combining fermentation with pervaporation is immediately recognised in the reduction of the fermentation time from 30 hours without pervaporation to 21 hours with pervaporation.

The system was operated as a batch reactor during the first 6 hours of fermentation to allow the ethanol concentration to reach a level of approximately 1.4 %(w/v). In both cases with and without pervaporation, the sucrose hydrolysis rate was approximately 30 g/(L h). This was due to the fact that most of the sucrose was hydrolyzed during the first 9 hours of fermentation.

The final fructose concentration at the end of the fermentation is 12.9 %(w/v), which gives us a fructose yield of 90.3 % compared to the 94.7% without pervaporation. One factor leading to a smaller fructose yield is probably due to the kestoses level, which remains at 0.5 %(w/v). Since kestoses are made of two fructose units and one unit of glucose, this may account for a lower fructose yield. One more reason for the decrease in the fructose yield may be due to the fact that when the ethanol inhibition is decreased, the biomass becomes more active, and may start consuming fructose. This may lead to a conclusion that when more ethanol is removed, more fructose is consumed. The biomass yield and the specific growth rate were 0.03 g/g and 0.099/h respectively.

The ethanol concentration in the reactor increases slowly as a function of time, which means that not all the ethanol in the reactor was removed by the membrane. This could be due to the smaller area of the membrane module. The actual ethanol concentration at the start of the pervaporation was around 1.4% (w/v), while it was 2.8% (w/v) at the end of the fermentation. The ethanol concentration in the permeate increased from 11 %(w/v) to 30 % (w/v).

According to the earlier pervaporation tests, the total flux should increase with time, since the ethanol concentration increased. But in fact it has decreased; i.e., the total flux decreased from 24 g/ (m^2h) to 20 g/ (m^2h). One reason for the flux decrease in the feed stream of fermentation/pervaporation is the presence of biomass. As the fermentation proceeds, the concentration of biomass in the reactor increases, and therefore more cells

deposit on the membrane surface, resulting in flux decrease with time. Figure 5.5 shows the membrane performance as a function of fermentation 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.

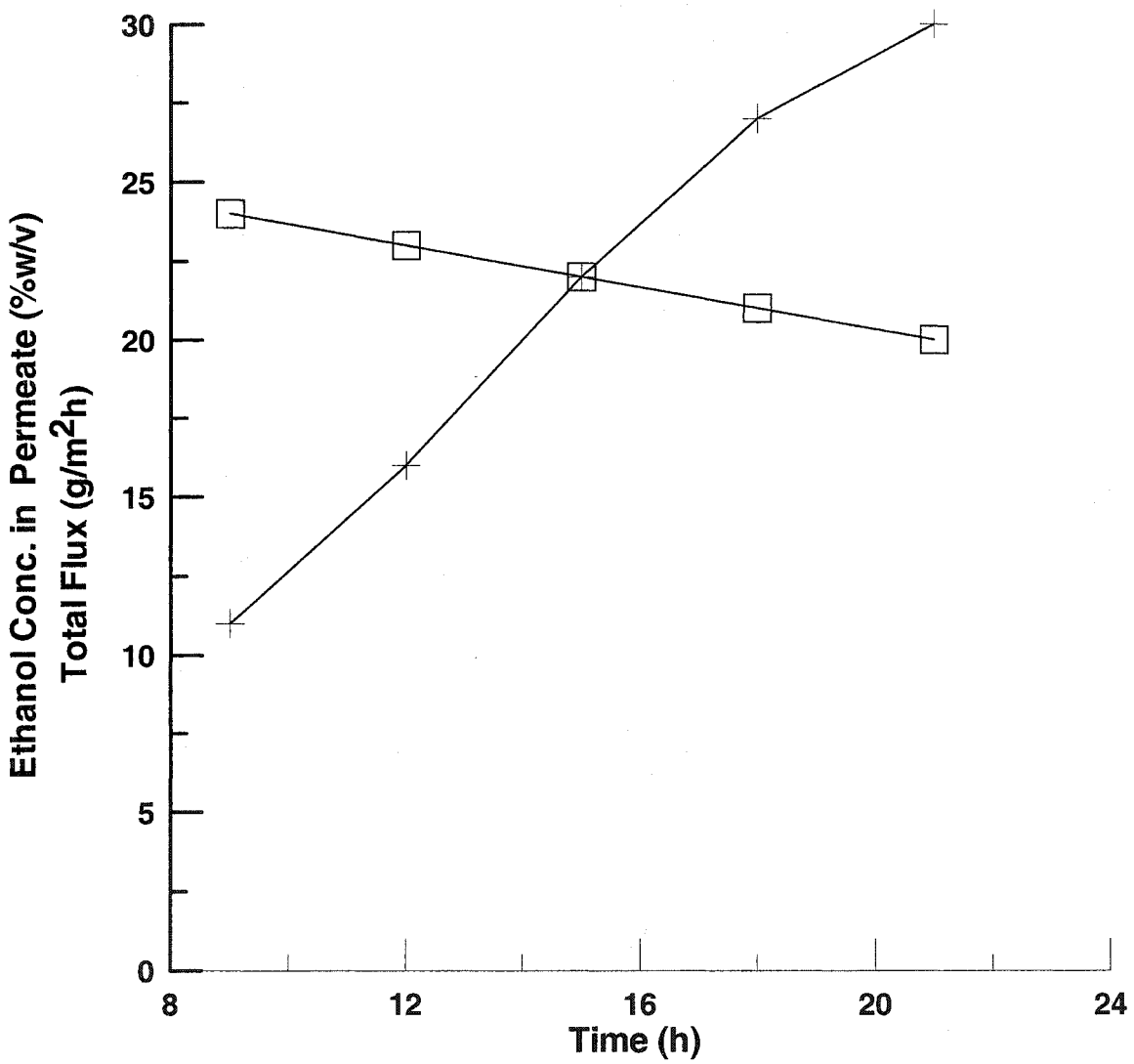

Figure 5.5 Membrane performance of the commercial small membrane module coupled to a bioreactor. Membrane was initiated after 6 hours of batch fermentation with a flow rate of 5 mL/min through the membrane module. (□) Total flux; (+) Ethanol concentration in the permeate.

Ethanol production from three different fermentation/pervaporation experiments is given in figure 5.6 together with the standard deviation.

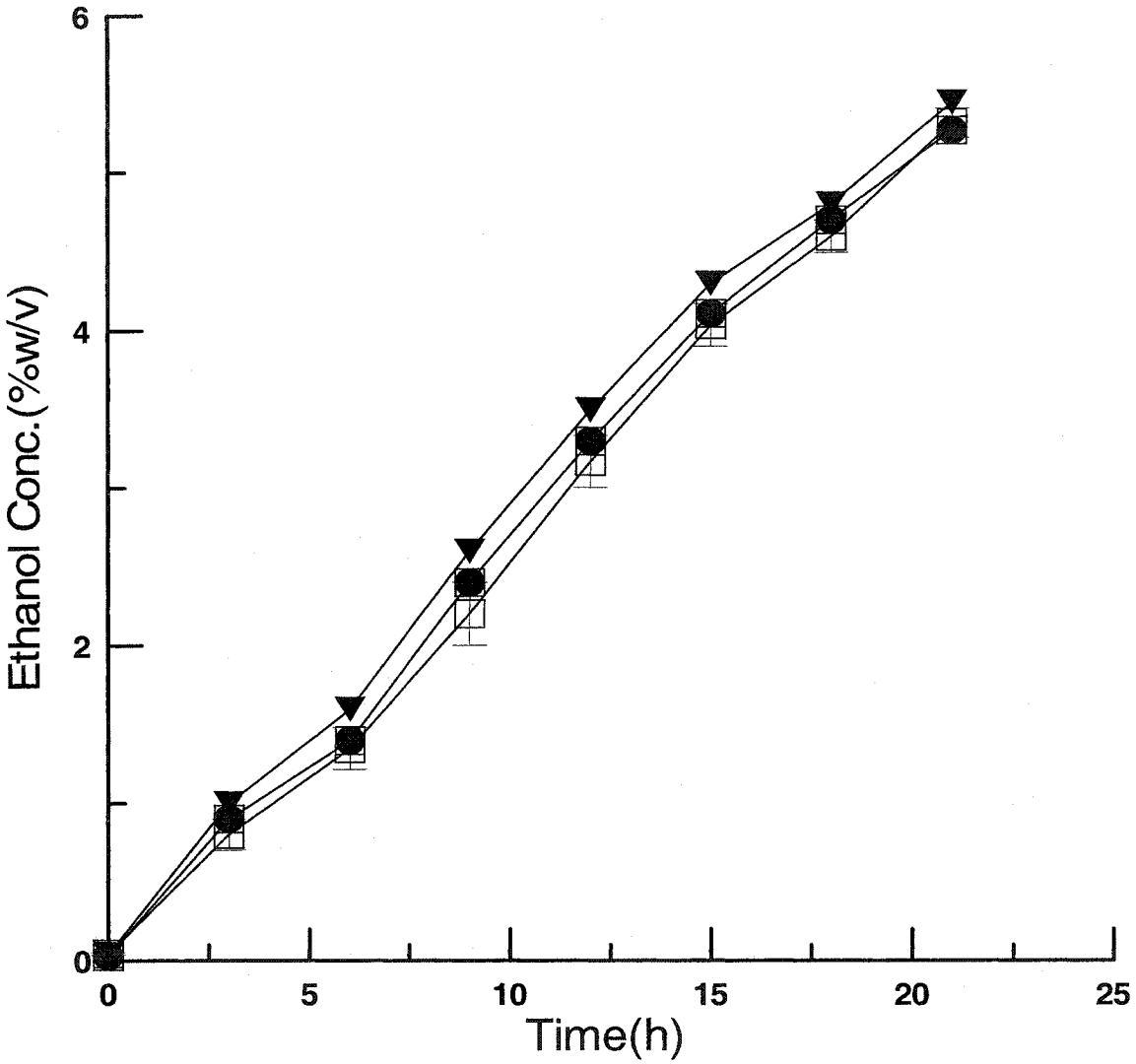

Figure 5.6 Ethanol production from three different fermentation/pervaporation experiments. (□) first run results; (●) second run results; (▼) third run results

5.3.2 Batch Fermentation coupled with Pervaporation B:

The second set of experiments was conducted using the commercial small membrane module under the following operating conditions: initial volume of the fermentation broth in the reactor 150 mL (sucrose medium along with the inoculum), an initial sucrose concentration of 27.1% (w/v), an initial biomass concentration of 3.5 g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation and a flow rate of 5 mL/min through the membrane module. The system was initially kept in batch mode operation for 3 hours, and then pervaporation was started. The second set of experiments was carried out to check whether the fermentation would finish at a faster rate by starting the pervaporation system 3 hours earlier than the previous experiment. It was also carried out to check whether there was an increase or decrease in the fructose and ethanol yields. Experimental results shown in Table 5.4 are volume corrected concentrations obtained at the end of the experiment. Figure 5.7 shows the actual reactor data as a function of time, and Figure 5.8 shows the volume corrected data as a function of time.

