Recovery of Xylitol from Fermentation of Model Hemicellulose Hydrolysates Using Membrane Technology

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(ABSTRACT)

Xylitol can be produced from xylose or hemicellulose hydrolysates by either chemical reduction or microbial fermentation. Current technology for commercial production is based on chemical reduction of xylose or hemicellulose, and xylitol is separated and purified by chromatographic methods. The resultant product is very expensive because of the extensive purification procedures.

Microbial production of xylitol is being researched as an alternative method for xylitol production. Apart from the chromatographic separation method and activated carbon treatment, no other separation method has been proposed for the separation of xylitol from the fermentation broth.

Membrane separation was proposed as an alternative method for the recovery of xylitol from the fermentation broth because it has the potential for energy savings and higher purity. A membrane separation unit was designed, constructed, tested, and successfully used to separate xylitol from the fermentation broth. Eleven membranes were investigated for xylitol separation from the fermentation broth. A 10,000 nominal molecular weight cutoff (MWCO) polysulfone membrane was found to be the most effective for the separation and recovery of xylitol. The membrane allowed 82.2 to 90.3% of xylitol in the fermentation broth to pass through while retaining 49.2 to 53.6% of the Lowry's method positive material (such as oligopeptides and peptides). Permeate from the 10,000 MWCO membrane was collected and crystallized. Crystals were analyzed by HPLC for xylitol and impurities and determined to have purity up to 90.3%.

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CHAPTER 1
INTRODUCTION

In August 1999, President Clinton issued an executive order to coordinate national efforts to accelerate the development of biobased industries that use trees, crops, and agricultural and forestry wastes to produce fuels, chemicals, and electricity. He created a permanent council to propose a biomass research program. The goal of this program is to triple the use of biomass for energy and raw materials for biobased products by 2010. The research described herein supports this goal.

The Polyols sweetener industry is experiencing a rapid growth because of the increasing consumer demand for sugar-free and reduced calorie products. The sweeteners experiencing this rapid growth are the sugar alcohols such as xylitol, sorbitol, mannitol, and maltitol. Xylitol ($C_5H_{12}O_5$) is not only a sugar-free sweetener, but also has unique properties that find applications in pharmaceutical, healthcare, and food industries (Gurgel *et al.*, 1995). It can be used as a sugar substitute for diabetic patients, has anticariogenic properties, and has skin smoothing properties (Chen, 1985). Clinical studies have shown that xylitol could prevent middle ear infection in children when administered in chewing gum. Currently xylitol sells for about $3 a pound and is used as a sweetener in food products, such as, chewing gum, candy, soft drinks, and personal health products such as mouth wash and tooth paste.

Xylitol can be found in some fruits and vegetables, but extraction of xylitol from these sources is uneconomical, due to the low concentrations present (Saha, 1997). Alternative methods of xylitol production are chemical or microbial reduction of D-xylose or hydrolysates of xylan-rich hemicellulosic materials. Commercial production of xylitol is currently from birch wood sulfite pulping liquor and other xylan-rich substrates. The production involves extraction and purification of xylose from the pulping liquor. Xylose is chemically hydrogenated to xylitol, and the chromatographic method is currently used to recover xylitol. This recovery process is extensive and therefore the final product is more expensive than other polyols.

It is known that certain molds, yeasts, and bacteria are capable of reducing xylose to xylitol as a first step in D-xylose metabolism (Heikkila *et al.*, 1992). In the past decade, yeasts have been more extensively studied than mold and bacteria for the production of xylitol. However, yields and productivities are low and no new purification methods other than chromatographic separation have been applied to the separation of the product.

Cation exchange resin columns are used for xylitol separations. Separation is effected by interactions between weakly acidic hydroxyl groups and cations such as calcium and strontium (Jandera, 1974). Following chromatographic separation the xylitol-rich solutions are crystallized at low temperatures.

The chromatographic method is particularly suitable for the chemical reduction route for xylitol purification. However, for fermentation processes there are different impurities, such as proteins and carbohydrates, introduced for microbial growth. These impurities and low xylitol concentrations in the broth pose new challenges for xylitol separation and purification.

Membrane separation has been used to separate fructose and glucose molecules in solution by membrane separation (Kim, 1985). The sugar molecules were complexed with salt molecules, such as $NaHSO_3$, to aid in the separation of fructose and glucose by membrane. This procedure could be used for xylitol separation, and there are different types of membrane methods ranging from reverse osmosis and nanofiltration to crossflow ultrafiltration that could be used to separate xylitol.

Crossflow ultrafiltration can be used for rapid removal of yeast cells from fermentation broth. Crossflow filtration allows fluid to flow parallel to the membrane and the shear force created prevents large microbial cell build up on the membrane surface. The clarified xylitol broth can be further treated with reverse osmosis, nanofiltration, or ultrafiltration membranes.

Reverse osmosis membranes do not allow xylitol to permeate the membrane. However, this process could be used as an alternative to evaporation for the concentration of xylitol. Alternatively, some membranes that xylitol can permeate are nanofiltration and ultrafiltration and these membranes can retain protein impurities. In addition, adding some substances that chemically react with the carbohydrate impurities in the fermentation broth can cause permeability differences through these membranes. Thus, xylitol would permeate the membrane, and impurities, such as proteins and the chemically reacted carbohydrates, would be retained. This would result in a purified xylitol-rich permeate stream.

1.1 Research Overview and Objectives

We propose to use ultrafiltration, reverse osmosis and nanofiltration membranes as an alternative methodology to separate xylitol from the fermentation broth. In addition to membrane separation the use of the Maillard reaction was investigated to improve the separation. The overall goal of this research is to develop a membrane separation technology to separate xylitol from a hemicellulose hydrolysate fermentation broth.

The specific objectives are:

1. To remove proteins and biomass from fermentation broth by crossflow ultrafiltration.
2. To investigate the use of reverse osmosis for concentration of xylitol for crystallization.
3. Determine the effect of chemical reactions with sugars on the separation of xylitol from fermentation broth using nanofiltration or ultrafiltration membranes.
4. To use crystallization to purify xylitol produced by fermentation.

CHAPTER 2
LITERATURE REVIEW

2.1 Natural Occurrences of Xylitol

Xylitol (Figure 2.1) is a naturally occurring five-carbon sugar alcohol that has the same sweetness and one-third the caloric content of conventional sugar (Heikkila *et al*., 1992).

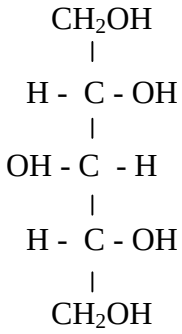

Figure 2.1 Chemical structure of xylitol.

Xylitol has been found in fruits, berries and vegetables (Table 2.1) and is produced in the human body during normal metabolism (Heikkila *et al.*, 1992). Xylitol can be produced by chemical or microbial reduction of D-xylose or xylan-rich hemicellulose hydrolysates. Birch wood, oats, corn fiber, cotton-seed hulls, corn cobs, sugar cane bagasse, rice straw and nut shells, are xylan-rich substrates, which could be used for xylitol production (Counsell, 1978).

Table 2.1 Natural occurrence of xylitol in fruits, vegetables and related products (Jaffe, 1978)

Product	Xylitol (mg/100 g dry substance)
Banana	21
Raspberry	268
Strawberry	362
Yellow Plum	935
Carrot	86.5
Endive	258
Onion	89
Lettuce	131
Cauliflower	300
Pumpkin	96.5
Spinach	107
Kohlrabi	94
Eggplant	180
Leek	53
Fennel	92
White Mushroom	128
Brewer's Yeast	4.5
Chestnut	14
Carrot Juice	12
Lamb's Lettuce	273

Table 2.2 Chemical composition of corn fiber hydrolysates

Hydrolysate	Xylose (%)	Glucose (%)	Arabinose (%)	Galactose (%)	Mannose (%)	Reference
Corn Fiber	17	37	11	4	-	Saha & Bothast (1999)
	30-41	25-38	21-28	4-6	-	Hespell (1998)
	20	20	10	-	-	Leathers (1996)
	20	16	11	2	-	Dien (1999)

2.2 Chemical Xylitol Production

The chemical method of xylitol production is based on the catalytic hydrogenation of D-xylose or xylose-rich hemicellulose hydrolysate (Figure 2.2). Melaja (1977) hydrogenated birch wood hemicellulose hydrolysate (Table 2.3) in the presence of Raney nickel catalyst in an autoclave at 135 ºC, and 40 atmospheres hydrogen pressure for 2.5 hours. The xylose and other carbohydrates were hydrogenated to their respective polyols. Approximately, 60% of the original xylan material was recovered as xylitol.

Table 2.3 Composition of xylose-rich solution for catalytic hydrogenation studies

Sugar	Dry Solids Basis (%)
Xylose	73
Arabinose	6.1
Mannose	9.0
Galactose	5.1
Glucose	6.8

The xylitol was crystallized from the hydrogenated solution and the uncrystallized xylitol fraction was separated by liquid chromatography method. Chromatographic separation was performed in a 1-meter high 94-cm diameter column containing cross-linked sulfonated polystyrene divinylbenzene cation exchange resin in the calcium or strontium form. Eluents collected from the chromatographic column had various proportions of polyols such as mannitol, arabitol, galactitol and sorbitol (Table 2.4). The fractions with very high xylitol concentrations were crystallized and the xylitol was recovered.

Table 2.4 Xylitol chromatographic fractions

Fraction	Arabinitol (g)	Mannitol (g)	Galactitol (g)	Xylitol (g)	Sorbitol (g)
1	0.65	0.85	-	-	-
2	1.85	1.70	-	-	-
3	1.95	1.40	0.3	-	-
4	0.10	0.45	1.0	1.4	-
5	-	-	0.9	10.5	-
6	-	-	0.1	14.3	0.3
7	-	-	-	10.1	0.65
8	-	-	-	4.8	0.85
9	-	-	-	2.15	0.5
10	-	-	-	0.85	0.2

(Counsell, 1978)

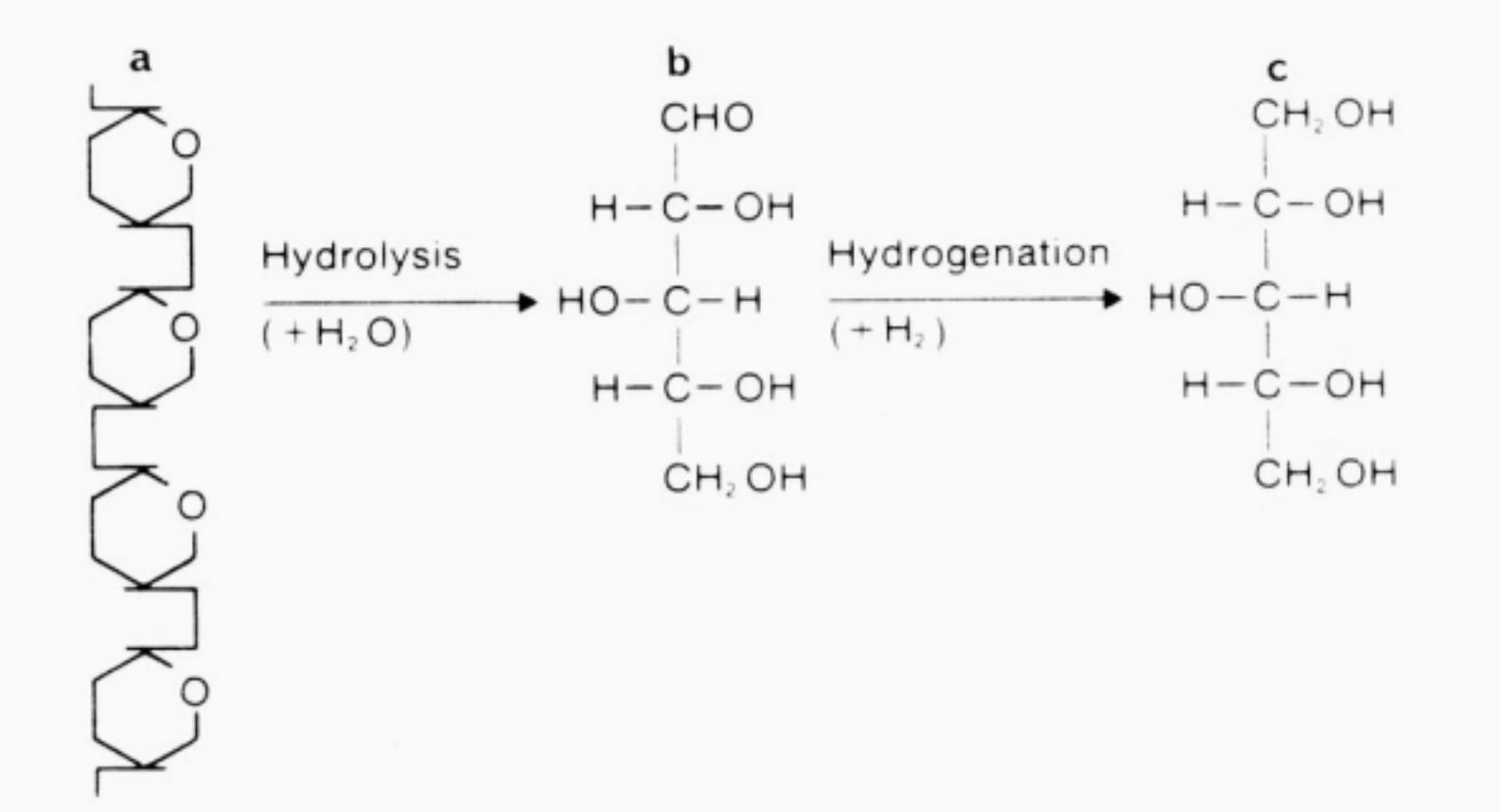

Figure 2.2 Hydrolysis and hydrogenation of xylan to xylitol a. xylan $(C_5H_8O_4)_n$, n~200, b. D-xylose, $C_5H_{10}O_5$, c. xylitol, $C_5H_{12}O_5$.