Table 5.4 Fermentation/Pervaporation B Parameters

Description	Fermentation/Pervaporation B
Initial Biomass Concentration (g/L)	3.5
Final Biomass Concentration (g/L)	7.7
Initial Sucrose Concentration %(w/v)	27.1
Fermentation Time (h)	21
Final Glucose Concentration %(w/v)	1.0
Final Fructose Concentration %(w/v)	12.3
Final Ethanol Concentration %(w/v)	5.9
Maximum Specific Growth Rate μ (1/h)	0.1059
Biomass Yield (g/g)	0.03
Ethanol Productivity P (g/ (L h))	2.8
Ethanol Yield %(w/v)	78.0
Fructose Yield %(w/v)	86.2
Sucrose Hydrolysis Rate (g/ (L h))	28.9

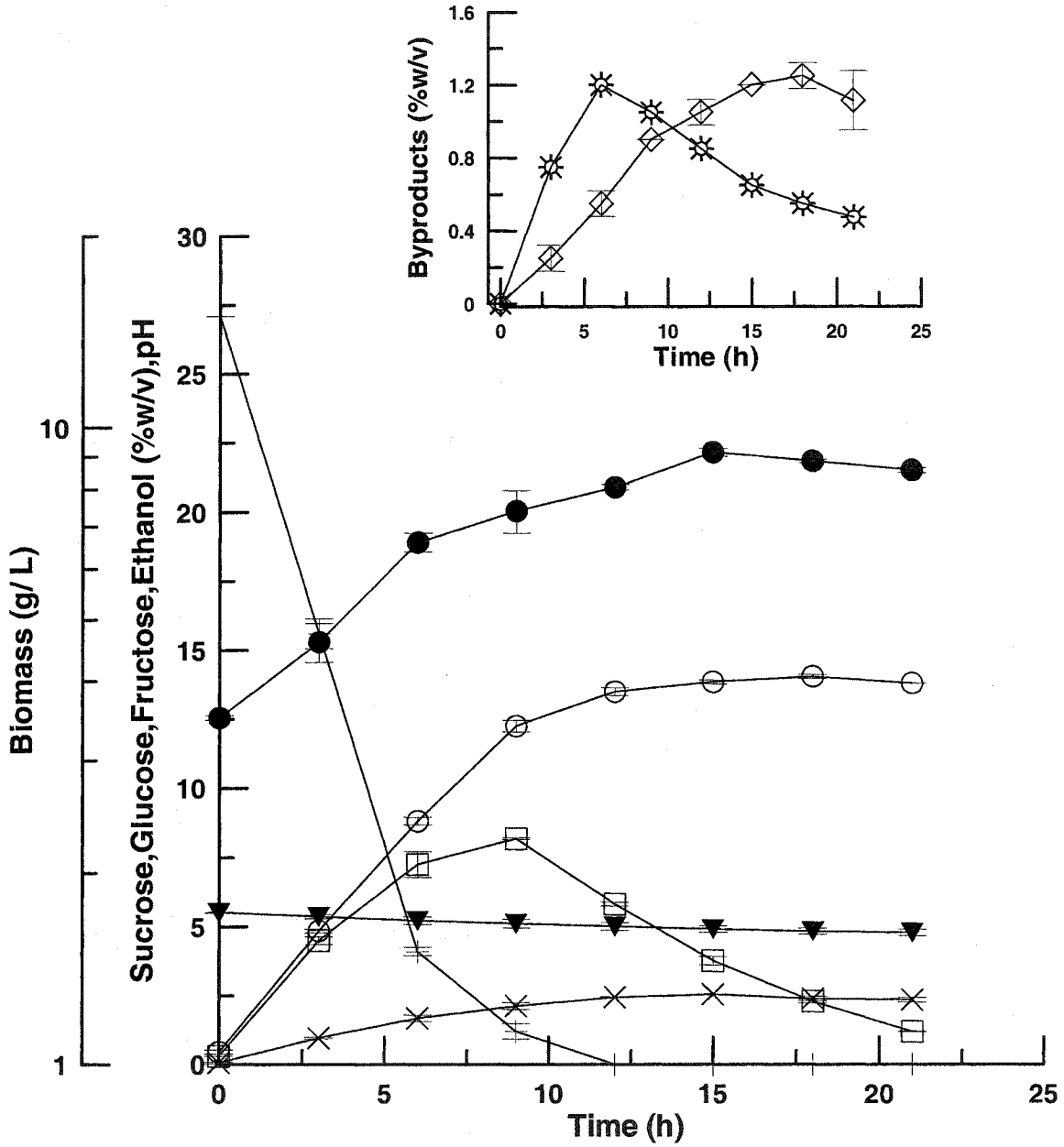

Figure 5.7 Actual reactor data of batch fermentation/pervaporation using the commercial small pervaporation module: Initial sucrose concentration, 27.1 % (w/v); initial biomass concentration, 3.5 g/L, temperature 33°C; initial volume, 150 mL; pervaporation initiated after 3 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

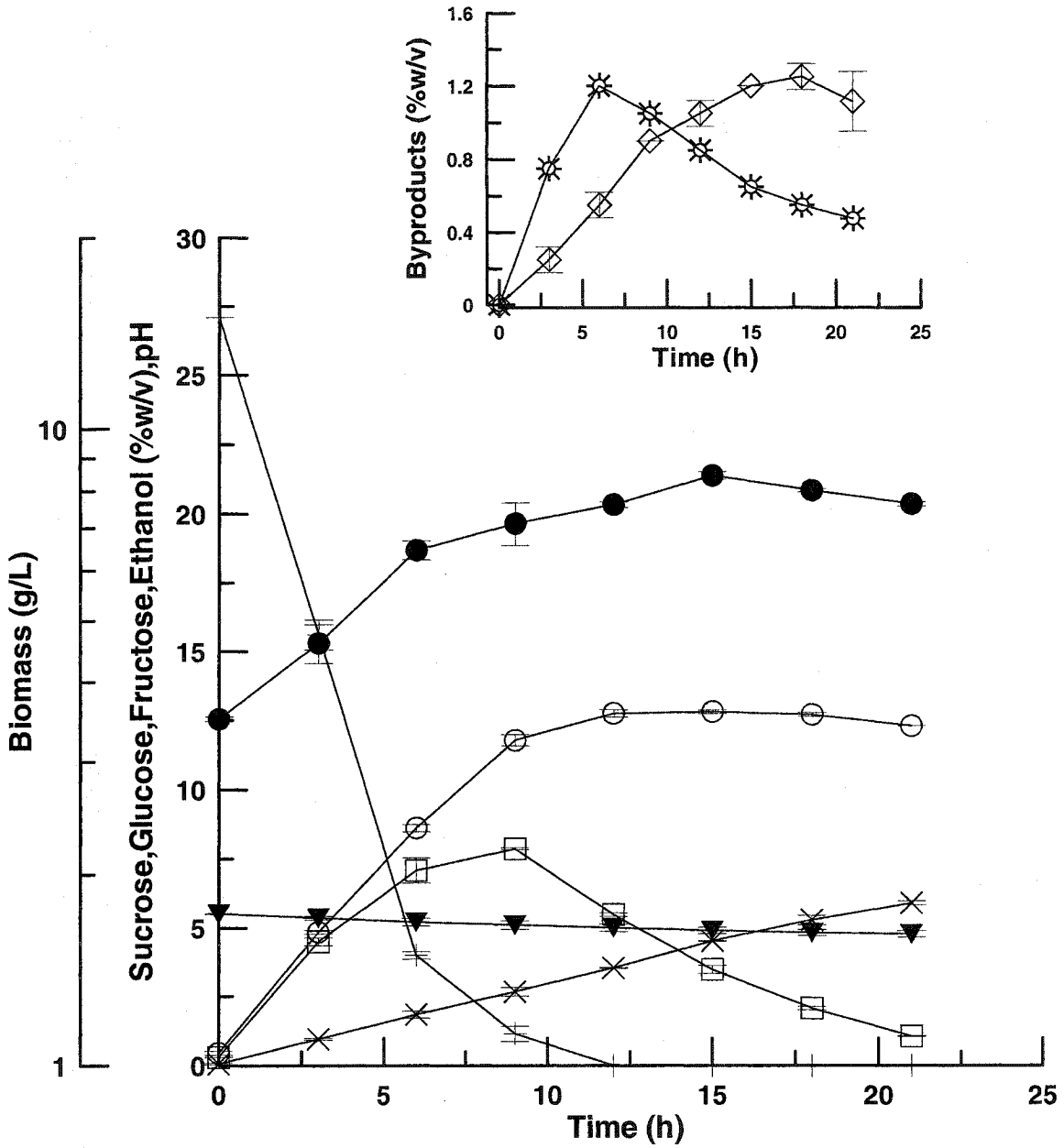

Figure 5.8 Volume corrected reactor data of batch fermentation/pervaporation using the commercial small pervaporation module: Initial sucrose concentration, 27.1 % (w/v); initial biomass concentration, 3.5 g/L, temperature 33°C; initial volume, 150 mL; pervaporation initiated after 3 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

The only difference between this experiment (B) and the previous one (A) is the length of the batch mode operation after which pervaporation was started. A decrease in fructose yield from 90.3 % (w/v) to 86.2 % (w/v) can be immediately noticed. It could have been due to the fact that more fructose has been converted to ethanol. This can be confirmed by the comparison of the final ethanol concentrations, which is 5.9 %(w/v) for experiment (B) compared to 5.3 % (w/v) in experiment (A). An important factor that should be noted here is that even with more ethanol being produced, the final ethanol concentration in the reactor is similar to the previous experiment. Since pervaporation was started after 3 hours of batch fermentation in this experiment, the ethanol was removed for a longer period. This is the reason why the ethanol concentration at the end of the reaction was similar even though a larger amount of ethanol was produced in the experiment. It should be noted that the yeast may have also utilised the fructose towards its growth.

The ethanol concentration in the reactor was 0.9 %(w/v) when pervaporation was initiated after 3 hours of batch reaction, while it was 1.4 %(w/v) after 6 hours of batch reaction. Lower ethanol concentration for the shorter batch reaction is quite expected. In Fermentation/Pervaporation B, the yeast was subjected to a lower ethanol level for a longer period of time and hence more fructose was consumed, lowering the fructose yield. If the fermentation had been stopped after 18 hours, then the fructose yield would have been higher.

Further comparing experiment (A) and (B), the bioreaction parameters are almost the same except for the higher ethanol yield. The flux decreased with time from 22 g/ (m^2h) to 14 g/ (m^2h) as in Figure 5.9, while the ethanol concentration in the permeate increased from 9 % (w/v) to 35% (w/v).Here, the concentration of ethanol in the permeate is larger than experiment A.

In conclusion, beginning of pervaporation after 3 hours instead of 6 hours would be beneficial because the bioreaction could be stopped earlier with only a small decrease in fructose yield. The process should not continue for a longer period of time, because the fructose yield would decrease. The velocity, which was calculated using the flow rate through the membrane module and the cross sectional area of the membrane module was found to be $1.049 * 10^{-4}$ m/s.

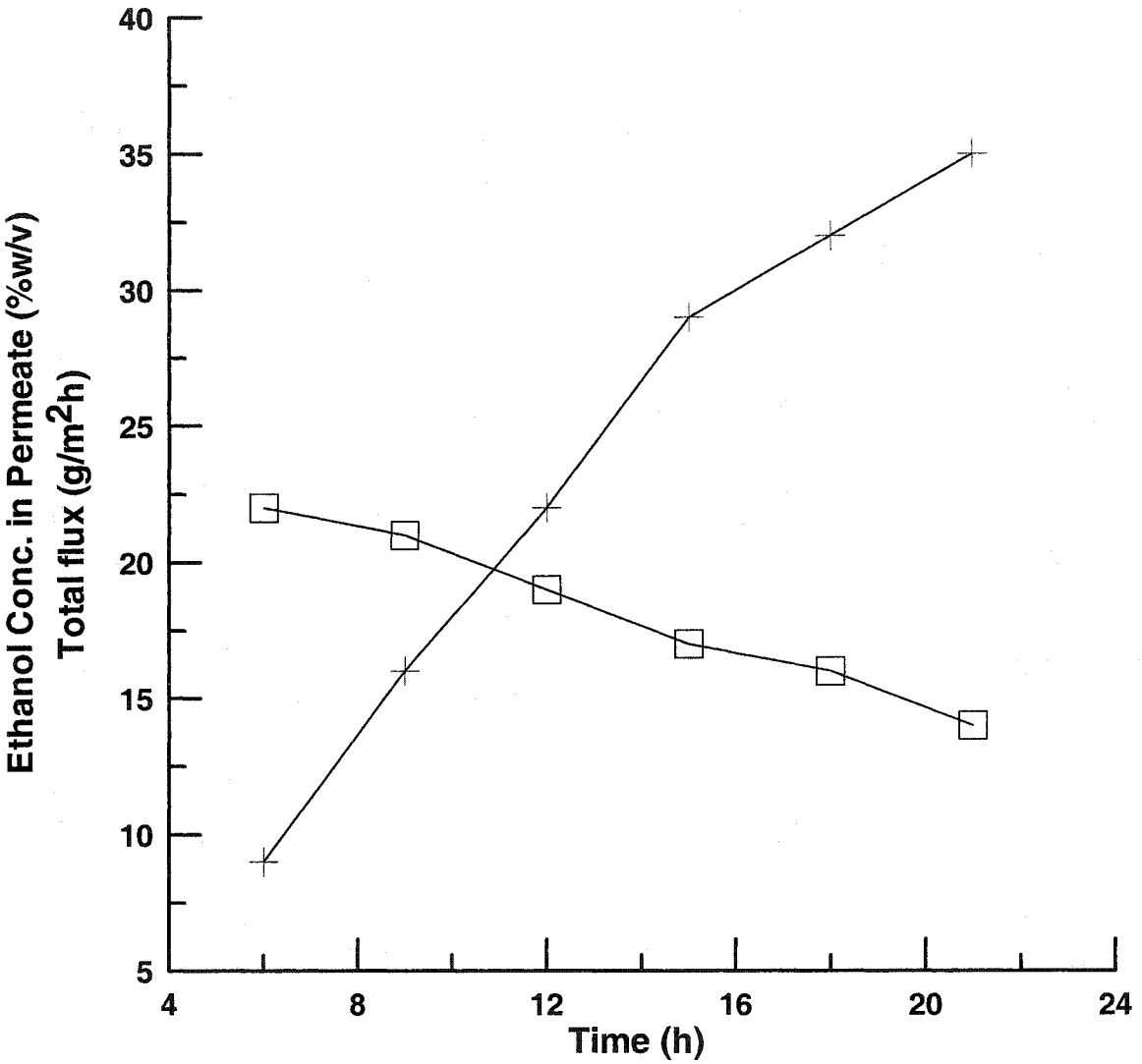

Figure 5.9 Membrane performance of the commercial small membrane module coupled to a bioreactor. Membrane was initiated after 3 hours of batch fermentation with a flow rate of 5 mL/min through the membrane module. (□) Total Flux; (+) Ethanol concentration in permeate.

Ethanol production from three different fermentation/pervaporation experiments is given in figure 5.10 together with the standard deviation.

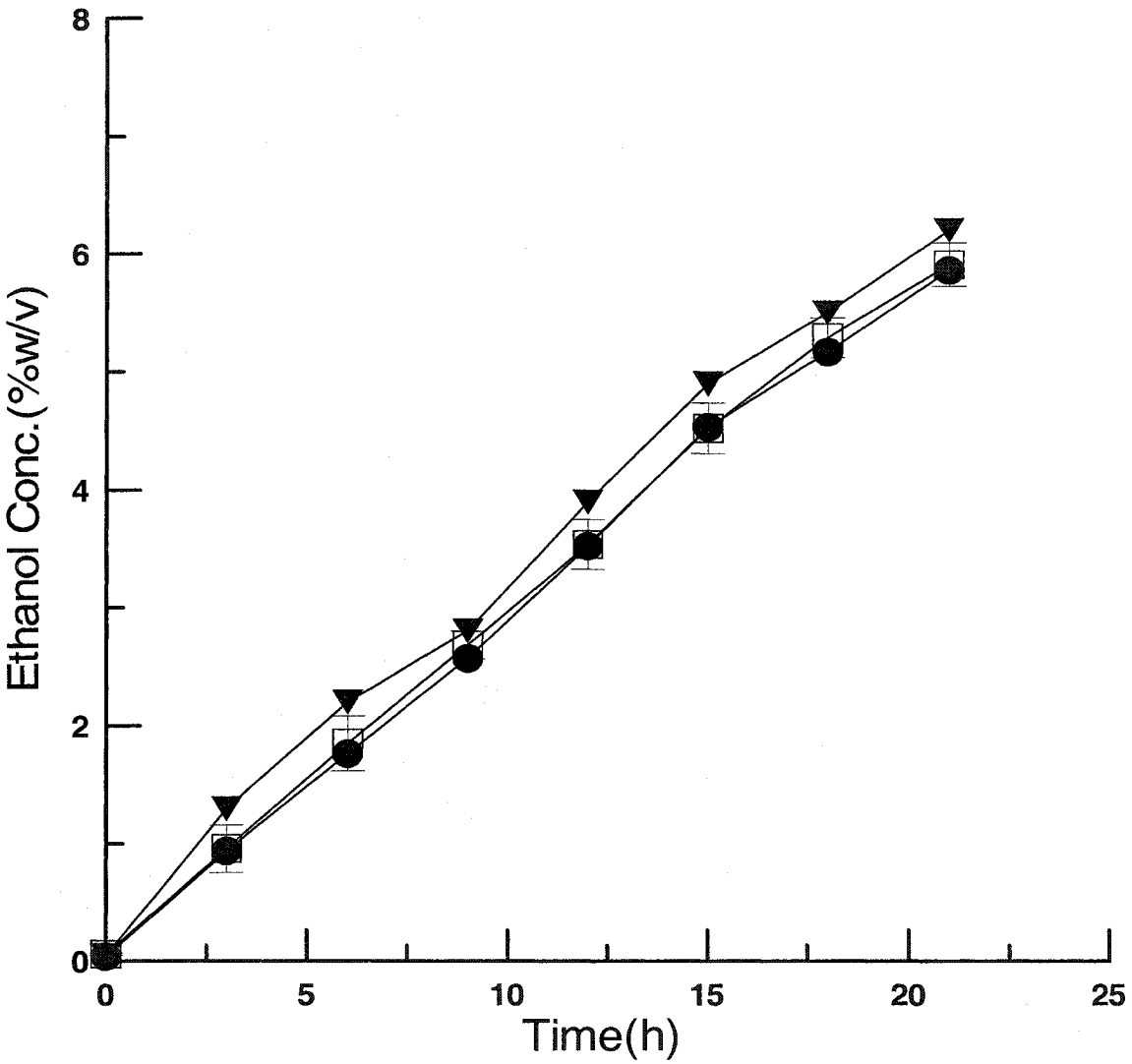

Figure 5.10 Ethanol production from three different fermentation/pervaporation experiments. (□) first run results; (●) second run results; (▼) third run results

5.3.3 Batch Fermentation coupled with Pervaporation C:

The third set of experiments was conducted using the commercial small membrane module under the following operating conditions: initial volume of the fermentation broth in the reactor 150mL (sucrose medium along with the inoculum), an initial sucrose concentration of 27.1% (w/v), an initial biomass concentration of 3.5 g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation and a flow rate of 5 mL/min through the membrane module. The system was never put under the batch mode operation, which means that in this particular case pervaporation was started simultaneously with batch fermentation. The third set of experiments was carried out to check whether all the ethanol produced in the reactor was completely removed because at the point when fermentation was started, there was no ethanol in the reactor. It was also carried out to check whether there was an increase or decrease in the fructose and ethanol yields. Experimental results are shown in Table 5.5 are volume corrected concentrations obtained at the end of the experiment. Figure 5.11 shows the actual reactor data as a function of time, and Figure 5.12 shows the volume corrected data as a function of time. The velocity, which was calculated using the flow rate through the membrane module and the cross sectional area of the membrane module was found to be $1.049 * 10^{-4}$ m/s.

Table 5.5 Fermentation/Pervaporation C Parameters

Description	Fermentation/Pervaporation C
Initial Biomass Concentration (g/L)	3.5
Final Biomass Concentration (g/L)	8.6
Initial Sucrose Concentration %(w/v)	27.1
Fermentation Time (h)	21
Final Glucose Concentration %(w/v)	0.5
Final Fructose Concentration %(w/v)	11.9
Final Ethanol Concentration %(w/v)	6.6
Maximum Specific Growth Rate μ (1/h)	0.1134
Biomass Yield (g/g)	0.03
Ethanol Productivity P (g/ (L h))	3.7
Ethanol Yield %(w/v)	80.1
Fructose Yield %(w/v)	83.0
Sucrose Hydrolysis Rate (g/ (L h))	29.0

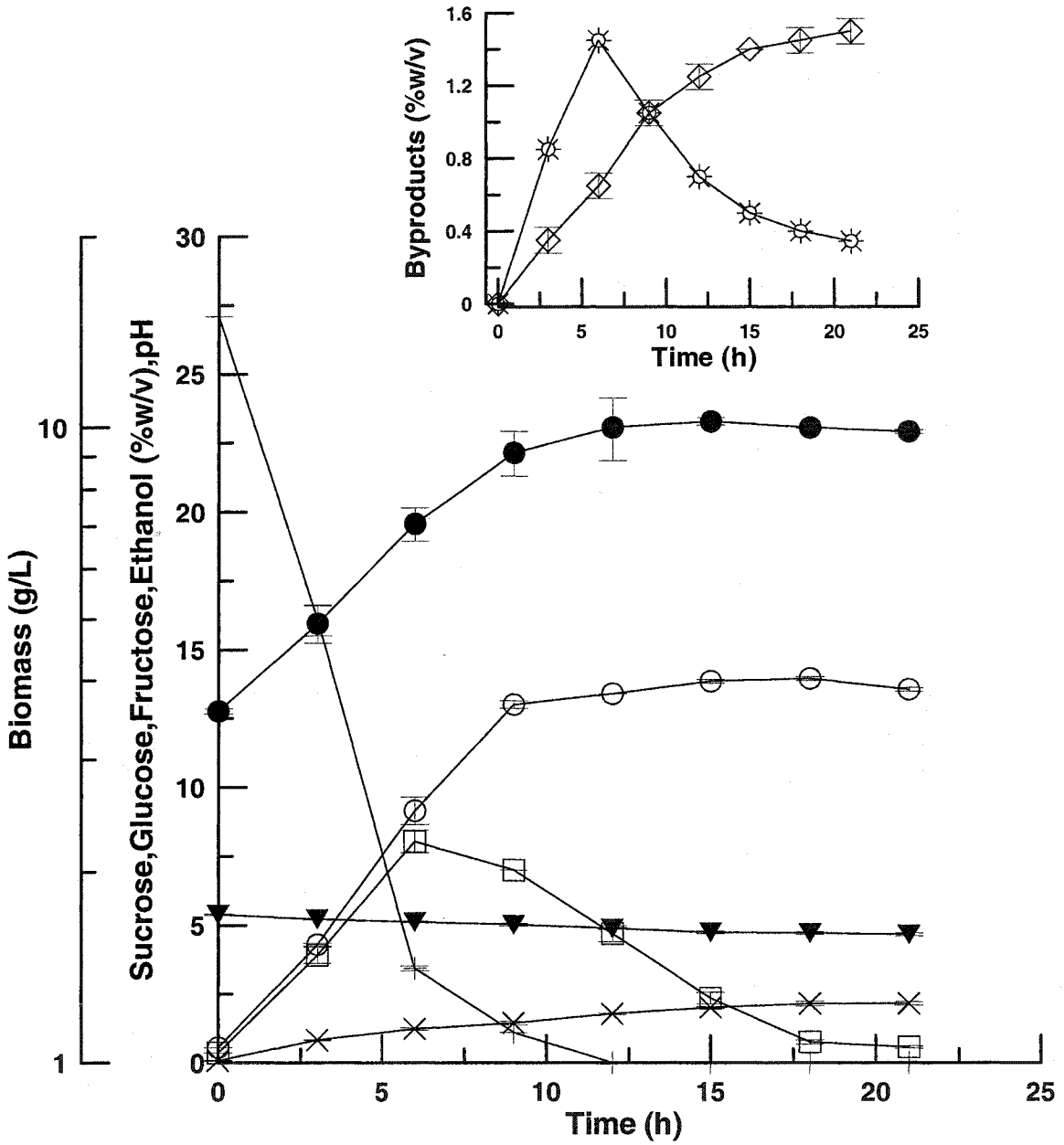

Figure 5.11 Actual reactor data of batch fermentation/pervaporation using the commercial small pervaporation module: Initial sucrose concentration, 27.1 % (w/v); initial biomass concentration, 3.5 g/L, temperature 33°C; initial volume, 150 mL; pervaporation initiated after 0 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