After crystallization the concentration of xylitol in the mother liquor was about 30 to 60 wt% xylitol (which was 5-10 wt% of the starting material) along with impurities such as arabinitol, sorbitol, galactitol, mannitol and other monomeric sugar alcohols (Munir, 1981). Similar to Melaja, a method for recovering xylitol from the mother liquor at the end of crystallization was proposed by Munir. The xylitol crystals were separated from the mother liquor by centrifugation and an example of the mother liquor content is given in Table 2.5.

Table 2.5 Mother liquor content following xylitol crystal removal

Polyol	Concentration (g/100 g dry substance)
Adonitol	0.6
Arabitol	14
Xylitol	46.2
Mannitol	0.3
Sorbitol	2.2
Galactitol	1
Oligo and polysaccharide alcohols	35.7

Chromatography was used to separate the mother liquor into fractions containing xylitol (Table 2.6). A strongly acidic, weakly crosslinked divinylbenzene cation exchange resin in the calcium form was used. Fraction 1 was recycled and reacted with Raney-nickel again. Fraction 2 was combined with several cycles of fraction 2 and subjected to the chromatographic separation again to obtain fractions containing a high percentage of xylitol that could be crystallized. Fraction 3 was combined with several cycles and concentrated to 85% dry weight and fed into a cooling crystallizer. The xylitol readily crystallized and was easily separated from the mother liquor in a wire basket and 34.4%

of the xylitol in the original mother liquor was recovered. The mother liquor obtained from the crystallization of fraction 3 was further treated with chromatography mentioned in the previous method to recover any remaining xylitol.

Table 2.6 Chromatographic fractions of mother liquor

	Fraction 1 (% dry substance)	Fraction 2 (% dry substance)	Fraction 3 (% dry substance)
Adonitol	-	2.1	-
Arabitol	-	19.6	16.0
Xylitol	0.3	2.0	78.4
Mannitol	-	1.2	-
Sorbitol	-	-	3.7
Galactitol	-	0.3	1.0
Oligo and polysaccharide alcohols	99.7	74.8	0.9

Xylitol was also crystallized from a concentrated aqueous solution containing 50 to 75 wt% xylitol, and no more than 5 wt% xylose (Jaffe, 1976). The solution was slowly cooled over 2 hours to 5 ºC and the precipitated xylitol crystals were recovered by filtration. The mother liquor was recycled and fractional crystallization of the xylitol solution was continued until the final product contained no more than 0.10 wt% xylose.

Crystalline xylitol from saturated aqueous solution is moisture sensitive and tends to cake and when added to gum tends to make the gum very soft and difficult to process. In addition, crystals produced in this manner tend to give chewing gum a coarse texture. Duross (1992) used melt-crystallized xylitol to produce crystals, which were moisture resistant and more easily formulated into chewing gum. A 70% xylitol solution was

made and heated to 170 °C. The xylitol melt was then cooled to 90 °C with agitation in a water bath (80 °C). The solution was seeded with one gram of xylitol crystals and the agitation was continued until noticeable increase in viscosity from crystal formation (50% complete). The solution was poured onto a tray and covered with aluminum foil to crystallize. The result was an agglomerated crystal structure with 99.5 wt% xylitol

2.3 Alternative Chemical Xylitol Production Procedures

Current methods use xylose obtained from hemicellulose hydrolysate of xylan containing materials. Sulfite cooking liquor from pulping contains xylose and xylonic acid and used to be discharged into the water system. Environmental regulations prevent this now, and methods for utilizing cooking liquor are needed. Heikkila et al. (1999) proposed a method to produce xylitol from xylonic acid. Xylonic acid crystals were produced and hydrogenated for 3 hours in an autoclave at 110 °C and 13,000 kPa using ruthenium on carbon catalyst. The initial concentrations and final product are shown in Table 2.7.

Table 2.7 Concentrations obtained from xylonic acid

	Composition of starting material (%/dry matter)	Composition of product (%/dry matter)
Xylonic acid	94.2	8.3
Xylitol	0	75.9
Arabitol	0	6.6
Xylose	1.1	0

The xylitol solution was filtered, concentrated to 92.2% xylitol and seeded with 0.05 g of xylitol crystals. Xylitol crystallized and was separated with centrifugation at 4500 rpm for 5 minutes. The yield was 0.297 g/g and the crystals were 68% pure.

It has recently been discovered that D-xylulose could be converted to xylitol by fermentation with *Mycobacterium smagematis* (Izumori, 1988). Leleu (1992) investigated D-xylulose conversion to xylitol by chemical methods. D-xylulose syrup (95% xylulose, 1% arabitol, 3% xylitol, 1% various) was percolated (at 65 °C and pH 7.7) over a column of immobilized isomerase glucose of SPEZYME. An isomerized syrup was obtained with the composition shown in Table 2.8.

Table 2.8 Isomerized xylulose syrup concentration

	Concentration (%)
D-arabitol	1
D-xylulose	25
D-xylose	70
D-xylitol	3
Various	1

The isomerized solution containing xylose was hydrogenated with Raney nickel at 120 °C for 3 hours. The product had a composition of 13.5% arabitol, 85.5% xylitol and 1 % various. The solution was concentrated to 84% dry matter, seeded with xylitol crystals and slow cooled from 60 °C to 25 °C over 30 hours. Xylitol crystals formed with a yield of 0.7 g/g and purity was generally above 97%.

Vuorinen (1996) produced xylitol from D-glucose, D-fructose, and D-galactose. D-glucose (1050 g) was oxidatively cleaved in an autoclave with an aqueous solution containing sodium hydroxide (18 g), water (264 g), methanol (100 g), and sodium arabinonate (16 g). The reactor was pressurized with oxygen (at 85 °C) to form sodium arabinonate crystals. Sodium arabinonate crystals were recovered by centrifugation, dissolved in water and acidified to arabinonic acid on an acid cation exchange resin. The

arabinonic acid was vacuum concentrated and crystallized to form D-arabino-1,4-lactone crystals. The D-arabino-1,4-lactone crystals were converted to arabitol in the presence of ruthenium-on-carbon catalyst with a yield of 90 mol% arabitol. The arabitol was crystallized. Then in a second reaction the arabitol crystals were converted to xylitol (90 mol% yield) in the presence of ruthenium-on-carbon catalyst. However, the procedure has been found to be too costly for large-scale production.

For chemical processing of 12-13 kilograms almond shells needed to obtain 1 kilogram of crystalline xylitol, about 11-12 kilograms of solid waste is produced (Beck, 1998). A procedure using gluconic acid as an alternative to almond shells was employed to decrease the amount of waste during the production of xylitol. Gluconic acid (218 g) was decarboxylated to arabinose by reacting with sodium hypochlorite for 45 minutes at 55 °C. The arabinose syrup was hydrogenated using Raney nickel catalyst and arabitol was formed. The pH was raised to 9 at 170 °C to isomerize the arabitol. The demineralized isomerizate was purified by chromatography to give xylitol yields as high as 77%.

2.4 Microbial Production of Xylitol

Xylitol is produced from D-xylose as a metabolic intermediate in many xylose utilizing microorganisms by converting D-xylose directly to xylitol by NADPH-dependent xylose reductase (Saha, 1997). The pathway for xylose utilization by microorganisms is shown in Figure 2.3.

(Saha, 1997)

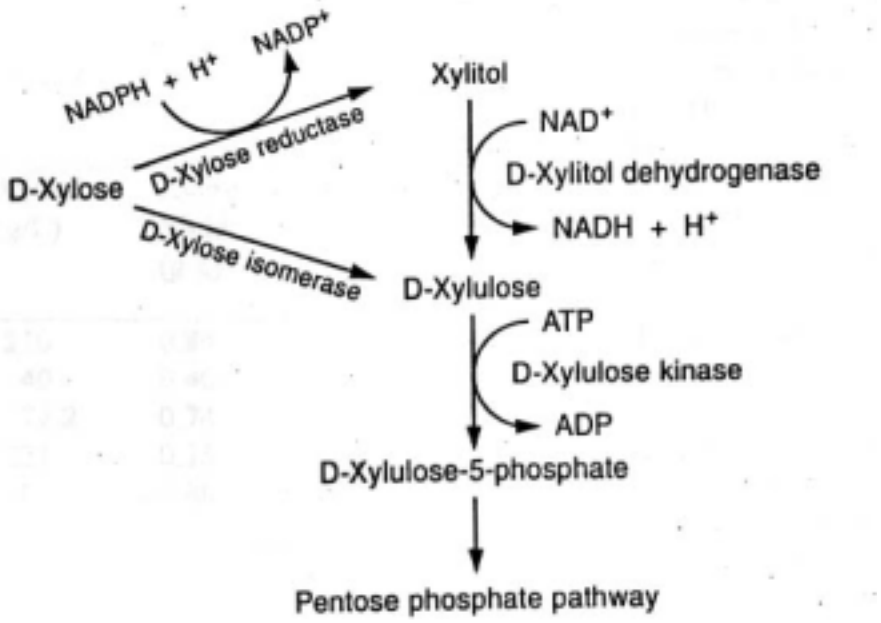

Figure 2.3 Pathway for microbial xylose utilization.

D-xylulose has been converted to xylitol by *Mycobacterium smagematis* (Izumori, 1988). D-xylulose solution (2%) was incubated with aerobic and anaerobic conditions to obtain concentrations of xylitol of 0.8% and 1.4% respectively. Yields obtained for xylitol were as high as 74%.

A number of yeast and filamentous fungi possess the enzyme xylose reductase and can produce xylitol. Some xylitol producing yeasts include *Candida pelliculosa, Candida boidinii, Candida guilliermondii,* and *Candida tropicalis*. Other genera of yeast investigated for xylitol production from xylose include: *Saccharomyces, Debaryomyces, Pichia, Hansenula, Torulopsis, Kloeckera, Trichosporon, Cryptococcus, Rhodotorula, Monilia, Kluyveromyces, Pachysolen, Ambrosiozyma,* and *Torula* (Saha, 1997). Bacteria species can also produce xylitol, such *as Enterobacter liqufaciens, Corynebacterium sp*., and *Mycobacterium smegmatis* (Horitsu *et al.*, 1992). The conversion of D-xylose to

xylitol by microorganisms is important for industrial production and has been studied extensively in yeasts.

2.4.1 Effect of Xylose Concentration on Xylitol Production

The initial xylose concentration can influence xylitol production by microorganisms. Horitsu et al. (1992) investigated the influence of D-xylose concentration on the production of xylitol by varying the concentration from 100 g/L to 300 g/L. The maximum D-xylose concentration found for *C. tropicalis* was 172.0 g/L. Rosa et al. (1998) investigated the effect of initial xylose concentration on *C. guilliermondii* FTI 20037 and found 15-60 g/L as the maximum concentration for the production of xylose reductase.