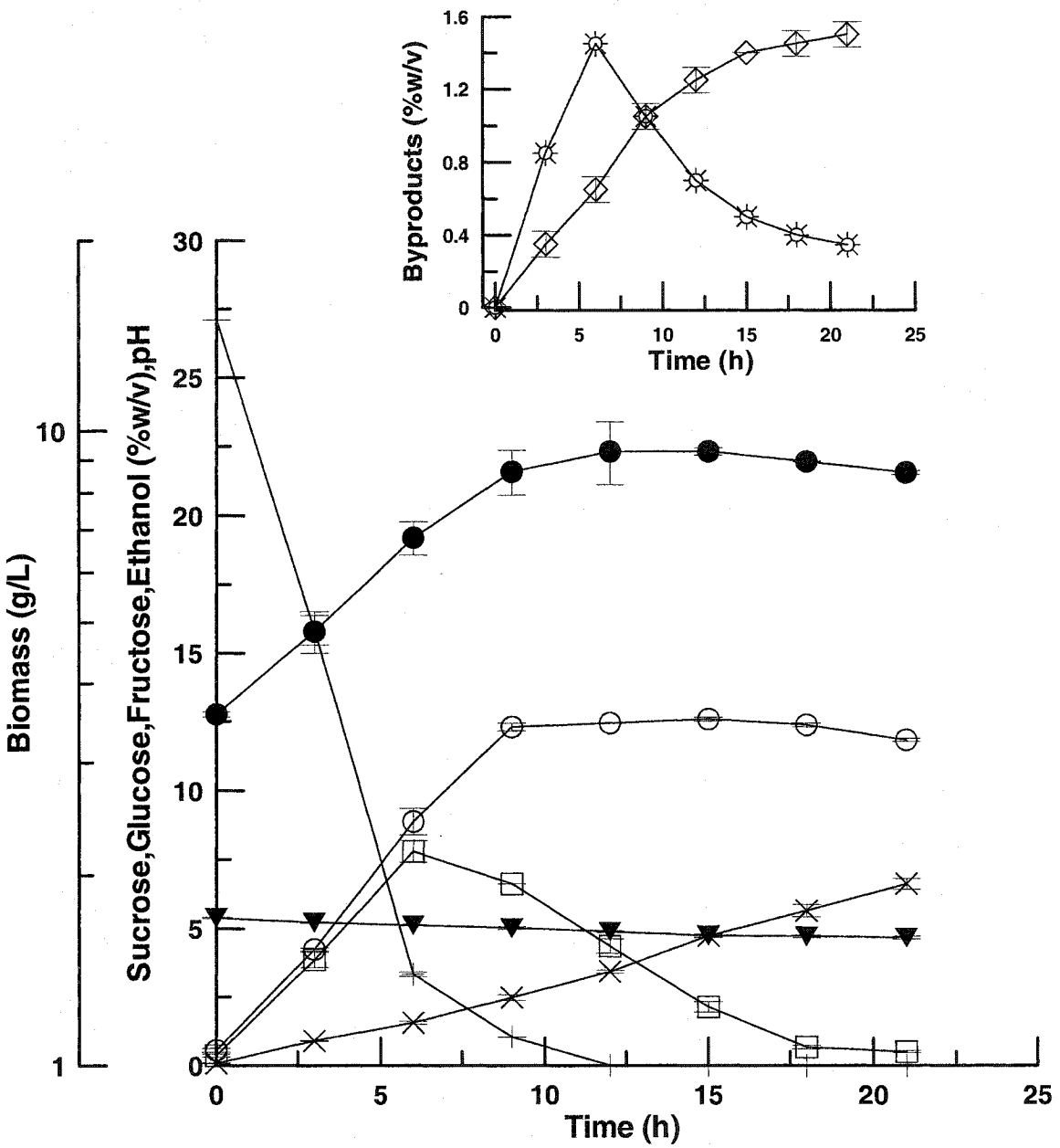

Figure 5.12 Volume corrected reactor data of batch fermentation/pervaporation using the commercial small pervaporation module: Initial sucrose concentration, 27.1 % (w/v); initial biomass concentration, 3.5 g/L, temperature 33°C; initial volume, 150 mL; pervaporation initiated after 0 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

This set of experiments will be compared with Fermentation/Pervaporation (A) and (B) since the only difference between all these experiments was the time at which pervaporation was started. Compared with (A) and (B), there was a large decrease in the fructose yield, which is now 83.0 % compared to 86.2 % with 3 hours of Fermentation/Pervaporation and 90.3 % with 6 hours of Fermentation/Pervaporation. As mentioned earlier, this was mainly due to the fact that a large amount of ethanol was being produced. The final ethanol concentration in experiment (C) was greater than both (A) and (B) experiments as can be seen in table 5.6. This is because the yeast became more active in experiment (C) with a longer period of pervaporation and started converting fructose to ethanol. The actual ethanol concentrations in the reactor at the end of fermentation were (A) = 2.8 % (w/v), (B) = 2.3 % (w/v), (C) = 2.1 % (w/v). The lowest ethanol concentration in experiment (C) indicates that ethanol was removed effectively during the entire period of pervaporation. The ethanol productivity was 3.7 g/ (L h) at the end of the fermentation in experiment (C).

Further looking into Table 5.6, the final fructose and glucose concentration are the lowest in experiment (C) which also indicates the higher consumption level of glucose and fructose by the active yeast. Both biomass and specific growth rate were greater in experiment (C) than (A) and (B).

Figure 5.13 shows that the total flux decreased from 18 g/ (m^2h) to 12 g/ (m^2h), and the ethanol concentration in the permeate increased from 6 % (w/v) to 38 % (w/v) during the period of fermentation. The ethanol concentration in the permeate covers a range larger than experiments (A) and (B) because there was no ethanol in the reactor when the pervaporation was started.

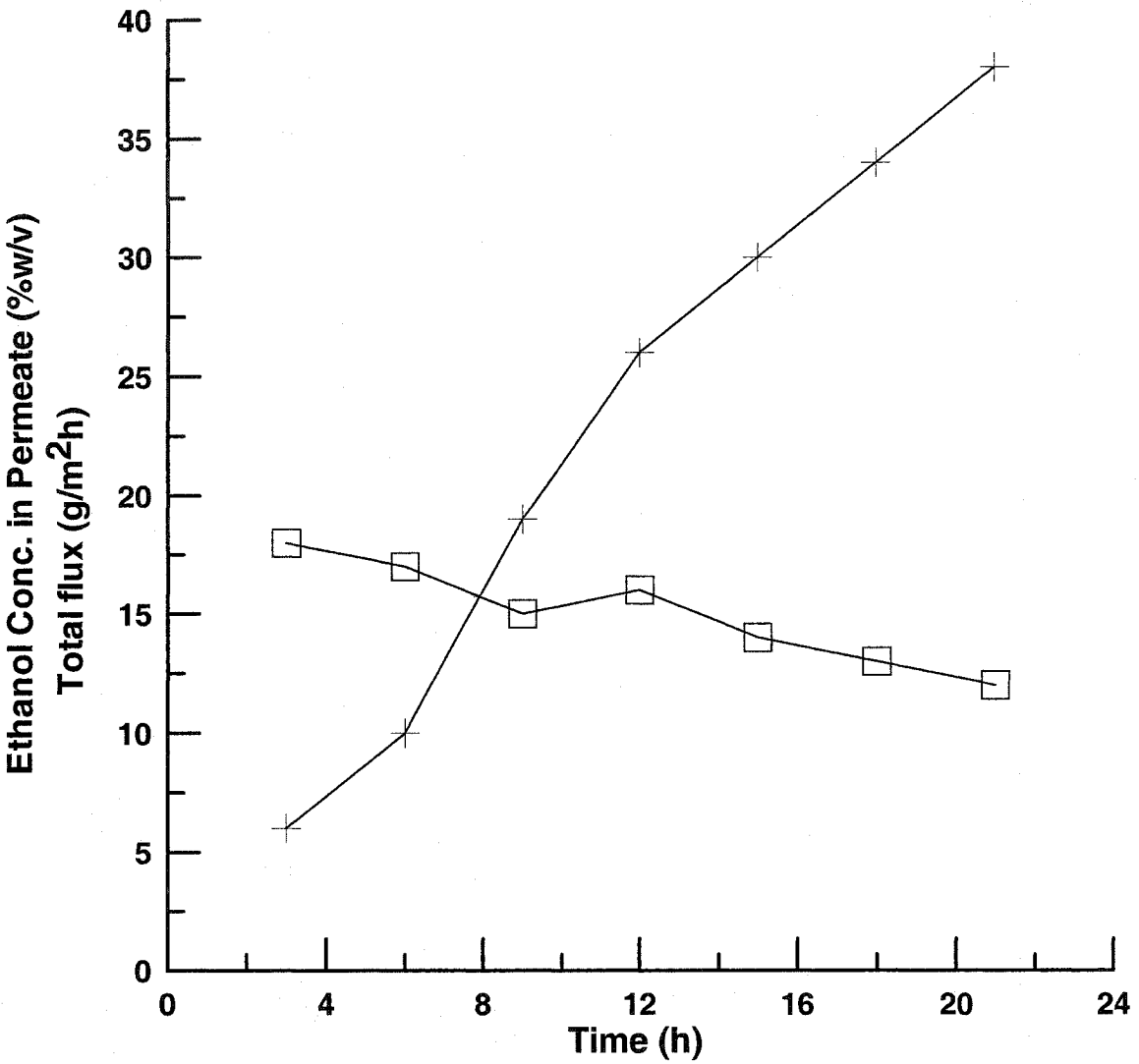

Figure 5.13 Membrane performance of the commercial small membrane module coupled to a bioreactor. Membrane was initiated after 0 hours of batch fermentation with a flow rate of 5 mL/min through the membrane module. (□)Total Flux; (+) Ethanol concentration in the permeate.

Ethanol production from three different fermentation/pervaporation experiments is given in figure 5.14 together with the standard deviation.

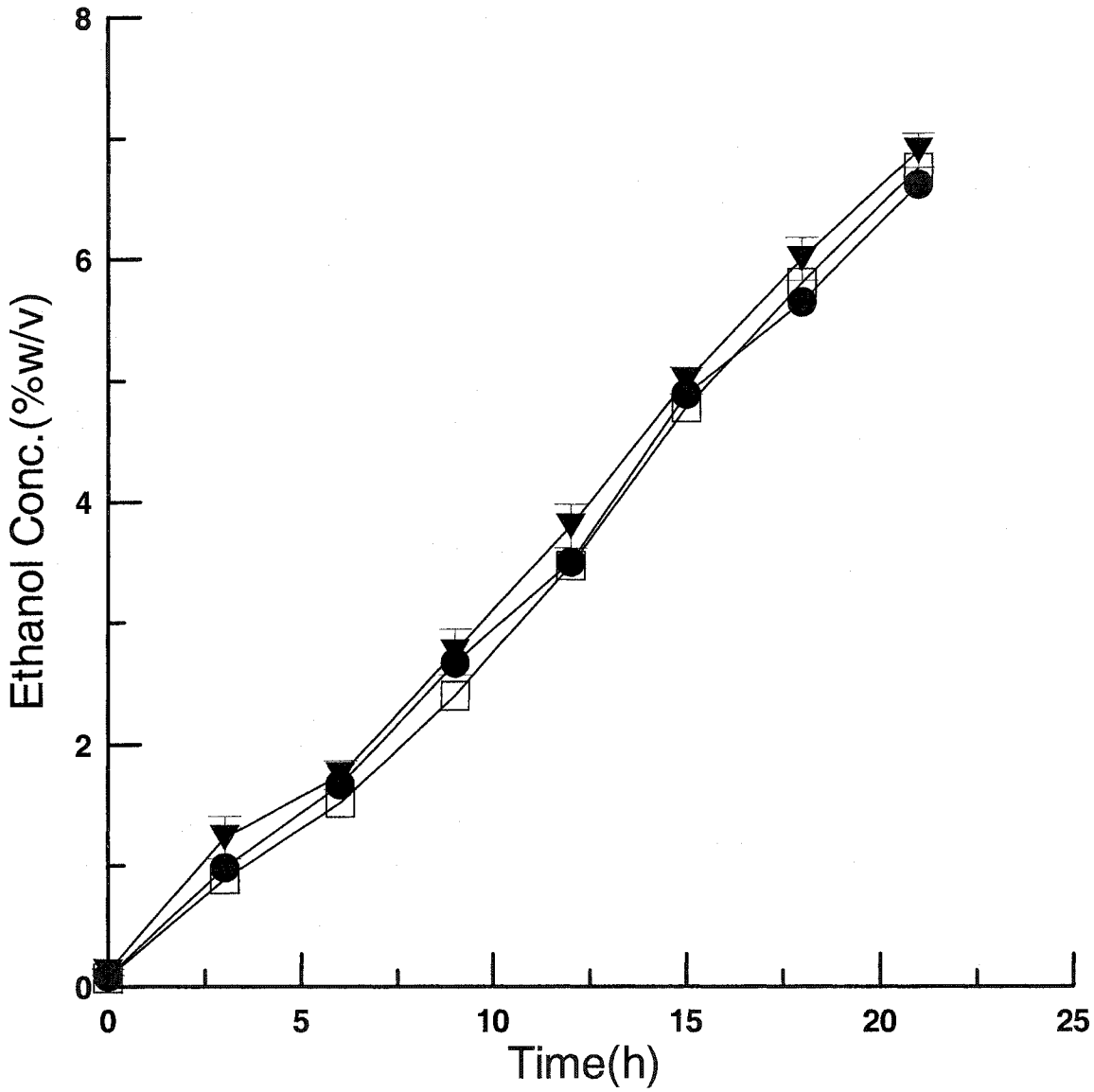

Figure 5.14 Ethanol production from three different fermentation/pervaporation experiments. (□) first run results; (●) second run results; (▼) third run results

Comparison of experiments A, B, C can be seen from table 5.6.

Table 5.6 Comparison of parameters between experiments (A), (B) and (C)[a]

Description	A	B	C
Initial Sucrose conc(%w/v)	27.1	27.1	27.1
Initial Biomass conc (g/L)	3.5	3.5	3.5
Final Biomass conc (g/L)	7.1	7.7	8.6
Final Fructose conc (%w/v)	12.9	12.3	11.9
Final Glucose conc (%w/v)	1.6	1.0	0.50
Maximum Specific Growth Rate μ (1/h)	0.099	0.1059	0.1134
Fructose Yield (%)	90.3	86.2	83.0
Ethanol Yield (%)	73.5	78.0	80.1
Biomass Yield (g/g)	0.03	0.03	0.03
Ethanol Productivity P (g/ (L h))	2.5	2.8	3.7
Final Ethanol conc (%w/v)	5.3	5.9	6.6
Sucrose Hydrolysis Rate(g/ (L h))	28.8	28.9	29
Time (h)	21	21	21

[a] All concentrations are volume corrected values

A = denotes pervaporation experiments initiated after 6 hours of batch fermentation.

B = denotes pervaporation experiments initiated after 3 hours of batch fermentation.

C = denotes pervaporation experiments initiated after 0 hours of batch fermentation.

5.4 Pervaporation of Ethanol/Water Mixtures using the Large Commercial Module (M60-4000)

In order to check the performance of the membrane module coupled to a bioreactor, pervaporation tests of ethanol/water mixtures at various concentrations were carried out.

The performance test was carried out with a larger hollow fiber membrane module at different ethanol concentrations in the feed. The operating conditions were as follows: initial volume of the ethanol/water mixture in the reactor 275 mL, downstream pressure of 1 torr, feed temperature of 33°C, flow rate of 2.5 mL/min through the membrane module and a turnover time of 110 min through the reactor. The only difference compared to the previous experiment with a smaller module was the increase in the flow rate. The larger area of the membrane module was the reason for the increase in the flow rate. The experimental results are summarized in Table 5.7 and Figure 5.15. The ethanol concentration in the permeate and the total flux kept increasing when the feed ethanol concentration was increased from 2 % (w/v) to 5 % (w/v), while the separation factor decreased slightly. The total flux increased from 5.1 g/ (m^2h) to 11.3 g/ (m^2h) and the ethanol concentration in the permeate increased from 26 % (w/v) to 45 % (w/v) as the ethanol concentration in the feed increased. The separation factor decreased from 17.1 to 15.5 with the increasing feed ethanol concentration. It was found that the data from the larger module was similar to that of the smaller module except for the total flux that was less than half of the smaller module.

Table 5.7 Experimental total, water, and ethanol flux as a function of ethanol feed concentration

Ethanol in Feed (% w/v)	Total Flux g/ (m^2h)	Ethanol Flux g/ (m^2h)	Water Flux g/ (m^2h)	Separation factor
2	5.1	1.3	3.8	17.21
3	6.6	2.2	4.4	16.65
4	9.1	3.7	5.4	16
5	11.3	5.0	6.3	15.54

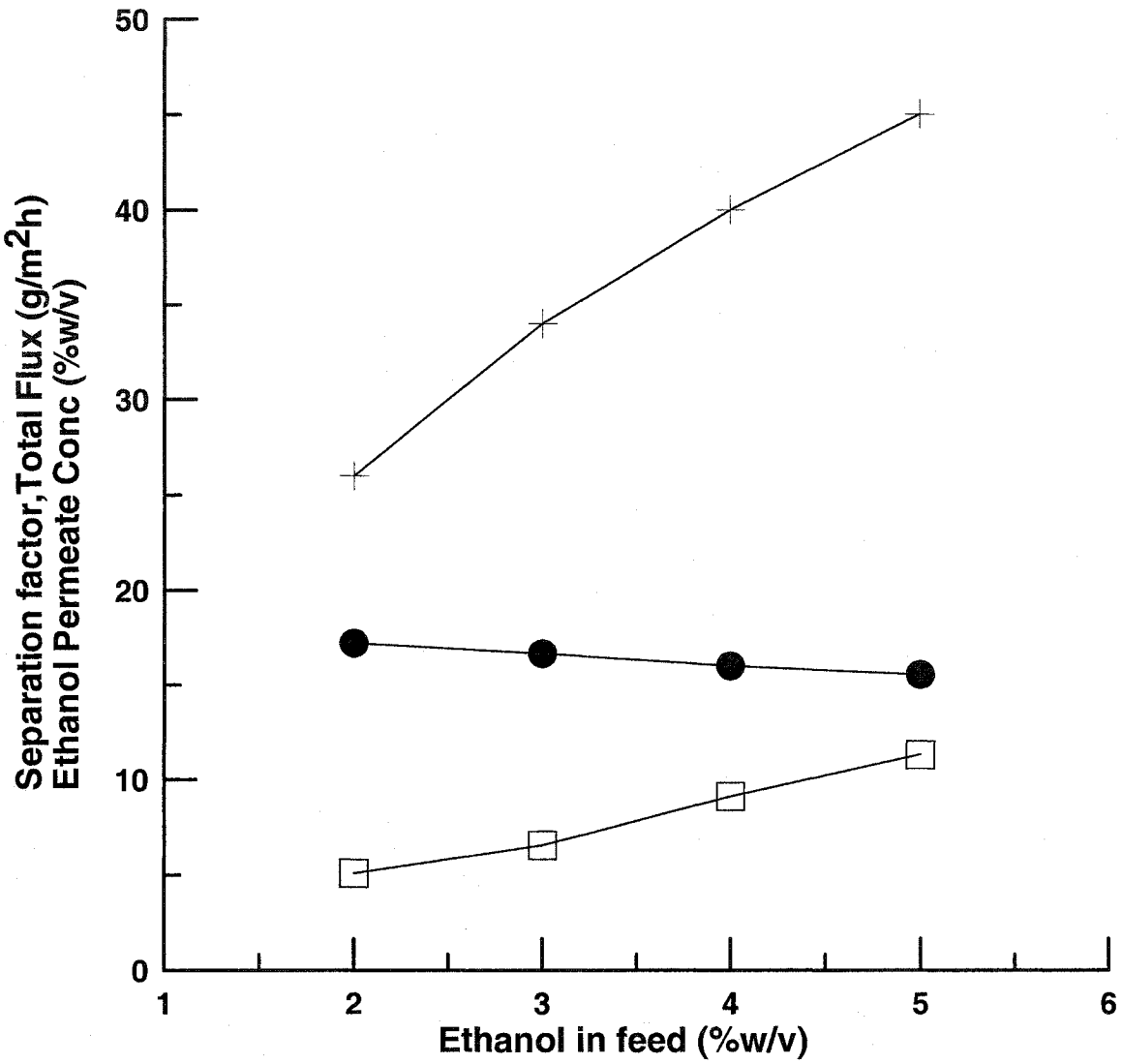

Figure 5.15 Effect of feed ethanol concentration on the membrane performance for pervaporation through silicone rubber hollow fibers (Large Membrane). A downstream pressure of 1 torr; temperature, 33°C; circulation rate, 2.5 mL/min. (●) Separation factor, (□) Total Flux, (+) Ethanol concentration in the permeate.

5.5 Batch Fermentation coupled with Pervaporation D:

The first set of experiments was conducted using the large commercial membrane module under the following conditions: initial volume of the fermentation broth in the reactor 275 mL (sucrose medium along with the inoculum), an initial sucrose concentration of 28.7 %(w/v), an initial biomass concentration of approximately 5.5 g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation and a turnover time of 55 min through the reactor. A flow rate of 5 mL/min was maintained through the membrane module. The increase in the reactor volume and the initial biomass concentration from the previous experiments was to due to the fact that more ethanol/water solution would be removed by pervaporation due to the use of a larger membrane module and to help the experiment proceed at a faster rate. Experimental results shown in Table 5.8 are volume corrected concentrations obtained at the end of the experiment. Figure 5.16 shows the actual reactor data as a function of time, and Figure 5.17 shows the volume corrected data as a function of time.

Table 5.8 Fermentation/Pervaporation D Parameters

Description	Fermentation/Pervaporation D
Initial Biomass Concentration (g/L)	5.2
Final Biomass Concentration (g/L)	7.7
Initial Sucrose Concentration %(w/v)	28.7
Fermentation Time (h)	22
Final Glucose Concentration %(w/v)	1.1
Final Fructose Concentration %(w/v)	13.7
Final Ethanol Concentration %(w/v)	5.2
Maximum Specific Growth Rate μ (1/h)	0.04
Biomass Yield (g/g)	0.02
Ethanol Productivity P (g/ (L h))	2.4
Ethanol Yield %(w/v)	66.3
Fructose Yield %(w/v)	90.6
Sucrose Hydrolysis Rate (g/ (L h))	30.3

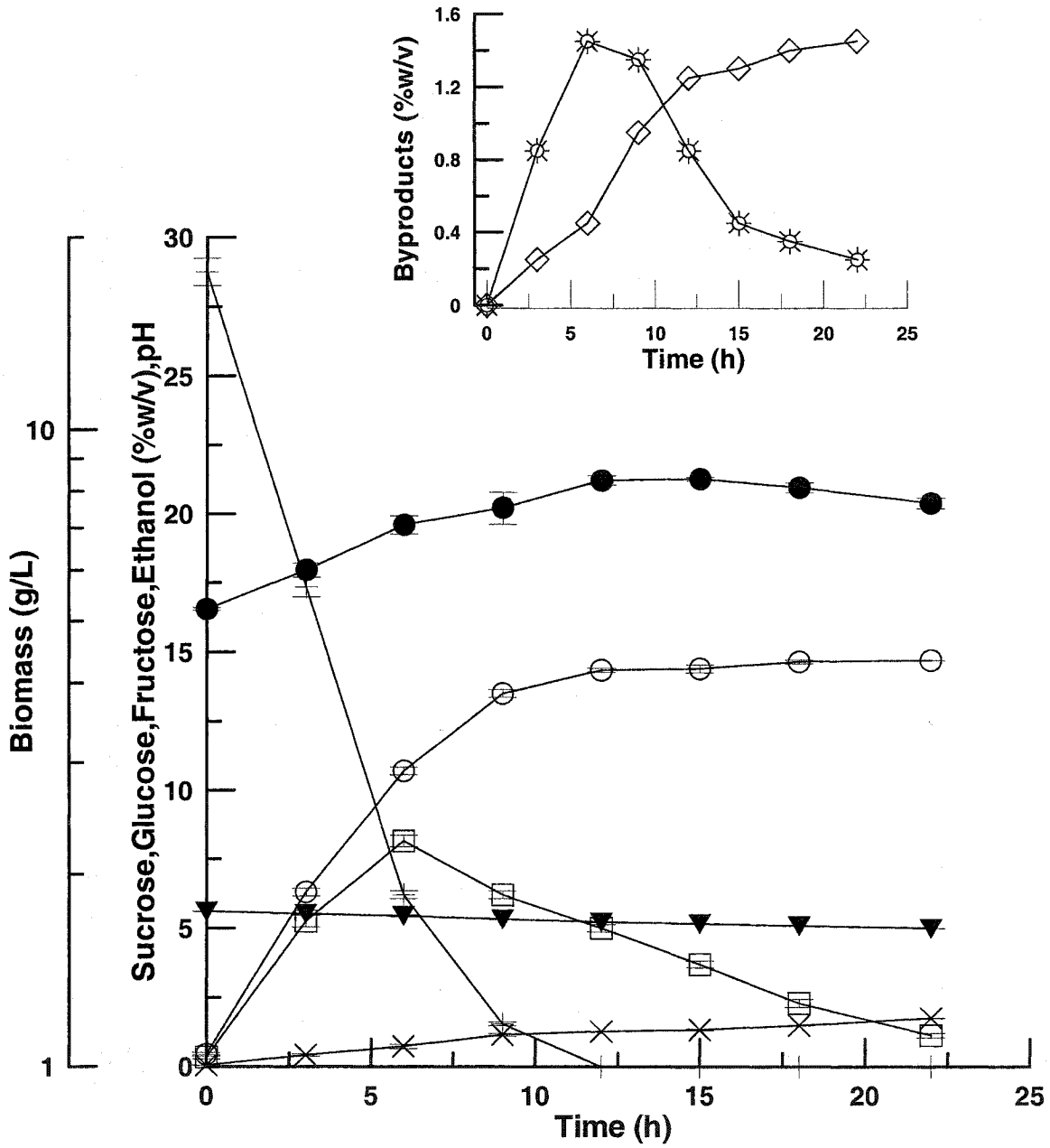

Figure 5.16 Actual reactor data of batch fermentation/pervaporation using the commercial large pervaporation module: Initial sucrose concentration, 28.3 % (w/v); initial biomass concentration, 5.3 g/L, temperature 33°C; initial volume, 275 mL; 5 mL/min flow through the membrane module; pervaporation initiated after 3 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●) Biomass, (▼) pH, (x) Ethanol (☼) Kestoses, (◊) Glycerol.