2.4.2 Effect of Other Sugars on the Production of Xylitol by Yeasts

Yahashi et al. (1996) supplemented D-xylose with D-Glucose during the cultivation of *C. tropicalis* yeast cells. D-Glucose was utilized more rapidly than D-xylose for cell growth. The addition of glucose to the fermentation media resulted in an increased xylitol yield and productivities were 1.2-1.3 times higher. The experiment produced 104.5 g/L xylitol in 32 h with a yield of 0.82 g/g. Silva et al. (1996) also investigated the addition of glucose to the fermentation medium during xylose fermentation by *Candida guilliermondii* FTI 20037. The yield was 0.66 g/g, but when glucose was added the yield decreased to 0.45 g/g.

2.4.3 Effect of Culture Conditions

The effect of inoculum age, inoculum level and hydrolysate composition have considerable impact on increasing the production of xylitol and have been studied using *C. guilliermondii* FTI 20037 (Felipe *et al.*, 1997). The xylose concentration in the hydrolysate was varied from 37.6 g/L to 74.2 g/L and inoculum level was varied from 0.1 to 6.0 g/L while the inoculum age was varied from 16 to 48 hours. At an initial xylose content of 54.5 g/L a maximum xylitol yield of 0.74 g/g and productivity of

0.75 g/L·h was reported for 3.0 g/L of 24-hour-old inoculum. It was reported that *C. guilliermondii* cells assimilated acetic acid, suggesting that these cells could be used to detoxify the growth medium. Horitsu (1992) investigated the influence of culture conditions on the production of xylitol by *Candida tropicalis*. The aeration rate and yeast extracts were varied respectively from 100 mL/min to 700 mL/min and 10 g/L to 30 g/L. A maximum production rate of 2.67 g/L·h was reported for 172.0 g/L D-xylose, 21.0 g/L yeast extract, and a K_La value of 451.5 h^{-1}.

2.4.4 Production of Xylitol from Hemicellulose Hydrolysate

Microorganisms containing the enzyme xylose reductase can ferment hemicellulose hydrolysate from woody plant materials. Chen and Gong (1985) used *Candida sp*. B-22 to produce xylitol from sugar cane bagasse hemicellulose hydrolysate with a yield of over 85% of the theoretical value. Final xylitol concentration was 94.74 g/L obtained from a hydrolysate with an initial xylose concentration of 105.35 g/L. Continuous adaption-selection technique was used to acclimatize the yeast cells. In this process, cells that grew well in dilute hydrolysate were repeatedly transferred to more concentrated hydrolysate media. The procedure was repeated until yeast cells were able to tolerate concentrated hydrolysate conditions and grew well.

Rice straw hydrolysate in the presence of ammonium sulfate and rice bran was converted to xylitol by *C. guilliermondii* at a yield of 0.68 g/g and productivity of 0.54 g/L·h (Roberto *et al*., 1995). Corn cob hemicellulose hydrolysate was also used as a substrate for *Candida sp.* 11-2 in the production of xylitol (Dominguez *et al*., 1997). Xylitol yield and productivities were 0.57 g/g and 1.94 g/L·h, respectively.

2.5 Xylitol Recovery From Fermentation Broth

Knowing the characteristics of the xylitol molecule is critical to understanding methods of recovery. The size of the xylitol molecule has been investigated and was found to be about 0.96-0.99 nm in length and 0.3-0.33 nm maximum radius (Kiyosawa, 1991).

Physical and chemical properties of xylitol that are critical for separation from fermentation media are given in Table 2.10.

The impurities found in the xylitol fermentation broth have a range of molecular sizes. Most of these impurities are residual nutrients from the fermentation and include yeast extract, polypeptides, sugars, sugar alcohols, and inorganic salts. The recovery of dilute concentrations of xylitol from such a complex mixture is a major challenge, which may explain why published literature shows only limited research in xylitol recovery from fermentation broths. Yeast extract, an impurity in the broth is composed of amino acids, peptides, oligopeptides and proteins (Table 2.9).

Table 2.9 Composition of yeast extract
(Sommer, 1996)

Fraction	% of total yeast extract	Molecular weight
Free amino acids	35-40	N/A
Peptides	10-15	<600
Oligopeptides	40-45	2000-3000
Other Oligopeptides and Proteins	2-5	3000-100,000

Table 2.10 Physical properties of xylitol
(Counsell, 1978, Jaffe, 1978)

Property	**Xylitol**
Formula	$C_5H_{12}O_5$
Molecular Weight	152.15
Appearance	White, crystalline powder
Odor	None
Solubility at 20 °C	169 g/100 g H_2O
pH in water (1 g/10 mL)	5 – 7
Melting Point (°C)	93 – 94.5
Boiling Point (at 760 mmHg)	216 °C
Density (bulk density) (15 °C)	1.50 g/L
Caloric value	4.06 cal/g (16.88 J/g)
Moisture absorption (%) (4 days, 20-22 °C)	
at 60% relative humidity	0.05
at 92% relative humidity	90
Density (specific gravity) of aqueous solution (20 °C)	
10%	1.03
60%	1.23
Heat of solution, endothermic	36.61 cal/g (153.76 J/g)
Viscosity (cP) (20 °C)	
10%	1.23
40%	4.18
50%	8.04
60%	20.63

Methods for xylitol recovery include ion-exchange resins, activated carbon, and chromatography. Gurgel et al. (1995) used both anion and cation exchange resins to purify xylitol from sugar cane bagasse hydrolysate fermentation broth. Xylitol had affinity for strong cation-exchange resin (Amberlite 200C) and weak anion-exchange resin (Amberlite 94S), which resulted in 40-55% loss of product because the xylitol adhered to the surface of the resin. The fermentation broth was also treated with activated carbon, which removed both color and proteins. The fermentation broth was treated with 200 g/L activated carbon at 80 °C, pH 6 for 60 min. This treatment removed color and proteins, but adsorbed about 20% of the xylitol. The solution was filtered, concentrated and crystallized. Crystal recovery was very difficult because the solution was colored and viscous. It took almost six weeks at –15 °C to crystallize the xylitol.

Xylitol production and recovery from the fermentation of birch wood waste sulfite pulping liquor and steam-exploded birch wood hydrolysate (Table 2.11) has been reported (Heikkila *et al.*, 1992). *Candida tropicalis* (ATCC 9968) was used for the fermentation and xylitol was separated by chromatographic methods (Melaja, 1997). A cation exchange resin was used to separate the xylitol from impurities in the solution, and the xylitol-rich fractions were crystallized to produce 99.4% xylitol crystals.

Table 2.11 Birch wood hydrolysate composition

Component	Concentration (g/L)
Xylose	110.0
Glucose	3.1
Rhamnose	3.5
Mannose	3.4
Galactose	1.5
Arabinose	1.6

2.6 Membrane Separation of Xylitol

Raw sugar syrups usually contain high molecular weight materials (Tragardh, 1988). Ultrafiltration has the advantage of removing color from these syrups and improving the purity of the sugar. Membrane separation for sugar refining has been studied for color removal (Cartier *et al*., 1997). Membranes with porosity ranging from 0.2 μm to 15 kilodalton (kDa) were tested to remove color from raw sugar cane solution. The permeate was decolorized by 50% at a flux of 65 L/h·m^2 using a 300 kDa membrane, which gave the best results. The 15 kDa membrane only removed 39% of the color and had a flux of 25 L/h·m^2.

Membrane filtration has been used to separate glucose (25 g/L) and fructose (25 g/L) (Kim, 1985). Kim added some substances that formed complexes with glucose or fructose and this produced permeability differences through membranes, which otherwise had no selectivity by themselves. Salts such as $NaHSO_3$ were added to the solution and these salts formed complexes with glucose or fructose, which aided the separation of the sugars. The increased size of the complexed molecule prevented it from permeating through the membrane. The separation of the sugars was described in terms of a separation factor as shown in equation 2.1.

$$S = \frac{(fructose/glu\cos e)_{product}}{(fructose/glu\cos e)_{Feed}} \qquad (2.1)$$

The maximum separation factor (1.5) was obtained when 20 g/L $NaHSO_3$ was added to the solution.

(Cheryan, 1998)

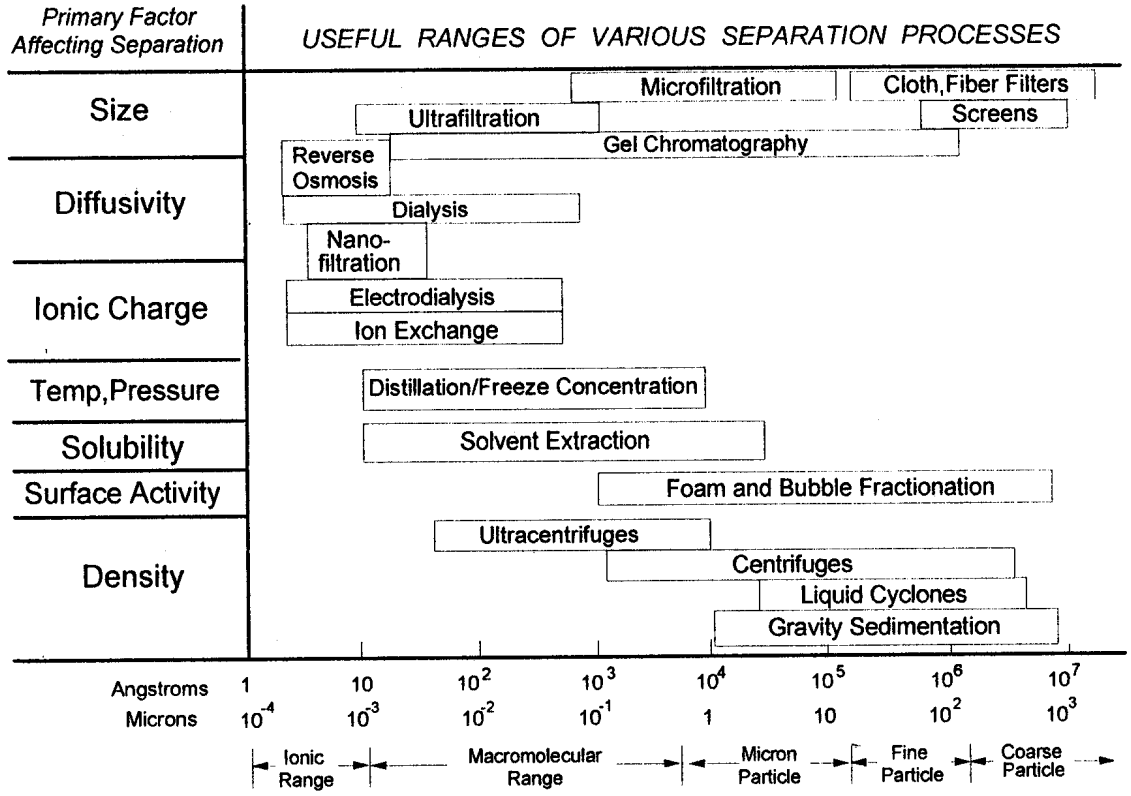

Figure 2.4 Ranges for separation processes.

There are several different types of membranes available and all vary in characteristics depending on the membrane material (Table 2.12) and conditions used during the manufacturing process (e.g. temperature and curing time). It is the nominal molecular weight cutoff and pore size that defines some membranes (Figure 2.4). Membranes are categorized into groups that will reject certain molecules. Each membrane category can be used to filter solutions and perform different separation tasks. Membranes are generally classified into the following categories: microfiltration, ultrafiltration, nanofiltration, and reverse osmosis (Figure 2.5). The major differences between each of these classes of membranes are the nominal molecular weight cutoff (MWCO). The MWCO is based on the spherical shape of the protein molecules and can change with different shape molecules such as, polysaccharides. Microfiltration membranes are classified with pore size and range from 0.1 μm to 5 μm. Ultrafiltration membranes are

used to reject molecules with molecular weight above 1000 with pore sizes up to 100 nm. Nanofiltration membranes have MWCO ranging from 300 to 1000, while reverse osmosis membranes are used for removing salts and larger impurities. A detailed description of these classes of membranes is given in the following sections.

(Cheryan, 1998)

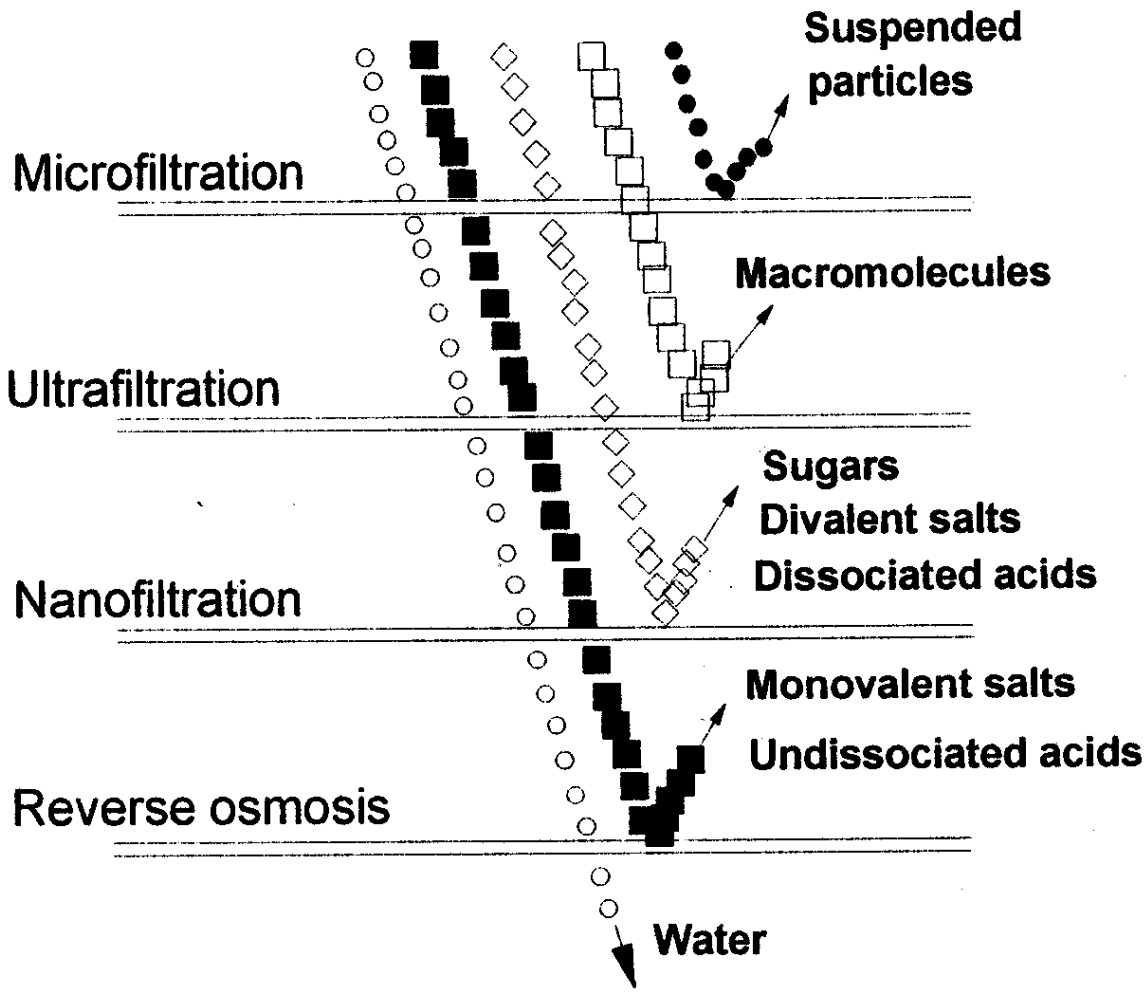

Figure 2.5 Separation characteristics for pressure driven membranes.

Table 2.12 Materials used in manufacturing membranes
(Cheryan, 1998)

Material	Microfiltration	Ultrafiltration	Reverse Osmosis
Alumina	X		
Carbon-carbon composites	X		
Cellulose esters (mixed)	X		
Cellulose nitrate	X		
Polyamide, aliphatic (e.g. Nylon)	X		
Polycarbonate (track-etch)	X		
Polyester (track-etch)	X		
Polypropylene	X		
Polytetrafluoroethylene (PTFE)_	X		
Polyvinyl chloride (PVC)	X		
Polyvinylidene fluride (PVDF)	X		
Sintered stainless steel	X		
Cellulose (regenerated)	X	X	
Ceramic composites (ziconia on alumina)	X	X	
Polyacrylonitrile (PAN)	X	X	
Polyvinyl alcohol (PVA)	X	X	
Polysulfone (PS)	X	X	
Polyethersulfone (PES)	X	X	
Cellulose acetate (CA)	X	X	X
Cellulose triacetate (CTA)	X	X	X
Polyamide, aromatic (PA)	X	X	X
Polyimide (PI)		X	X
CA/CTA blends			X
Polybenzimidazole (PBI)			X
Polyetherimide (PEI)			X

2.6.1 Microfiltration Membranes

Microfiltration can be used to separate suspended particles from solutions. The membranes are designed to reject particles in the micron range (0.1 μm to 5 μm). Microfiltration can be used for removing particles from liquid or gas streams, purification of water, clarification (e.g. apple juice) and wastewater treatment. Materials used to make microfiltration membranes include polypropylene, regenerated cellulose and polyvinyl chloride.

Achieving high cell concentrations during fermentation is a major objective and yeast cell concentrations up to 300 kg/m^3 (dry weight) have been achieved with microfiltration (Lafforgue, 1987). Microfiltration can be used to replace the less economical centrifugation methods for glutamic acid recovery (Huang, 1995). *Corynebacterium crenatum* was used to produce L-glutamic acid and microfiltration was used to concentrate cells for the fermentation (78 %w/v) as well as clarify broth for further processing. Microfiltration has also been used to concentrate yeast cells in the production of ethanol (Groot *et al*., 1992). Fermentation productivity depends on the biomass concentration, and productivity was increased 12-fold to 55 kg/m^3 at a biomass concentration of 135 kg/m^3 using microfiltration.

2.6.2 Ultrafiltration Membranes

Ultrafiltration can be broadly defined as a method for concentrating and fractionating macromolecules where a membrane acts as a selective barrier (Krishnan *et al*., 1994). Ultrafiltration employs membranes whose pore size typically ranges from 5 to 100 nm, with a MWCO above 1,000 (Boye, 1993). Polysulfone and polyethersulfone are commonly used to make ultrafiltration membranes.

Some factors that affect the separation in ultrafiltration membranes are the membrane type and characteristics, transmembrane pressure, pH of the feed, and the protein concentration in the feed (Krishnan *et al*., 1994). Membrane types and materials are shown in Table 2.12 and the characteristics of the membrane are controlled by the conditions they are made under (e.g. temperature and curing time). Materials and

conditions used can control how large the pores of the membrane are and consequently what molecules and particles can pass through the membrane. The transmembrane pressure is the driving force for flux and is measured as the average of the inlet and outlet pressure, minus the pressure on the permeate side of the membrane (Cheryan, 1998). Permeate rates are measured in flux, which is the amount of fluid passing through the membrane and is usually given in terms of volume per unit time per unit membrane area. The pH is important for membrane service life. In water treatment applications using cellulose acetate membranes, the membrane service life is about 4 years at pH 4-5, 2 years at pH 6 and a few days at pH 1 or 9. Protein concentration is important because initially proteins are allowed to pass through the membrane, but build up of proteins on the membrane surface and in the pores can decrease the amount of proteins that permeate the membrane. This build up of proteins and other particles on the membrane surface and in the pores is called fouling, which can impact how large a flux can be obtained for a membrane.

Fouling of ultrafiltration membranes can be severe in dead-end filtration that involves flow of fluid perpendicular to the membrane surface because there is a large build up of particles on the membrane. The main causes of the resistance to permeation are plugging of the membrane pores and formation of microbial cake on the membrane (Tanaka *et al*., 1994). Fouling can be overcome by crossflow filtration, which involves flow of fluid tangentially over the membrane surface. The shear force created by the fluid flow during crossflow filtration reduces the amount of particles deposited on the membrane surface and in the pores.

Crossflow ultrafiltration has been used to separate microbial cells from fermentation broths (Tanaka, 1994). At the initial stage of crossflow filtration the yeast cells and other particles were deposited on the membrane to form a cake similar to dead-end filtration. The flux through the ultrafiltration membrane rapidly decreased in the first 15 minutes of filtration and then steady state was achieved after the initial microbial cake was deposited on the membrane. The permeation flux equation used for this experiment is given in

Equation 2.2. The flux (10^{-4} to 10^{-5} m/s) was found to be independent of the membrane pore size (0.45 to 5 μm).

$$J = \frac{\Delta P}{\mu(R_m + \alpha\omega)} \qquad (2.2)$$

Where

J	- Permeation Flux	(m/s),
ΔP	- Transmembrane Pressure	(Pa),
μ	- Viscosity of the Permeate	(Pa·s),
R_m	- Membrane Resistance	(m^{-1}),
α	- Specific Resistance	(m/kg), and
ω	- Weight of cells deposited on the membrane per unit area	(kg/m^2).

Permeation flux was sometimes described as the permeation velocity (Krishnan *et al.*, 1994). The separation of monoclonal (IgM) antibodies by crossflow ultrafiltration membranes was used to concentrate the proteins in the retentate using feed flow rates of 800 and 1200 mL/min. The permeation velocity was calculated from equation 2.3.

$$V = kN \frac{\pi \ d_m^4 \Delta P}{128 \ \varepsilon \ \eta \ \tau \ LA} \qquad (2.3)$$

Where

V	- Permeation velocity	(m/s),
k	- Correction factor to account for non-uniformity in pore diameter	(dec),
N	- Number of pores,	
d_m	- Mean pore diameter	(m),
ΔP	- Transmembrane pressure	(Pa),
ε	- Membrane surface porosity	(dec),
η	- Permeate viscosity	(Pa·s),
τ	- Pore tortuosity	(=1),
L	- Thickness of the membrane layer	(m), and
A	- Total area membrane filtration area (at time t)	(m^2).

2.6.3 Nanofiltration

Nanofiltration refers to a filtration process with a membrane MWCO of 300 to 1,000 (Boye, 1993). For such membranes, the MWCO falls in the separation domain situated between reverse osmosis and ultrafiltration. Unlike reverse osmosis, the retention of salts in nanofiltration is low for molecular weight below 100; it is high for organic molecules of molecular weight above 300.

Nanofiltration membranes are produced commercially by companies such as Osmonics (Minatanka, MN) and Millipore (Bedford, Mass). Boye (1993) patented a method for producing mechanically strong, thermally and chemically resistant composite nanofiltration membranes. An inorganic substrate was used to support the nanofiltration membrane, which had an elastomeric polyphosphazene on one side of the inorganic support. The resulting membrane had a pore size of 0.2 to 2 nm. The xylitol molecule (0.9 nm) falls in the middle of this range and cannot easily permeate this type of membrane because nanofiltration pores can foul and water can pass through the membrane more easily and dilute the xylitol concentration in the permeate.

Nanofiltration membranes are capable of concentrating sugars, divalent salts, bacteria, proteins, particles, dyes, and other particles with molecular weight greater than 1000. Nanofiltration membranes reject molecules based on size when the particles are too large to pass through the pores. In addition, nanofiltration membranes can also use charge to reject molecules, much like reverse osmosis. From the above description, it appears these membranes could be ideal for the fermentation broth because not only will they reject the large molecular weight materials, but they will also reject charged particles like phosphates and sulfates, while allowing xylitol to permeate.

2.6.4 Reverse Osmosis

Osmosis is the spontaneous flow of pure water into an aqueous solution, or from a less to a more concentrated aqueous solution, when separated by a semipermeable membrane (Sourirajan, 1970). Reverse osmosis (RO) is the process of forcing water through a membrane from a more concentrated to less concentrated aqueous solution. Reverse

osmosis utilizes extremely fine pores in the membranes that are typically made from cellulose acetate. The pores are believed to be less than 0.001 micron (μm) in diameter (Byrne, 1995). However, reverse osmosis is not filtration. Filtration is the removal of particles by size exclusion or the particles are too large to go through physical pores. In the case of reverse osmosis, such pores have never been viewed with a microscope. It is more likely that the small molecules permeate the reverse osmosis membrane by diffusive forces.