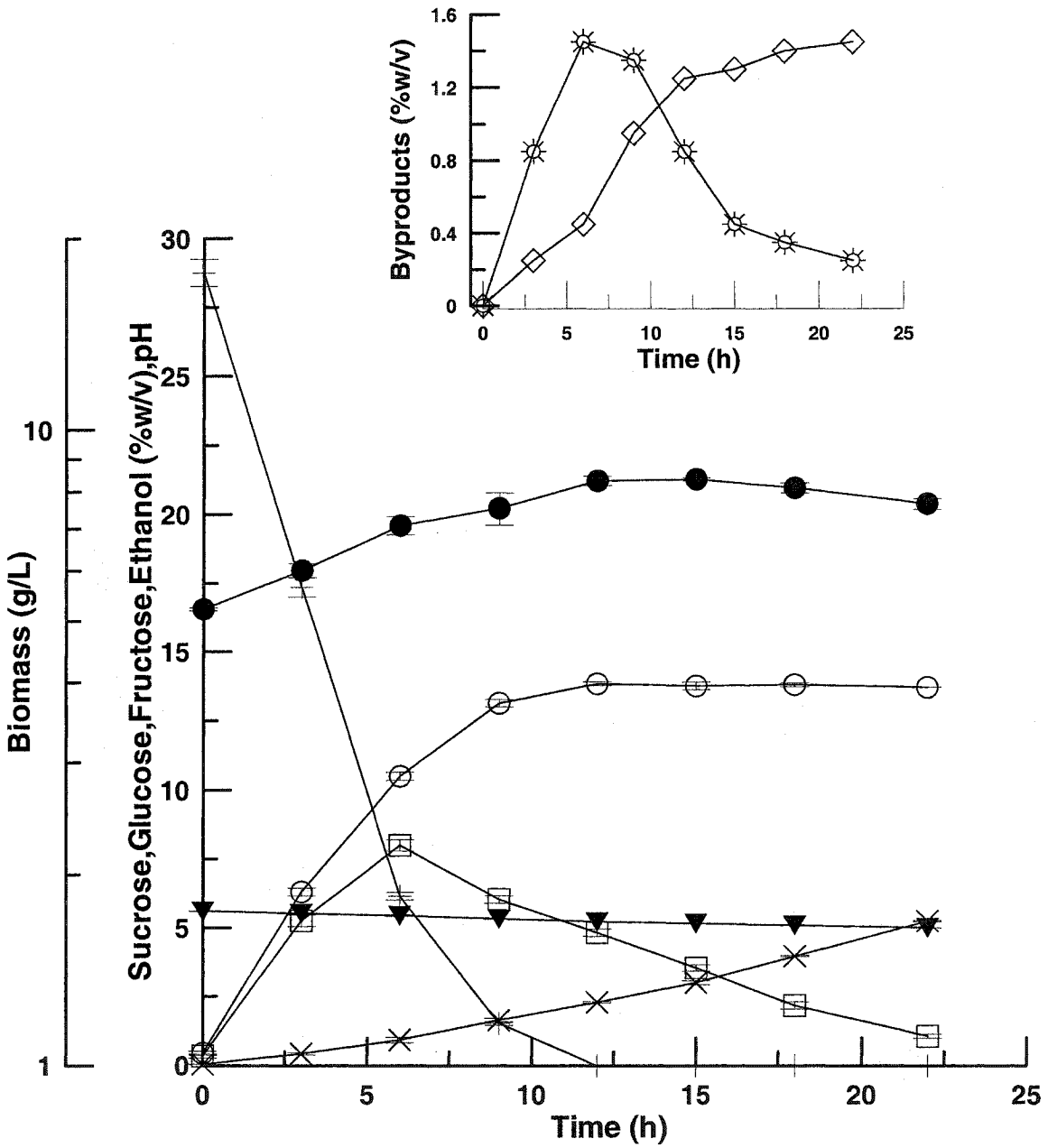

Figure 5.17 Volume corrected reactor data of batch fermentation/pervaporation using the commercial large pervaporation module: Initial sucrose concentration, 28.3 % (w/v); initial biomass concentration, 5.3 g/L, temperature 33°C; initial volume, 275 mL; 5 mL/min flow through the membrane module; pervaporation initiated after 3 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

The bioreactor was kept in a batch mode for 3 hours, before pervaporation was started in order to make sure that at least some ethanol was being produced in the reactor. At the point when pervaporation was initiated, the vacuum pump was started with the peristaltic pump circulating the feed medium from the top to the bottom of the membrane module. It was noticed that there was no retention of the medium at the top of the membrane module. It may suggest that all the hollow fibers in the membrane module were not entirely utilized. The feed after passing through the membrane was re-circulated back into the reactor.

The experiment was carried out for 22 hours, until the glucose concentration in the reactor became almost negligible or similar to the set of experiments with the small membrane module. The final fructose concentration was 13.7 % (w/v), which gave a fructose yield of 90.6 %. The ethanol concentration at the end of the experiment was 5.2 % (w/v), which gave an ethanol yield of 66.3 % and an ethanol productivity of 2.4 g / (L h).

The maximum specific growth rate and the biomass yield were 0.0312/h and 0.02 g/g respectively. The biomass started increasing slowly in the reactor from 5.2 g/L and finally reached 7.7 g/L. Figure 5.18 shows that the ethanol concentration in the permeate increased from 13 % (w/v) to 52 % (w/v). The increase in the final value of the permeate shows that more ethanol has been removed from the reactor. The flux decreased from 3.7 g/ (m^2h) to 1.9 g/ (m^2h). One of the reasons for the decrease in the flux could be due to fouling. Fouling tended to happen when biomass settled on both the top and the bottom of the membrane module even though the module was flushed with 80 % ethanol solution after every single fermentation/pervaporation experiment. Flushing did not remove all the biomass settled on the top and bottom of the module. A different method for cleaning the membrane module would help maintain an increased flux.

The advantage of increasing the volume of the feed medium in the reactor was to show that more liquid could be removed from the reactor with the help of a larger membrane module at the same time. The velocity, which was calculated using the flow rate through the membrane module and the cross sectional area of the membrane module was found to be $1.562 * 10^{-4}$ m/s.

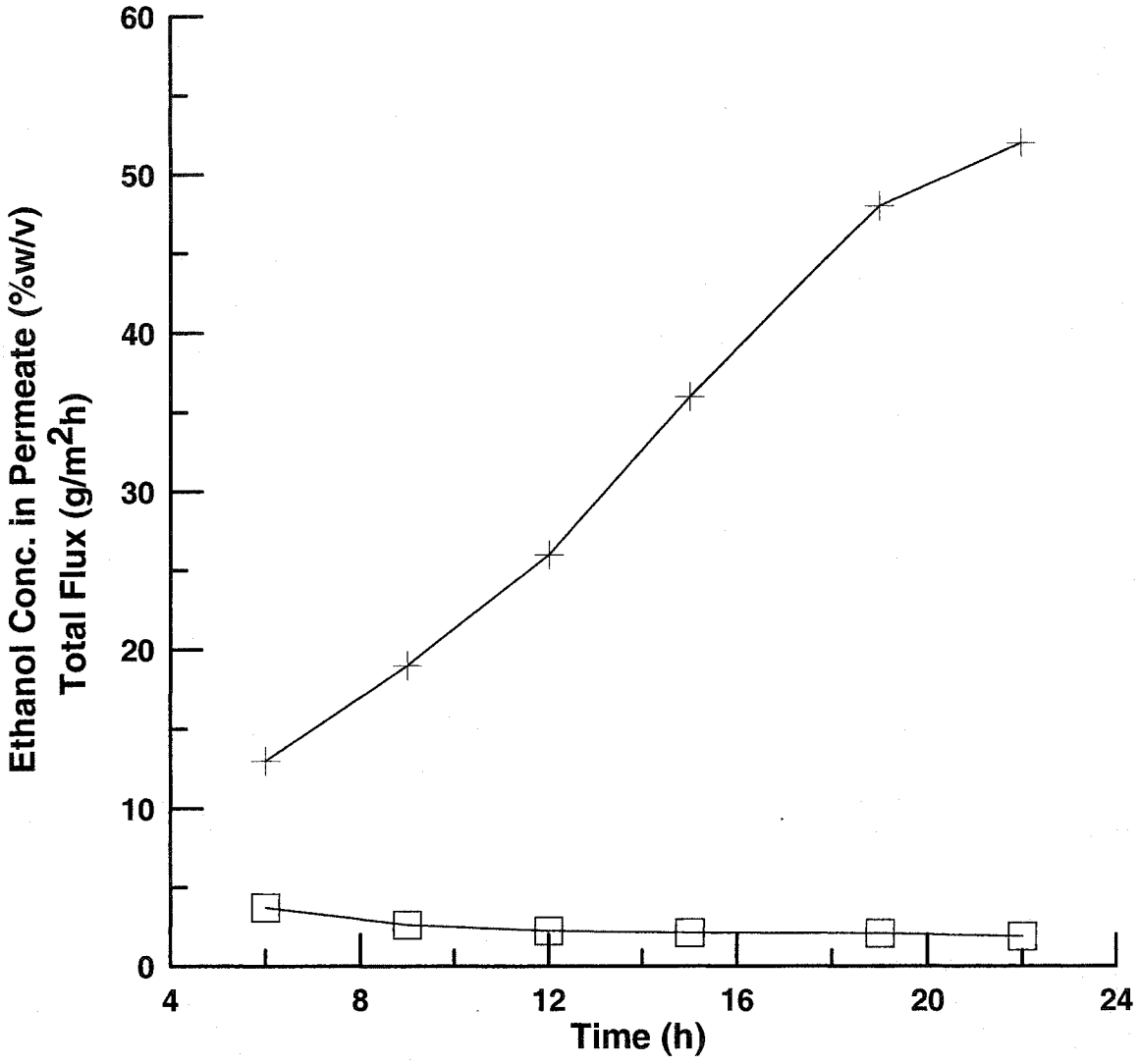

Figure 5.18 Membrane performance of the commercial large membrane module coupled to a bioreactor. Membrane was initiated after 3 hours of batch fermentation with a flow rate of 5 mL/min through the membrane module. (□) Total Flux; (+) Ethanol concentration in permeate.

Ethanol production from three different fermentation/pervaporation experiments is given in figure 5.19 together with the standard deviation.

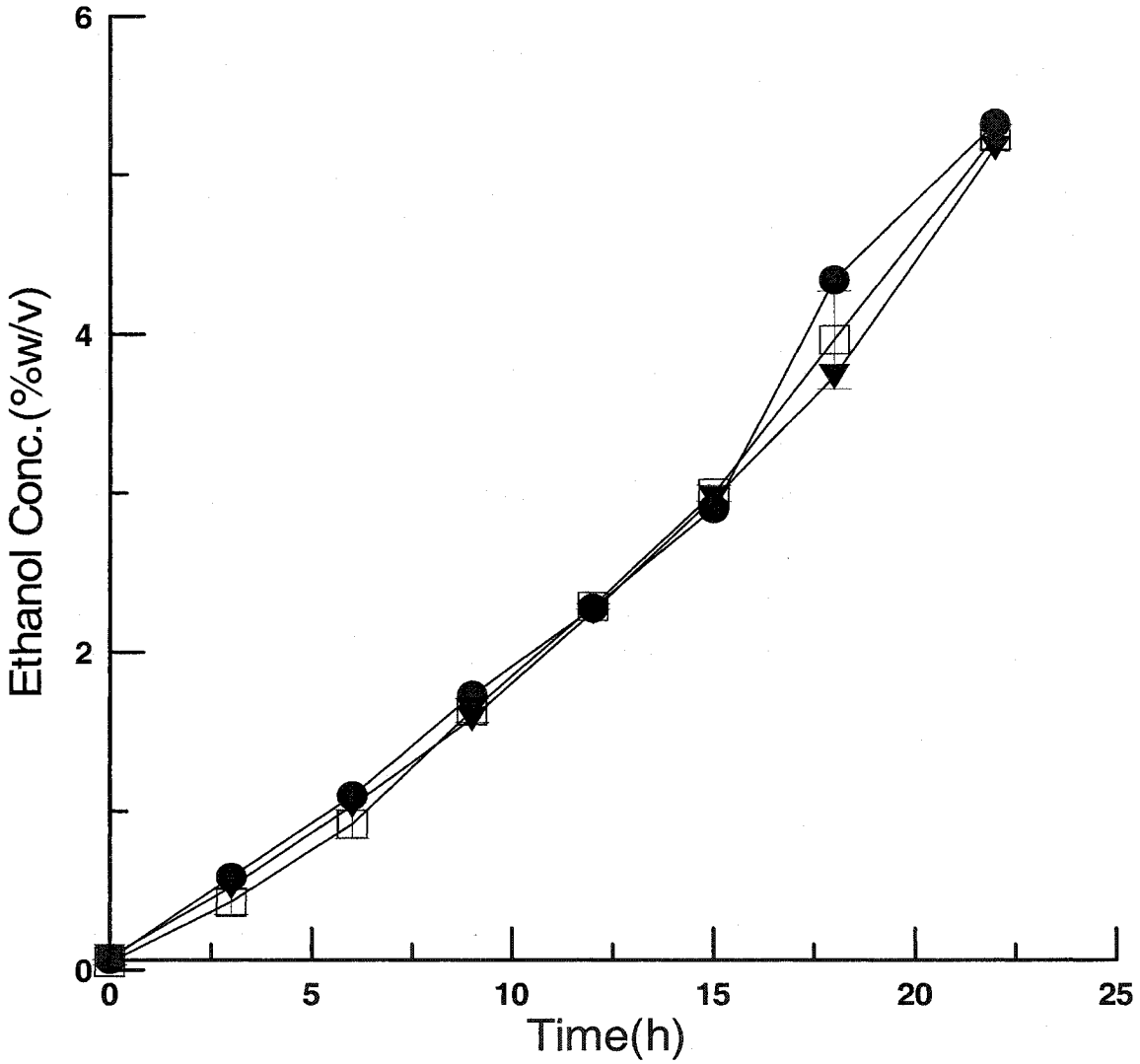

Figure 5.19 Ethanol production from three different fermentation/pervaporation experiments. (□) first run results; (●) second run results; (▼) third run results