Some applications of reverse osmosis include desalination of brackish and sea waters, removal of natural organic matter for disinfection by-product control, separation of specific dissolved inorganic and organic contaminants, and rejection of pathogenic microorganisms (Urama, 1997). Because these membranes are easily fouled, increased mixing can decrease deposition of particles on the surface.

The use of RO to recover and concentrate sugar solutions from various food processes has grown rapidly in the past decade (Byrne, 1995). Detailed reverse osmosis studies on glycerol-water, sucrose-water, and urea-water have been reported. In addition, Matsuura (1971) studied reverse osmosis for the concentration of glucose-water (concentrated 0.1 to 1.5 M), maltose-water (0.03 to 0.11 M), and lactose-water (0.04 to 0.22 M).

In China, xylose is concentrated by evaporation with the Three Boiling System prior to chemically producing xylitol (Yurong *et al.*, 1987). The xylose solutions were concentrated from 3% to 15% using reverse osmosis as an alternative to evaporation. Rutskaya (1989) used reverse osmosis to concentrate xylitol for crystallization. The reverse osmosis procedure was used to concentrate a solution from 5-6% soluble solids to 15-16% soluble solids.

Reverse osmosis has some advantages over evaporation when concentrating sugar solutions. It prevents carmelization and saves energy. Yurong et al. (1987) found that reverse osmosis membranes allowed acid in the feed to permeate the membranes, so the

acidity of the solution decreased, thus increasing the service life of the ion-exchange resins used for further purification.

2.7 Chemical Reactions Used to Enhance Membrane Separation

Membrane separation of sugar solutions can be considerably enhanced by the addition of suitable compounds that form complexes with the sugar molecules. The formation of the sugar complexes can result in permeability differences of sugar molecules through the membrane. In order to separate molecules of similar molecular weight, some differences in the molecules have to exist. If both molecules have the same weight and both are neutral molecules, either size must be increased or a charge added to the molecule to effect membrane separation.

Sodium bisulfite ($NaHSO_3$) adds to carbonyl groups and the reaction is shown in Figure 2.6. Bisulfite adducts were used mainly for the purification of reactive carbonyl compounds (Adam, 1979). The increased bulk of the molecule can aid in membrane separation.

(Solomons, 1992)

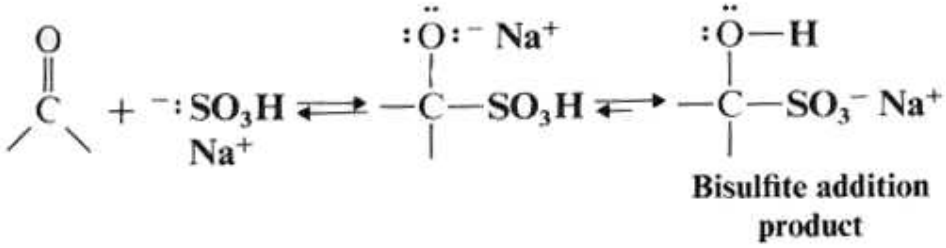

Figure 2.6. Sodium bisulfite reaction.

Urea can react with arabinose to form more bulky molecules for membrane separation. Arabinose was reacted with urea at 50 °C for seven days (Naito *et al.*, 1961). 1-D-arabinopyranosylurea was formed from 46.5% of the arabinose.

Another potential reaction that could be used to improve the separation of sugar alcohols from fermentation broth is the Maillard reaction. The Maillard reaction is responsible for the "brown" color that appears in many cooked and baked foods (Bedinghaus, 1995). The reaction is the result of interactions between amino-bearing groups, commonly proteins or amino acids, and reducing sugars. The molecular weight distribution of Maillard reaction products (MRP) of glucose and ε-amino groups of lysine were determined by gel permeation chromatography with the majority of molecular weights ranging from 1000-2000 (Labuza *et al.*, 1994). Other abundant components were found to have molecular weight between 172 and 406, with molecular weight distribution up to 200,000. Glucose-tryptophan Maillard reaction products were produced by refluxing glucose and tryptophan at 100 °C for 10 hours at pH 11.0 (Yen, 1994). Six membrane filters were used to separate the Maillard reaction products into fractions. The six fractions ranged in molecular weight from: below 3,000, 3,000 to 10,000, 10,000 to 30,000, 30,000 to 50,000, 50,000 to 100,000, above 100,000. The experiment was performed to look for the greatest inhibitory activity of the six fractions against the mutagenicity of 2-Amino-3-methylimidazo(4,5-f)quinoline, but showed that Maillard reaction products could be separated using membranes on the basis of molecular weight. Maillard reaction products have also been prepared by refluxing D-xylose with lysine at 100 °C for ten hours at pH 9 and separated into fractions by membrane separation (Yen, 1993). Again, the antimutagenic affect of the fractions on 2-amino-3-methylimidazo(4,5-f)quinoline was observed in this experiment. Membranes were used to separate Maillard reaction products into five molecular weight fractions: below 10,000, 10,000 to 30,000, 30,000 to 50,000, 50,000 to 100,000, and 100,000 and above. Estimation of the molecular weight was performed by an elution curve using Sephadex G-100 column and known molecular weight compounds such as ribonuclease A (MW 13,700) and blue dextran 2000 (MW 2,000,000). The membranes were used to separate the Maillard reaction products based on molecular weight distribution, but the experiment was not concerned with how much Maillard reaction product was in each fraction.

CHAPTER 3
MATERIALS AND METHODS

3.1 Fermentation

Candida tropicalis (ATCC 96745) was obtained from the American Type Culture Collection (Rockville, MD), stored at 8 °C on YM (Yeast-Malt) agar slants and subcultured once a month.

The preculture medium consisted of D-xylose, 60 g/L; yeast extract, 10 g/L; KH_2PO_4, 15 g/L; $(NH_4)_2HPO_4$, 3 g/L; $MgSO_4 \cdot 7H_2O$, 1 g/L and two drops of Sigma 289 antifoam agent (Yahashi *et al.*, 1996). The pH was adjusted to 5.0 with 1 M HCl. The preculture was incubated in a 500 mL Erlenmeyer flask containing 100 mL of medium. All samples were agitated at 130 rpm on a rotary platform shaker (Innova 2050) for 14 hours at 30 °C.

The production medium consisted of a model corn fiber hemicellulose hydrolysate defined by Walther (1999): D-xylose, 90 g/L; glucose, 17.4 g/L; arabinose, 23.2 g/L; galactose, 2.9 g/L; yeast extract, 20 g/L; KH_2PO_4, 15 g/L; $(NH_4)_2HPO_4$, 3 g/L; $MgSO_4 \cdot 7H_2O$, 1 g/L and two drops of antifoaming agent. The pH was adjusted to 5 with 1 M HCl throughout the fermentation time. The model hemicellulose hydrolysate was fermented in a BioFlo III fermentor with a 1 L working volume (New Brunswick Scientific Co.). The following fermentation conditions were used: agitation rate (130 rpm), fermentation temperature (30 °C), aeration rate (1.5 vvm (volume of air per volume of medium per min)), reaction time (170 hours).

All chemicals used for the fermentation were reagent grade purchased from Sigma Chemical Co. (St. Louis, MO). Throughout the fermentation process, the following parameters were monitored: dissolved oxygen (%), pH, agitation (rpm), and temperature (°C).

After 170 hours of fermentation, the reaction was stopped and the biomass was harvested. The fermentation broth was vacuum filtered using 10 μm Whatman filter paper

(1001 070). The filtrate was transferred to a 1 L Nalgen sample container for membrane treatment or frozen at -20 °C for further analysis.

Dry cell mass was measured using optical density of the yeast cells at 640 nm using a spectronic 1001 spectrophotometer (Milton Roy Company). A calibration curve was created using four fermentation samples containing *C. tropicalis* yeast cells at various optical densities, the optical densities were recorded and the samples were placed in a tare weighed 2 mL plastic centrifuge tubes (previously dried at 90 °C for 24-hours). The cells were centrifuged at 19,000 g, the liquid was removed and the centrifuge tubes containing yeast cells were dried in an oven at 90 °C for 24-hours. After 24-hours, with no change seen in the mass of the tubes and yeast cells, the dry cell mass was recorded and the calibration curve plotted for dry cell mass versus optical density. Any fermentation sample taken could then be measured for the optical density at 640 nm and the dry cell mass could be calculated using the calibration curve.

3.2 Cross Flow Ultrafiltration

The fermentation broth was filtered using an OPTISEP ® (North Carolina SRT, NC) cross-flow membrane unit with a 150,000 nominal molecular weight cutoff (MWCO) polyethersulfone membrane (North Carolina SRT, NC) with a surface area of 0.0213 m^2. Fermentation broth was pumped from a 1 L plastic sample bottle into the OPTISEP ® system using a peristaltic pump (Masterflex, model 7529-00). Tygon tubing (5/16 in. I.D. and 3/32 in. thick) was used to transport the fermentation broth from the holding tank to the peristaltic pump, through the OPTISEP ® unit (connected by hose clamps) and recycled back to the holding tank. The following conditions were used for the cross-flow ultrafiltration separation: flowrate (0.5 L/min), pressure (13.8 kPa), pH (5), temperature (ambient 25 °C). All permeate was collected in a 250 mL beaker and the volume was measured with a 250 mL graduated cylinder. The permeate was stored in 1 L Nalgene plastic bottles at –20 °C.

3.3 Activated Carbon Treatment

Fermentation samples were acquired from a flask culture experiment performed by Patcharee Hensirisak, a fellow graduate student. The yeast cells were removed from the broth by vacuum filtration using 10 μm Whatman (1001 070) filter paper. Samples (4 mL) were taken and analyzed by HPLC for initial sugar, xylitol, and relative UV absorbing material. Three filtered broth samples (100 mL) were placed in 250 mL Erlenmeyer flasks and activated carbon (2.5 g, 50-200 mesh) was added to each flask and the pH was adjusted to 6.0 with 1 M ammonium hydroxide. The flasks were placed in a shaker bath (Precision Scientific Model 25) at 80°C for one hour at 100 rpm. The mixture was cooled to room temperature and the activated carbon was vacuum filtered with 0.2 μm Whatman glass fiber filters. Filtrate samples (4 mL) from each flask were taken for analysis. Filtrate was concentrated to 70 wt% xylitol by evaporation at 40 °C under reduced pressure using a Buchi Rotavapor R-124. The concentrated mixtures were placed on the lab bench and cooled to room temperature (25 °C), seeded with xylitol crystals (0.5 mg), and placed in the refrigerator at 8 °C for 1 week. Crystals were recovered by vacuum filtering through 10 μm Whatman (1001 070) filter paper. The crystals were dissolved in deionized water and analyzed by HPLC for final sugar, xylitol, and relative UV absorbance at 260 nm.

3.4 Membrane Testing Apparatus

A membrane separation apparatus was constructed for testing the separation of xylitol from sugars with different membranes such as reverse osmosis, nanofiltration, and ultrafiltration that had various nominal molecular weight cutoffs (MWCO). A flow diagram of the membrane separation apparatus is shown in Figure 3.1 and a picture of the apparatus is shown in Figure 3.2.

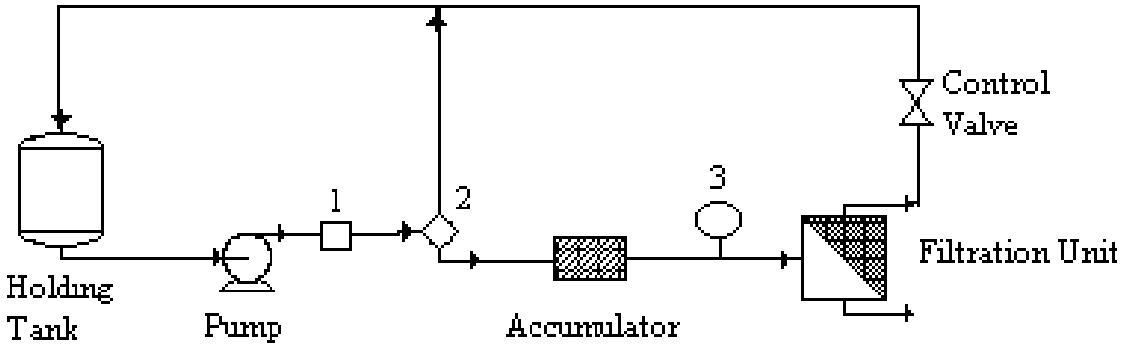

Figure 3.1 Flow sheet of membrane testing apparatus. Numbered components of membrane testing apparatus 1. Check valve 2. Relief valve 3. Pressure gauge.