5.6 Batch Fermentation coupled with Pervaporation E:

The next set of experiments performed using the large commercial membrane module were under the following conditions: initial volume of the fermentation broth in the reactor 275 mL (sucrose medium along with the inoculum), an initial sucrose concentration of 28.3 %(w/v), an initial biomass concentration of approximately 5.5 g/L, a fermentation temperature of 33°C, a downstream pressure of 1 torr for pervaporation and a turnover time of 27.5 min through the reactor. Experimental results shown in Table 5.9 are volume corrected concentrations obtained at the end of the experiment. Figure 5.20 shows the actual reactor data as a function of time, and Figure 5.21 shows the volume corrected data as a function of time. The only difference between Batch Fermentation/Pervaporation experiments (D) and (E) is the flow rate through the membrane module, which was increased from 5 mL/min to 10 mL/min. This was done to examine whether all the hollow fibers in the membrane module were being used.

Table 5.9 Fermentation/Pervaporation E Parameters

Description	Fermentation/Pervaporation E
Initial Biomass Concentration (g/L)	5.3
Final Biomass Concentration (g/L)	8.2
Initial Sucrose Concentration (%(w/v)	28.3
Fermentation Time (h)	19
Final Glucose Concentration %(w/v)	1.1
Final Fructose Concentration %(w/v)	13.2
Final Ethanol Concentration %(w/v)	6.0
Maximum Specific Growth Rate μ (1/h)	0.05
Biomass Yield (g/g)	0.02
Ethanol Productivity P (g/ (L h))	3.2
Ethanol Yield %(w/v)	76.4
Fructose Yield %(w/v)	88.8
Sucrose Hydrolysis Rate (g/ (L h))	30.3

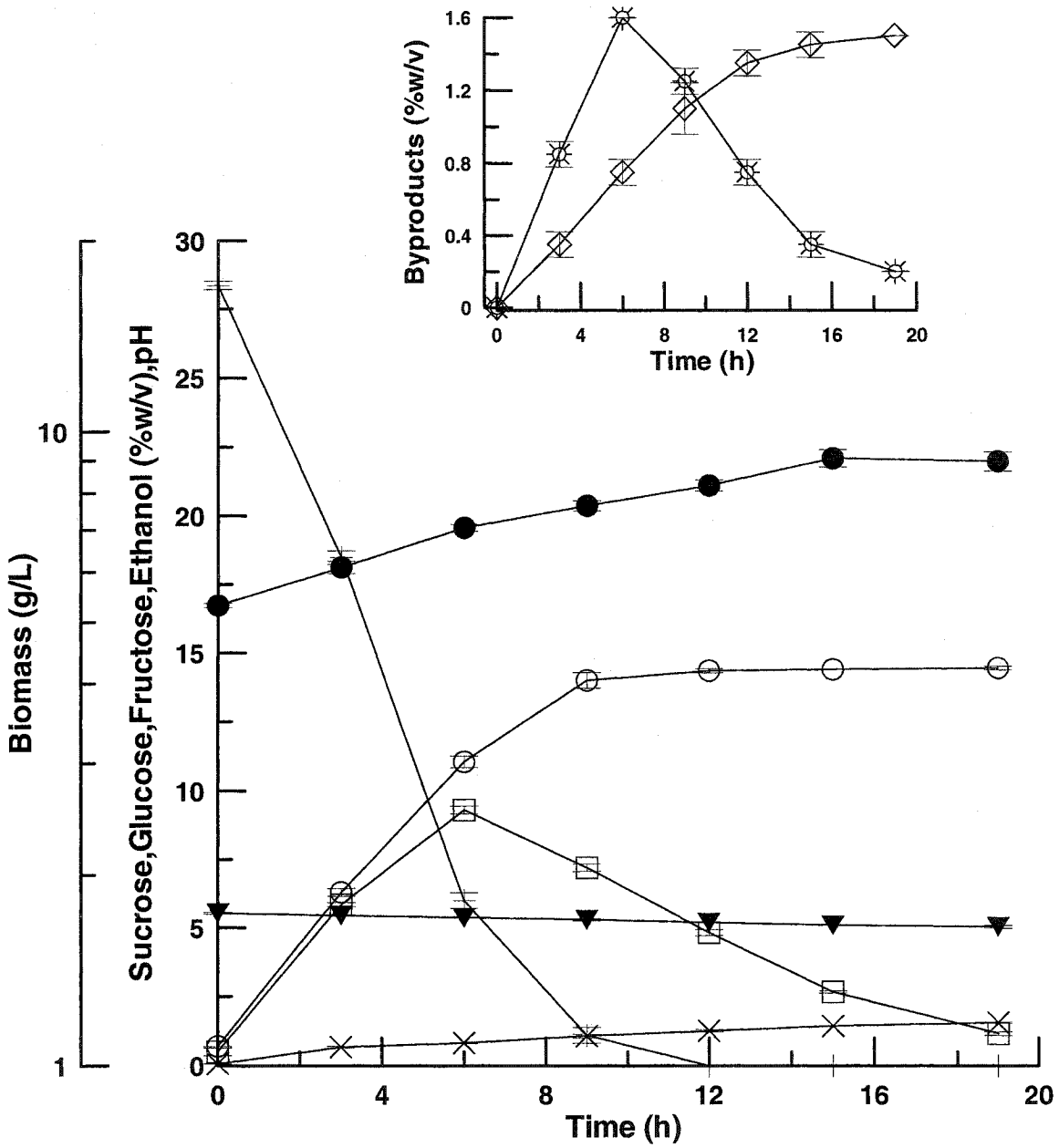

Figure 5.20 Actual reactor data of batch fermentation/pervaporation using the commercial large pervaporation module: Initial sucrose concentration, 28.3 % (w/v); initial biomass concentration, 5.3 g/L, temperature 33°C; initial volume, 275 mL; 10 mL/min flow through the membrane module; pervaporation initiated after 3 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

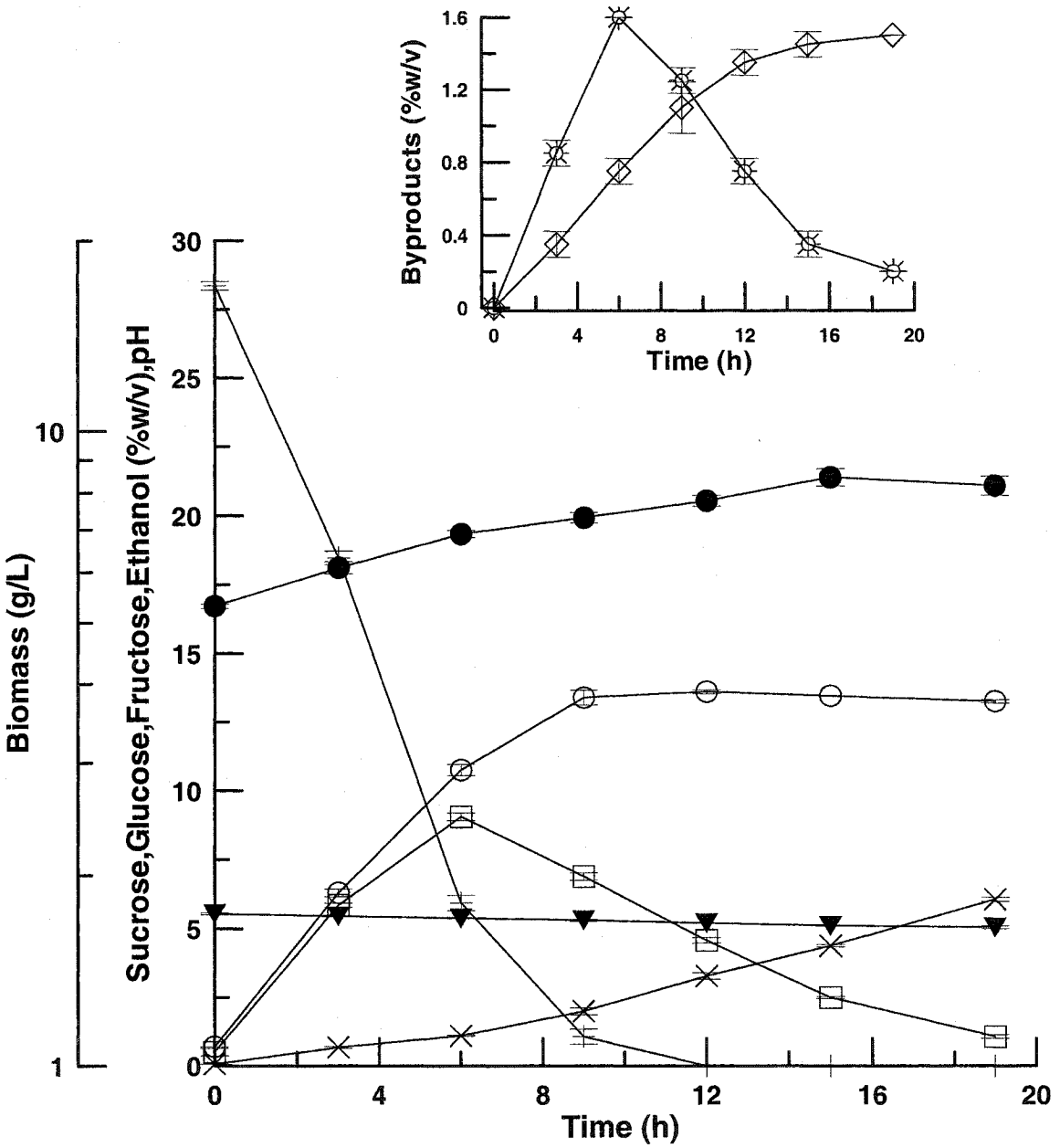

Figure 5.21 Volume corrected reactor data of batch fermentation/pervaporation using the commercial large pervaporation module: Initial sucrose concentration, 28.3 % (w/v); initial biomass concentration, 5.3 g/L, temperature 33°C; initial volume, 275 mL; 10 mL/min flow through the membrane module; pervaporation initiated after 3 hours of batch fermentation; (†) Sucrose, (□) Glucose, (○) Fructose, (●)Biomass, (▼) pH, (x) Ethanol, (☼) Kestoses, (◊) Glycerol.

As the previous experiment, the pervaporation setup was started after 3 hours of running the system in a batch mode environment. When the flow of the feed medium through the hollow fibers began, it was noticed that there was a large amount of stagnant liquid medium on top of the module and it took a long time for the liquid to flow from the top to the bottom part of the module.

To achieve the same glucose concentration (1.1 % (w/v)) as in the batch fermentation/pervaporation (D), it took about 19 hours in this experiment, whereas it took 22 hours in experiment (D). The final fructose concentration at the end of the experiment was 13.2 % (w/v), which gives a fructose yield of 88.8 %. This was less by 2 % (w/v) than the experiment (D) where the turnover time was 55 min through the module. The ethanol concentration at the end of experiment was 6.0 % (w/v), which gave an ethanol yield of 76.4 % and an ethanol productivity of 3.2 g/ (L h). This was far better compared to the previous experiment which gave an ethanol yield of 66.3 %. It was an increase of almost 10 % with respect to the ethanol yield.