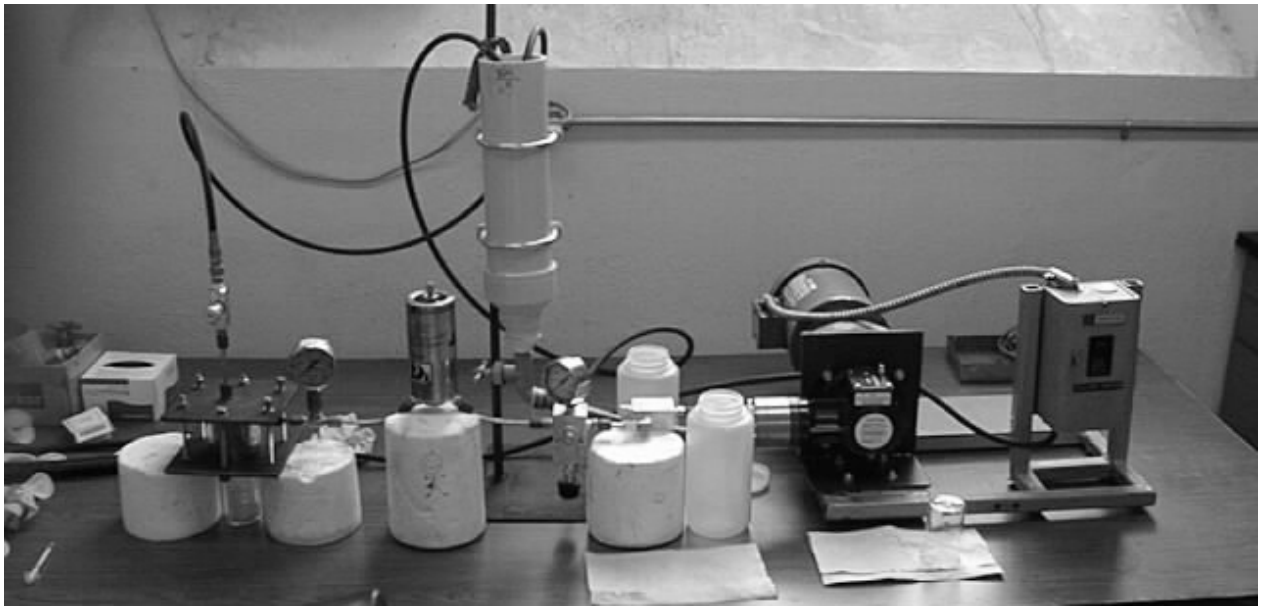

Figure 3.2 Membrane test apparatus for recovery of xylitol.

The specifications for the design of the membrane separation unit are as follows:

Motor - three phase motor
½ horsepower
1725 RPM
Dayton Model # 2N916M (Dayton, Chicago, IL)

The motor drive was controlled with a motor starter (GE electric starter) to turn the motor on and off. The motor was bolted directly to a 4:1 speed reduction gearbox (Boston Gear, Cat. No. F611B-4B5, Quincy, Mass). The diaphragm pump, which had the specifications below, was directly coupled to the gearbox.

Hydra-Cell Diaphragm Pump-	max pressure 6.9 MPa Flowrate 0.2-1 gpm / 0.76-3.8 Lpm brass casing model F20XABGSNECG (Texas Pump, Minneapolis, MN)

The pump, motor, gearbox and electric starter were all mounted on a 1-inch steel tubing frame.

The liquid entered the system through a check valve (Sun Hydraulics, Part No. GAA) to prevent flow back into the pump and to reduce pressure fluctuations. A relief valve (Sun Hydraulics, Part No. FEA) was used to prevent excessive pressure buildup in the system and to control the flow rate. A needle valve (Parker, Model N400B, Elyria, OH) was used to control the pressure inside the system by throttling the valve. The pressure fluctuations caused by the Hydra-Cell diaphragm pump were dampened with an accumulator (Liquid Dynamics, Part No. PIG-TW61B44x75NIT100P1/4-1/4NPT316, Liquid Dynamics, Hampstead, NC).

The pump, check valve, relief valve, accumulator, pressure gauge, and test cell were connected with ¼-inch O.D., 1/16-inch thick stainless steel piping and stainless steel connectors (4-4 FBU-SS, 4-6 FBU-SS at the pump fitting, and 4 F5BU-SS at the test cell, Applied Fluid Power, Richmond, VA).

The membrane test cell was constructed in the Biological Systems Engineering machine shop. The design specifications of the membrane test chamber are shown in Figures 3.3 and 3.4. Figure 3.5 shows how the top and bottom parts of the membrane chamber fit together to hold the membrane for testing.

A 1 ½-inch diameter porous stainless steel frit from Mott Industrial Corporation (Farmington, CN) was used to support the filtration membrane and nitrile o-rings were used to seal the test cell chamber. The membrane was cut with scissors in house to fit the test cell, with a surface area of 0.000507m^2. The shiny membrane surface was placed facing the top of the test cell and the dull side on the porous metal frit. A small film of high pressure vacuum grease (Dow Corning, Midland, MI) was applied to the o-ring seals and set into the o-ring grooves. Two ¼-inch steel plates and 6 (¼-inch) bolts and nuts were used to clamp the test cell together to form a water tight seal.

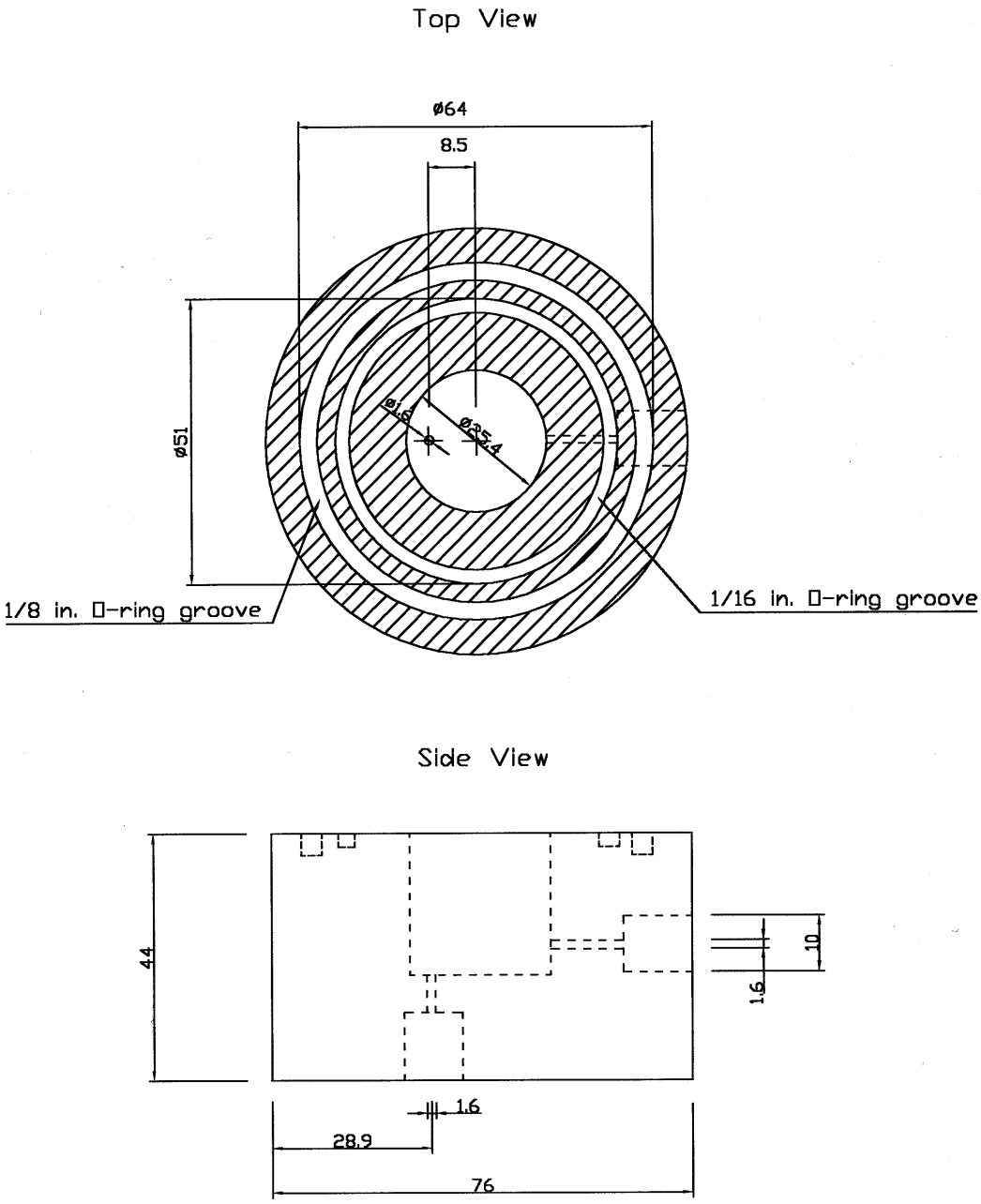

Figure 3.3 Design of top to test cell, constructed from three inch diameter stainless steel cylinder.
(All dimensions in mm)

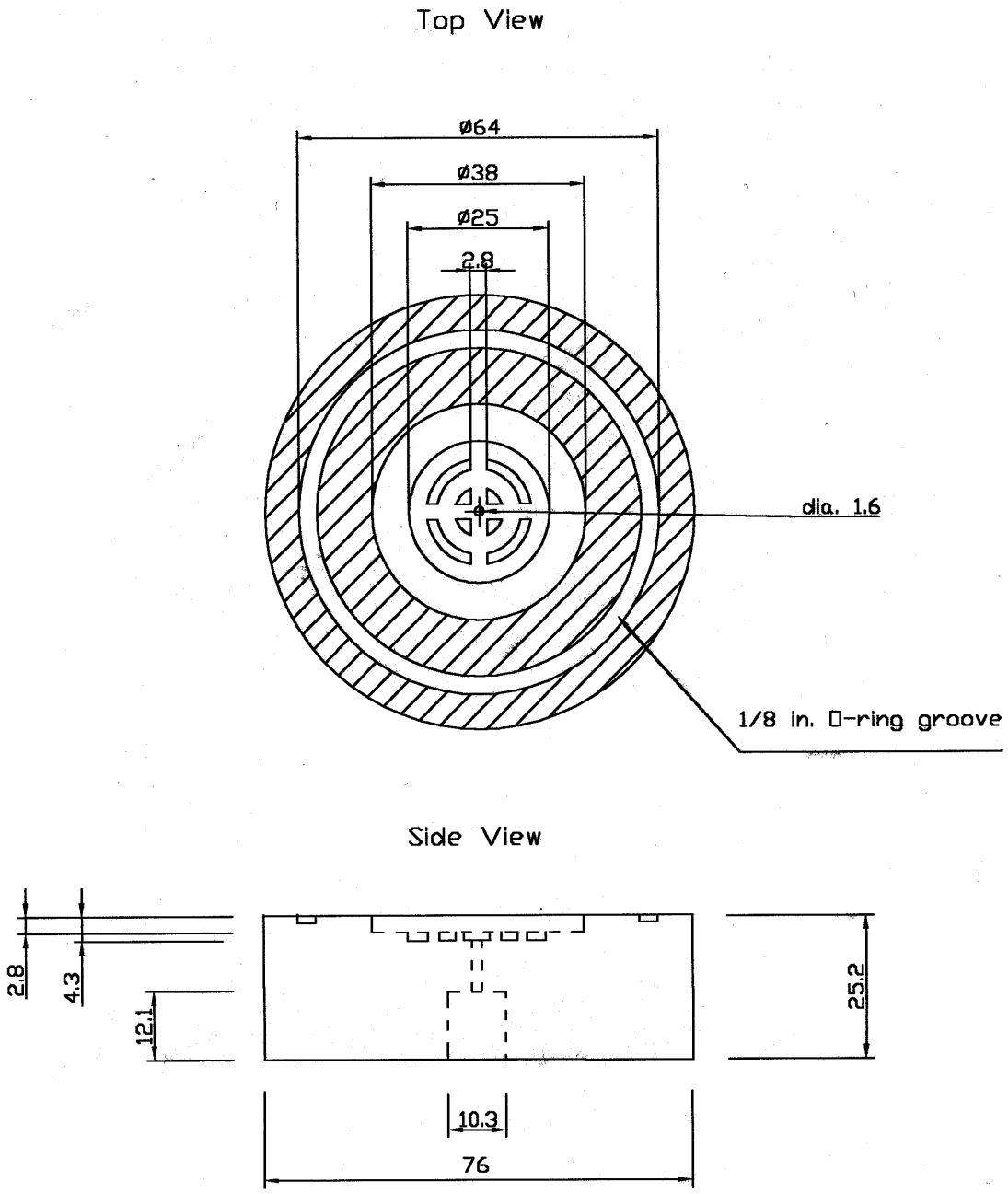

Figure 3.4 Design of test cell bottom, with depression cut for 1 ½-inch diameter porous stainless steel frit.
(All dimensions in mm)