The maximum specific growth rate and the biomass yield were 0.0434/h and 0.02 g/g respectively. The biomass concentration increased from an initial value of 5.3 g/L to 8.2 g/L. Figure 5.22 shows the ethanol concentration in the permeate increased from 13 % to 53 % and the flux decreased from 4.45 g/ (m^2h) to 2.92 g/ (m^2h) as the fermentation progressed. As earlier mentioned, fouling could be the reason for the decrease in the flux rate. Higher ethanol concentration in the permeate and a little better flux may be due to either less concentration polarization for higher circulation rate or more utilization of the hollow fibers.

Increasing the flow rate through the membrane module was better in many aspects. The ethanol yield and the productivity were improved. It also helped in keeping the ethanol level in the reactor slightly less than the previous experiment. Increasing the flow rate more than 10 mL/min through the membrane module could eventually rupture the membrane and the membrane module may start to leak. However, for the given module, there was a limit in increasing the flow rate. The velocity, which was calculated using the flow rate through the membrane module and the cross sectional area of the membrane module was found to be $3.125 * 10^{-4}$ m/s.

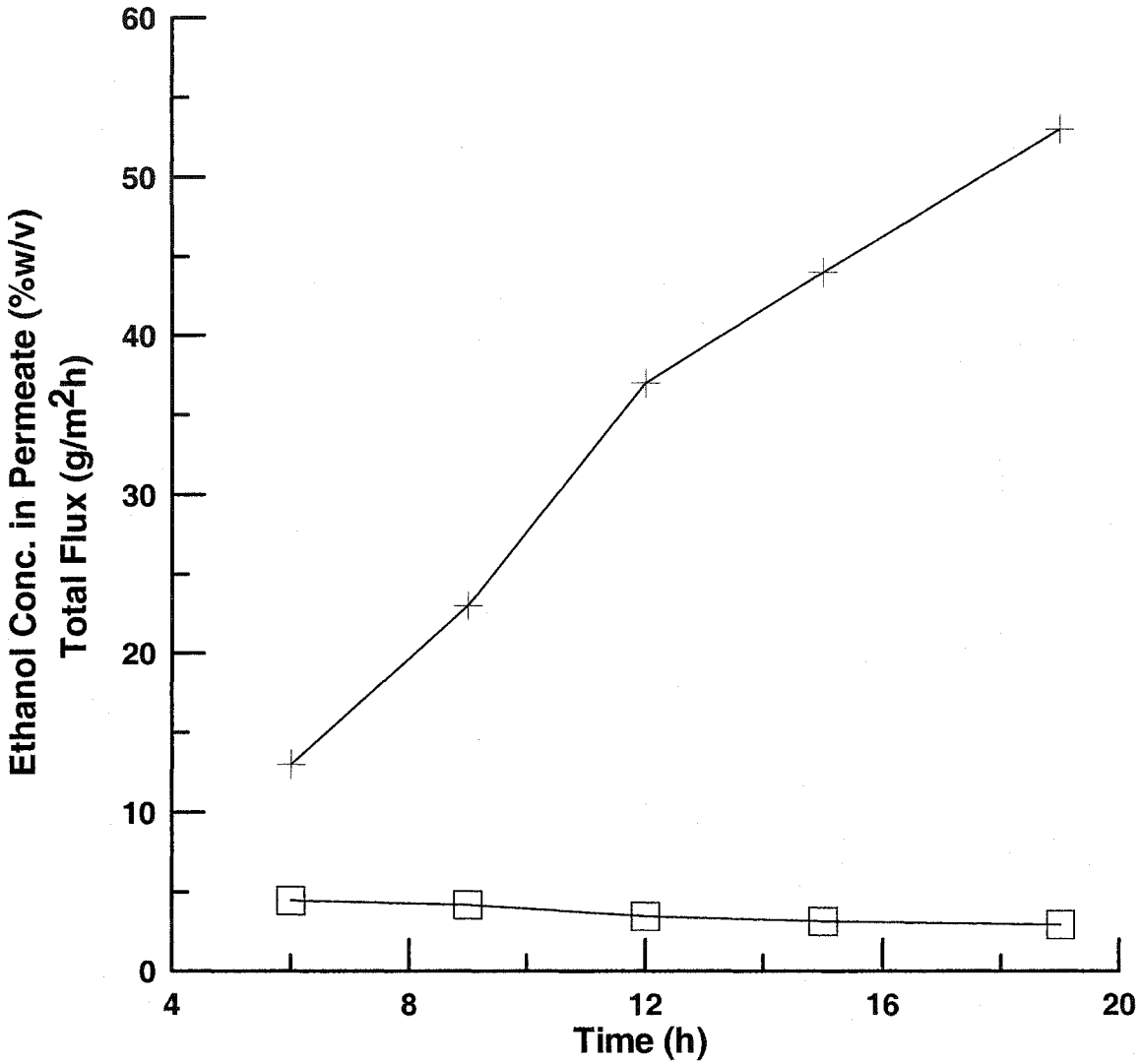

Figure 5.22 Membrane performance of the commercial large membrane module coupled to a bioreactor. Membrane was initiated after 3 hours of batch fermentation with a flow rate of 10 mL/min through the membrane module. (□) Total Flux; (+) Ethanol concentration in the permeate.

Ethanol production from three different fermentation/pervaporation experiments is given in figure 5.23 together with the standard deviation.

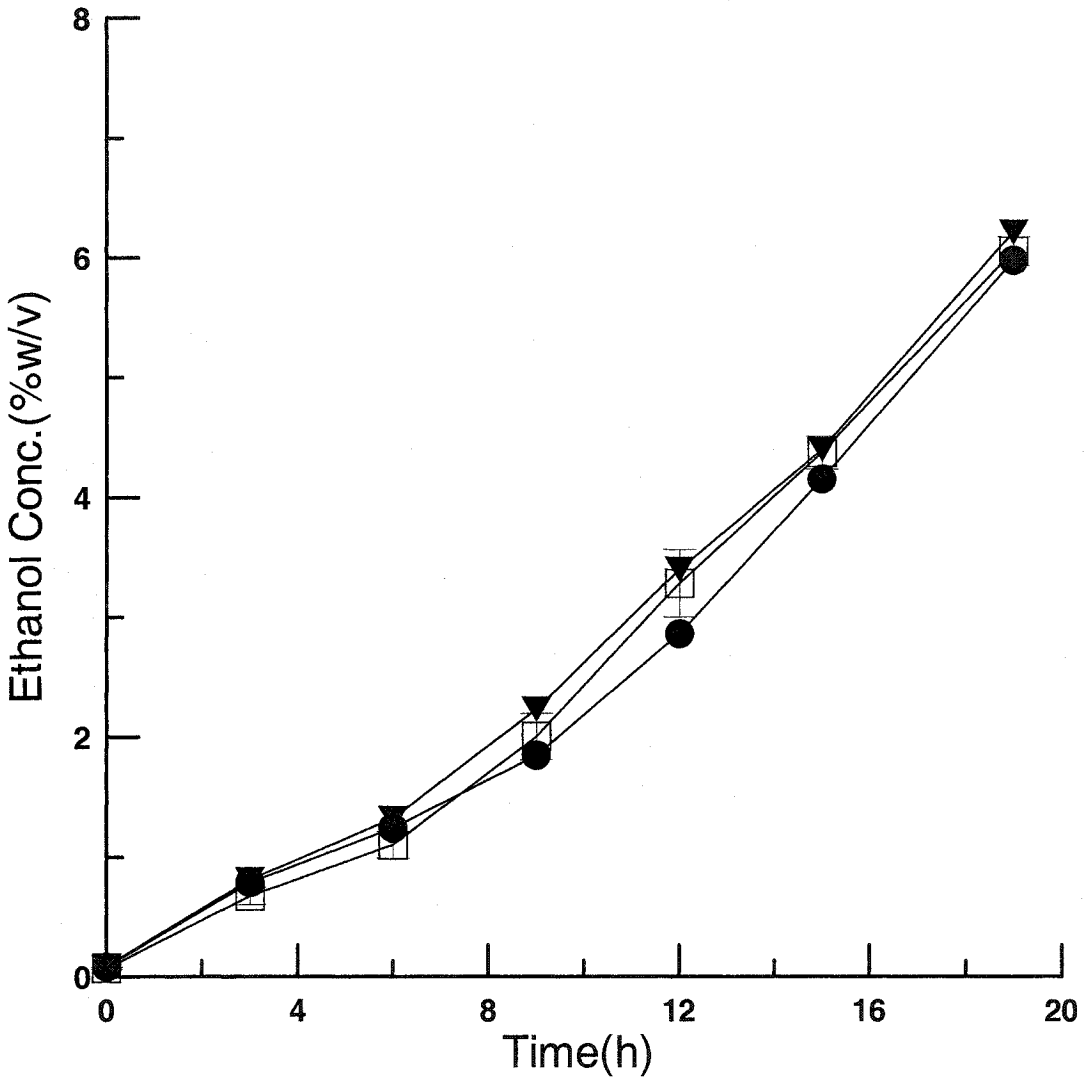

Figure 5.23 Ethanol production from three different fermentation/pervaporation experiments. (□) first run results; (●) second run results; (▼) third run results

The comparison results of experiments (D) and (E) is shown below in Table 5.10.

Table 5.10 Comparison between experiments (D) and (E)

Description	**Exp (D)**	**Exp (E)**
Initial Sucrose Conc %(w/v)	28.7	28.3
Initial Biomass Conc (g/L)	5.2	5.3
Final Biomass Conc (g/L)	7.7	8.2
Final Fructose Conc %(w/v)	13.7	13.2
Final Glucose Conc %(w/v)	1.1	1.1
Maximum Specific Growth Rate μ(1/h)	0.04	0.05
Fructose Yield (%)	90.6	88.8
Ethanol Yield (%)	66.3	76.4
Biomass Yield (g/g)	0.02	0.02
Ethanol Productivity g/(L h)	2.4	3.2
Final Ethanol Conc %(w/v)	5.2	6.0
Sucrose Hydrolysis Rate g/(L h)	30.3	30.3
Time (h)	22	19

D = denotes batch fermentation/pervaporation experiments performed with a flow rate of 5 mL/min through the membrane module.

E = denotes batch fermentation/pervaporation experiments performed with a flow rate of 10 mL/min through the membrane module.

6. Conclusions

- The simultaneous production of fructose and ethanol can be enhanced by coupling the bioreactor to a pervaporation unit.

- The total flux and ethanol concentration in the permeate increased with an increase in the ethanol concentration in the feed, while the selectivity tended to decrease with an increase in feed ethanol concentrations in ethanol/water mixtures.

- Higher glucose consumption rate and higher ethanol yields were observed when pervaporation was initiated after 0 hours of batch fermentation with the smaller membrane module (M60 - 75S) than when pervaporation was initiated after 3 or 6 hours of batch fermentation.

- Lower fructose yields were obtained when pervaporation tests were initiated after 0 hours of batch fermentation than when pervaporation was initiated after 3 or 6 hours of batch fermentation. The experiment could be stopped when the glucose concentration in the reactor was around 1.0 %(w/v) as in the other pervaporation/fermentation tests to obtain higher fructose yields.

- The large commercial module (M60 – 4000) removed more ethanol than the small commercial membrane module (M60 - 75S) and improved the process considerably, which could be seen from the high ethanol concentration in the permeate and the low ethanol concentration in the reactor.

- The flow rate through the membrane module played a significant role in the overall performance of the process. Higher ethanol yields and shorter fermentation times required to complete the fermentation process were observed with a flow rate of 10 mL/min than with a flow rate of 5 mL/min through the membrane module.

7. Recommendations

- Efficient methods for cleaning the membrane should be found in order to flush the entire amount of biomass in the hollow fibers after the conclusion of the experiments. Eighty percent ethanol-water solution seemed to remove the biomass to a certain extent, but not completely.

- When more ethanol is being removed from the system, whether more fructose is being consumed should be verified by performing individual experiments with fructose and ethanol solutions.

- The whole pervaporation system needs to be automated in order to perform fed-batch fermentations.

- Perform extensive fouling studies.

- Membrane materials different from silicone rubber need to be tried in order to find the membrane's compatibility with the bioreactor. Membranes with high flux and selectivities could be tried.

- Decreasing the turnover time even further through the reactor in the large commercial membrane module should be done in order to check the ethanol yield.

- Two membrane modules could be connected in a series or a parallel mode to remove more ethanol at the same period of time and increase the speed of the fermentation process.

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