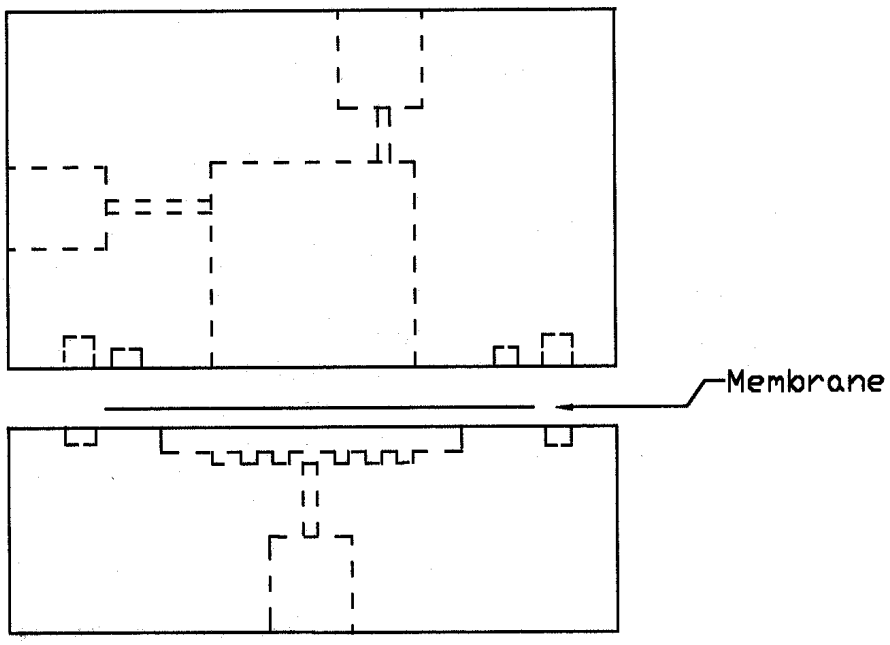

Figure 3.5 Side view of top and bottom of membrane test chamber as assembled.

3.5 Membrane Selection

The main focus of membrane selection was to find a suitable membrane that would allow xylitol to pass through the membrane while retaining a large portion of the impurities found in the fermentation broth. Ten membranes for reverse osmosis, nanofiltration, and ultrafiltration were purchased from Atlantic Technologies Group Inc. (Richmond, VA) for testing (Table 3.1).

Prior to testing the membrane, the piping system was thoroughly rinsed with 4 L deionized (DI) water to clean. The unit was then run under test conditions (pressure 1.4 MPa, flowrate 1 L/min and 25 °C) for 1 hour with total recycling of 1 L of DI water. This procedure allowed water to permeate the membrane and flush out impurities from the porous frit and permeate port. After the run, the DI water was completely removed from the system.

Table 3.1 Reverse osmosis, nanofiltration, and ultrafiltration membranes which were evaluated

Designation	Polymer Type	Nominal Molecular Weight Cutoff (MWCO) Range	% NaCl Rejection	Recommended Pressure (MPa)	Maximum Pressure (MPa)	Recommended pH range	Maximum Temp. (°C)
MS19	Polyamide	125-200	≥99	1.4	6.8	2-12	80
ST10	Cellulose Acetate	150-220	≥95	2.8	6.8	2-8	50
SR10	Cellulose Acetate	200-300	≥92	2.8	6.8	2-8	50
SF10	Cellulose Acetate	250-400	≥85	2.1	6.8	2-8	50
SX01	Cellulose Acetate	300-500	50-70	1.4	3.4	2-8	50
SX10	Cellulose Acetate	300-500	50-70	1.4	3.4	2-8	50
MX07	Polyamide	300-600	50-70	0.7	6.8	2-12	80
SV10	Cellulose Acetate	400-600	30-50	1.4	2.7	2-8	50
BQ01	Anion Rejection	500-1000	20-30	0.7	6.8	0.5-11	100
HG19	Polysulfone	2500-10000	-	0.7	2.7	0.5-13	100

3.5.1 Reverse Osmosis Filtration

After the previously mentioned cleaning with DI water, a model fermentation broth (1 L) was added to the holding tank. The model fermentation solution contained 10 g/L yeast extract, 7.5 g/L KH_2PO_4, 1.5 g/L $(NH_4)_2$ HPO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 4.5 g/L xylose, 25g/L arabinose, and 88 g/L xylitol. The model fermentation solution was pumped through the system for one hour under test conditions to flush out any residual DI water from the porous frit and permeate port. Feed samples of the model fermentation solution were taken prior to any sample collection. Sample collection began after 1 hour of operation.

For reverse osmosis membranes, three pressures were tested (1.4 MPa, 3.4 MPa, and 4.8 MPa) to determine the effect of pressure on the flux and the separation of Lowry positive material from the xylitol. A new solution was used for each membrane tested. All three pressures were tested during the same run period by changing the pressure during the membrane test. At 1.4 MPa, permeate was collected in a 100 mL beaker and one (1 mL) sample was taken after 10 minutes for analysis. One sample was taken to note the effect of pressure on flux, but three samples were taken in the recommended operating pressure region. The pressure was then raised to 3.4 MPa. The porous frit and permeate port were rinsed for 10 minutes with model fermentation solution to flush out any residual solution. Samples (1 mL) were collected every 10 minutes for 30 minutes. The pressure was then raised to 4.8 MPa and the permeate port was rinsed with model fermentation solution for 10 minutes. Then one sample (1 mL) was collected for 10 minutes to note the effect of pressure on the flux. The feed solution at the end of the run was also sampled and analyzed. During sampling any excess permeate collected was returned to the feed tank to maintain solution concentration. The samples were frozen at –20 °C for later analysis by HPLC. Table 3.2 shows the times and pressures used for reverse osmosis membrane testing.

Table 3.2 Reverse osmosis times for pressure change and sample collection

Time (minutes)	Pressure (MPa)	Samples taken
0	0	2 feed samples
60	1.4	1 hour cleaning
70	1.4	1 mL
70-80	3.4	10 min cleaning
90	3.4	1 mL
100	3.4	1 mL
110	3.4	1 mL
110-120	4.8	10 min cleaning
130	4.8	1 mL
130	0	1 end feed sample

An end feed sample was a sample taken from the recycled feed solution in the system at the end of a membrane test. After each membrane test, the unit was cleaned by circulating 1 L of DI water through the system. This water was immediately discarded with no recycling. This process was repeated 3 or 4 times until the water came out of the system clear. The system was then rinsed with 1 L of DI water under test conditions, recycled for 5 minutes and discarded. The test cell was then removed from the system and the steel plates were opened. The cell and porous frit were rinsed with DI water and dried at room temperature for the next membrane test.

3.5.2 Nanofiltration and Ultrafiltration

Similar to the reverse osmosis filtration, the system was rinsed with DI water and the model sugar solution was added. Three pressures were tested (1.4 MPa, 2.1 MPa, and 3.4 MPa) to determine the effect of pressure on the flux and the separation of Lowry

positive material from the xylitol. Three pressures were tested during the same run period by changing the pressure during the membrane test. Three samples (1 mL) at 20 minute intervals were taken during the 1.4 MPa run. The pressure was then increased to 2.1 MPa and the porous frit and permeate port were rinsed for 10 minutes with model fermentation solution. One sample was collected after 10 minutes at 2.1 MPa to note the effect of pressure on the flux. The pressure was then raised to 3.4 MPa and the permeate port rinsed with model fermentation solution for 10 minutes. After 10 minutes at 3.4 MPa, one sample was collected to determine flux effect. At the end of the run, a feed sample was taken for analysis and comparison. The samples were frozen at –20 °C for later HPLC analysis. Table 3.3 shows the times at which the pressure was changed and samples were taken. The clean-up procedure mentioned in the reverse osmosis filtration section was applied to the nanofiltration and ultrafiltration tests as well.

Table 3.3 Ultrafiltration and nanofiltration times for pressure change and sample collection

Time (minutes)	Pressure (MPa)	Samples taken
0	0	2 feed samples
60	1.4	1 hour cleaning
80	1.4	1 mL
100	1.4	1 mL
120	1.4	1 mL
120-130	2.1	10 min cleaning
140	2.1	1 mL
140-150	3.4	10 min cleaning
160	3.4	1 mL
160	0	1 end feed sample

3.6 Membrane Filtration of Fermentation Broth

The selected membranes (HG19 10,000 MWCO polysulfone, and 150,000 MWCO polyethersulfone) were used to filter 200 mL of model corn fiber hydrolysate fermentation broth. The rinsing of the apparatus prior to the filtration of the sample were similar to those used for the model fermentation studies. In these tests, the filtration was conducted for 4 to 6 hours and permeate was collected for HPLC analysis and crystallization. Sample sizes were about 20 mL for 150,000 MWCO membrane, HG19 membrane, and Maillard treated solution using HG19 membrane.

3.7 Crystallization

About 20 mL of xylitol permeate solution was placed in a 50 mL round bottom flask and concentrated to 35-45 wt% xylitol, at 40 °C under reduced pressure using a Buchi Rotavapor (Model R-124). The concentrated solution was cooled for 30 minutes at ambient temperature (25 °C) and placed in the refrigerator at 8 °C. Due to impurities in the solution, crystallization was slow and required 14 days to crystallize. After 14 days, the crystals were separated from the mother liquor by vacuum filtration using a medium porosity glass crucible filter. The crystals were washed with 80 mL of 99.9% methanol. Crystals were collected in a glass vial and 5 to 50 mg were dissolved in 1 mL of DI water for HPLC analysis.

3.8 Maillard Reaction

Maillard testing was performed for reaction between reducing sugars and proteins in the fermentation broth. Testing was conducted in a convection oven at 90 °C for 24 hours with model sugar solutions containing 10 g/L and 40 g/L yeast extract (Pitotti, 1995). Model sugar solution (100 mL) was placed in a 250 mL Erlenmeyer flask and samples were taken at 0,1,3,6, and 24 hours.

In a following Maillard experiment, samples (10 mL) of fermentation broth with yeast cells and fermentation broth with yeast cells removed (by 150,000 MWCO membrane filtration) were placed in 20 mL vials and heated for one to two hours at 121 °C.

In further Maillard experiments, samples (10 mL) of fermentation broth with cells, fermentation broth with cells removed, and fermentation broth with cells and yeast extract added (20 g/L, 40 g/L, or 60 g/L) were heated to 125 °C for three hours. Samples were taken prior to and following heating. All samples were analyzed by HPLC.

3.9 Analytical Methods

3.9.1 HPLC

All samples were analyzed using HPLC (Shimadzu Company, Columbia, MD) equipped with a refractive index detector (Shimadzu RID-10A) and an ultraviolet/visible (UV/visible) detector (Shimadzu SPD-10AV vp). A Benson Polymeric Ca^{++} carbohydrate column (Benson Polymeric inc., Reno, NV) was used for the analysis. The column was operated in an oven (Shimadzu CTO-10AS vp) at 80 °C and DI water was used for the mobile phase. The flow rate was lowered linearly from 1 $mL \cdot min^{-1}$ for 25 minutes until the flow rate was equal to 0.7 $mL \cdot min^{-1}$. Sugars and polyols were detected and quantified by comparing their retention times to authentic standards. Samples were prepared for analysis by diluting 200 µL samples with 1800 µL DI water in a 2 mL micro centrifuge tube. The diluted samples were filtered using 0.45 micron (µm) syringe filters and 10 µL was injected and analyzed. Data were retrieved and analyzed using Shimadzu Class VP software.

The UV/visible detector (Shimadzu SPD-10AV vp) was used (at 260 nm) to measure the absorption of impurities such as, proteins, peptides, and amino acids. UV absorption of proteins at 260 nm can have interference from nucleic acids, peptides, and amino acids (Vernon, 1977). The absorption area at 260 nm was recorded for fermentation feed

samples, the membrane treated samples and the relative protein, peptide, and amino acid permeation of the samples was calculated using equation 3.1.

$$P = \frac{absorption\ \ area\ \ of\ \ \ permeate\ \ sample}{absorption\ \ area\ \ of\ \ \ feed\ \ sample} \bullet 100 \qquad (3.1)$$

Where,

P – Relative peptide, protein, and amino acid permeation

3.9.2 Lowry Method

Impurities (such as proteins, amino acids, and peptides) were quantified using the Lowry method (Sigma protein assay kit). While Lowry's method can analyze proteins, the method can also have interference from the sugars, amino acids, and peptides in the fermentation solution and therefore Lowry's method was used as a quatitative measure of the relative retention of amino acids, peptides, and proteins in the permeate.

A calibration curve was developed using bovine serum albumin. Samples (20 μL) were diluted with 980 μL of deionized water for a total volume of 1 mL. Maillard reaction samples (5 μL) were diluted with 995 μL of DI water. Lowry's reagent (1 mL alkaline cupric tartrate solution) was added to the diluted samples and allowed to sit for 20 minutes at ambient temperature. After 20 minutes 0.5 mL of Folin & Ciocalteus (phenol solution) reagent was added to the solution and allowed to sit for another 30 minutes at ambient temperature. The samples were then analyzed at 750 nm using a spectronic 1001 spectrophotometer (Milton Roy Company).

3.9.3 SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed on the fermentation broth. Stock acrylamide – ultra pure protoGel 30% (w/v) acrylamide, 0.8% (w/v) bisacrylamide stock solution (National diagnostics) was used.

MINI-PAGE (12% running gel) was made from 16 mL stock acrylamide, 10 mL of 1.5 M Tris (pH 8.8), 13.4 mL of DI H_2O, 0.4 mL of 10% SDS, 0.2 mL of 10% ammonium persulfate, and 0.02 mL of tetramethylethylenediamine (TEMED). The stock acrylamide, 1.5 M Tris (pH 8.8), DI H_20 and 10% SDS were combined and degassed to remove O_2. Then the ammonium persulfate and TEMED components were added and the gels were poured. DI H_2O-saturated butanol was poured over the top of the gel.

A stacker gel was used to load the protein samples and was made from 0.65 mL stock acrylamide, 1.25 mL of 0.5 M Tris (pH 6.8), 3.1 mL of DI H_2O, 0.05 mL of 10% SDS, 0.025 mL of 10% ammonium persulfate, and 0.005 mL of TEMED. When the running gel had polymerized the stock acrylamide, 0.5 M Tris (pH 6.8), DI H_2O and 10% SDS were mixed and degassed. Ammonium persulfate and TEMED were added after degassing. The butanol was rinsed off the running gel with DI H_2O and the stacker gel was poured. A comb was inserted into the stacker gel at an angle to avoid bubbles and create wells for protein solution. When the stacker gel was polymerized, the comb was removed and the wells were rinsed out with distilled water. Electrophoresis buffer with pH 8.8 containing 3 g/L Tris, 14.4 g/L glycine, 1 g/L SDS was added and the samples were loaded. The samples were run at 30 mA per gel for 90 minutes in the electrophoresis unit. Cooling water was run for 90 minutes to cool the system.

Protein samples were diluted 1:1 with 100% isopropanol and placed at –20 °C for 30 minutes. The precipitated proteins were centrifuged at 25,000 g in an Eppendorf centrifuge. The precipitated proteins were redissolved in 0.05 mL sample buffer and heated in a sand filled heat block to 100 °C for 5 minutes. Sample buffer solution was made by mixing 4.0 mL DI H_2O, 1.0 mL of 0.5 M Tris-HCl, 0.8 mL glycerol, 1.6 mL 10%(w/v) SDS, 0.4 mL of β-mercaptoethanol, and 0.2 mL of 0.2% bromophenol blue.

After samples were run on the SDS-PAGE gel the gels were cooled and stained with coomassie blue R-250 protein stain to show the protein markers. Coomassie blue R-250

was made by combining 1.0 g comassie blue R-250, 400 mL methanol, 100 mL glacial acetic acid, and 500 mL DI H_2O.

After staining the gels were placed in a destain solution to remove excess stain on the gel. The first destain solution contained 800 mL methanol, 200 mL glacial acetic acid, and 1000 mL of DI H_2O. The second destain solution contained 100 mL methanol, 140 mL of glacial acetic acid, and 1760 mL DI H_2O.

Protein molecular weight markers were used to determine molecular weights of the protein bands in the yeast extract and xylitol crystal samples. The molecular weight markers used were phosphorylase b (97,400), serum albumin (66,000), ovalbumin (45,000), carbonic anhydrase (31,000), trypsin inhibitor (21,500), and lysozyme (14,400).

3.9.4 Phosphate Analysis

Crystals were analyzed for salt content when unknowns were detected in the crystals after HPLC analysis. Quantitation of the phosphate salt was done using spectrophotometry. The procedure for microdetermination of phosphate was used (Chen, 1956). Crystal samples were dissolved in 3 mL of water and then diluted 1:100 (10 μL to 1 mL) in water. Reagent was prepared by mixing 1 volume of 6 N sulfuric acid with 2 volumes of distilled water and 1 volume of 2.5% ammonium molybdate, then adding 1 volume of 10% ascorbic acid and mixing well. The samples (50 – 100 μL) were mixed with the reagent and heated to 80 °C for 20 minutes. The samples were removed from the water bath and the absorbance was read using a spectrophotometer at 820 nm. A standard curve was created using known amounts of KH_2PO_4 and plotted versus absorbance. The GC-MS laboratory of the Biochemistry Department at VA TECH conducted the phosphate analysis.

3.9.5 Ash Content

Xylitol crystals recovered from the polyethersulfone membrane (150,000 MWCO) were analyzed for the ash content to validate to presence of inorganics in the crystals such as, phosphate salts. Ceramic crucibles were ignited under open flame for one minute and placed in a desiccator. The crucibles were cooled at room temperature and the tare weight was taken to the nearest 0.1 mg. Approximately 0.1 g of crystal sample was placed in each crucible and the mass of the crucibles with crystals was recorded. The samples were heated to 575 °C for two hours in a cold muffle furnace. After two hours the samples were placed in a desiccator and allowed to cool. The samples were massed and returned to the furnace. The samples were heated for 30 minutes and massed again. The process of heating and massing the samples continued until there was constant weight, within 0.2 mg. The percentage of ash in the samples was calculated by equation 3.2.

$$ash\ \% = \frac{w_2 - w_c}{w_1 - w_c} \bullet 100 \qquad (3.2)$$

Where,

w_c – weight of crucible (g),
w_1 – weight of sample and crucible (g),
w_2 – weight of ash and crucible (g).

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Xylitol Production

Fermentation experiments were run to produce xylitol for the membrane separation. A yield of 0.6 g xylitol/g xylose and a productivity of 0.26 g/L·h were obtained from the fermentation of model corn fiber hemicellulose hydrolysate using *Candida tropicalis* ATCC 96745. A typical time-course of changes in products and reactants concentrations during fermentation is shown in Figure 4.1. Cell mass changes versus time is shown in Figure 4.2. These time-courses are typical of fermentation of model sugars using *C. tropicalis* (Walther, 1999).

During fermentation, it was found that increasing the airflow rate improved the production of xylitol by *Candida tropicalis*. It appeared that the improvement in xylitol yield was probably due to the reduction of ethanol concentration in the fermentation broth because the air evaporated the ethanol. The glucose in the model sugar fermentation broth was initially converted to ethanol (Figure 4.1). By increasing the airflow rate, the ethanol concentration decreased rapidly. At very low ethanol concentrations (1 g/L), the xylitol production rate showed a rapid increase relative to when ethanol concentration was high. It is interesting to note that the time for rapid increase in xylitol concentration corresponded to a higher cell concentration (6 g/L). To further show the effect of ethanol concentration on xylitol yield, the airflow rate was reduced from 1.5 vvm to 0.5 vvm. At this rate, the ethanol concentration remained almost constant throughout the fermentation (See Figure 4.3). Xylitol production rate for the low airflow (0.5 vvm) was 0.07 g/L·h as shown by the slope of the xylitol concentration with time, which compares to a high airflow (1.5 vvm) productivity of 0.26 g/L·h. The xylitol yield was also lower (0.2 g/g). Furthermore, the high airflow rate also made other positive contributions to the fermentation. About 35 to 40% of the water was evaporated with the high airflow, thus increasing the concentration of the xylitol in the fermentation broth. This effect could be very important in reducing evaporation costs during xylitol concentration. (Figures 4.1-4.3 adjusted for evaporation)

C. tropicalis cell mass grew rapidly during the exponential growth rate phase and then cell mass concentration fluctuated (Figure 4.2). This fluctuation in cell mass concentration was attributed to the evaporation of the fermentation broth, which caused cells and medium components to stick and cake on the fermenter wall and lid. Over time the cell mass fell back into the broth due to agitation and there was additional cell growth. During the fermentation time, this process continued with cells sticking to the fermenter walls or falling off. As a result cell mass concentrations fluctuated with time. Several runs were required to accumulate enough fermentation broth for the separation experiments. The time-course changes of reactant and products concentrations can be found in the Appendix B.

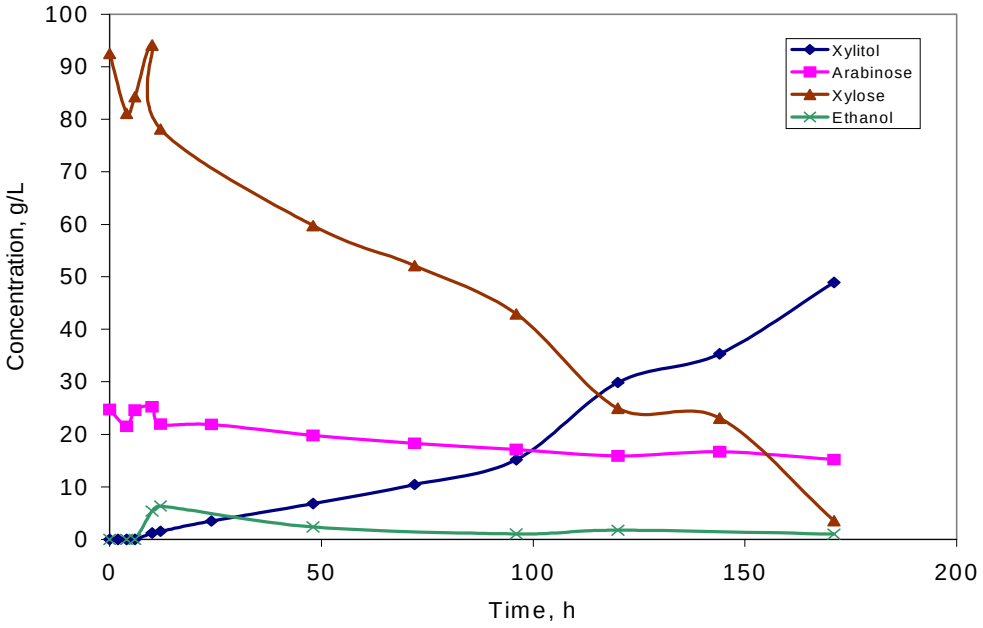

Figure 4.1 Arabinose, xylose, ethanol, and xylitol concentrations over time during fermentation at 1.5 vvm aeration rate.

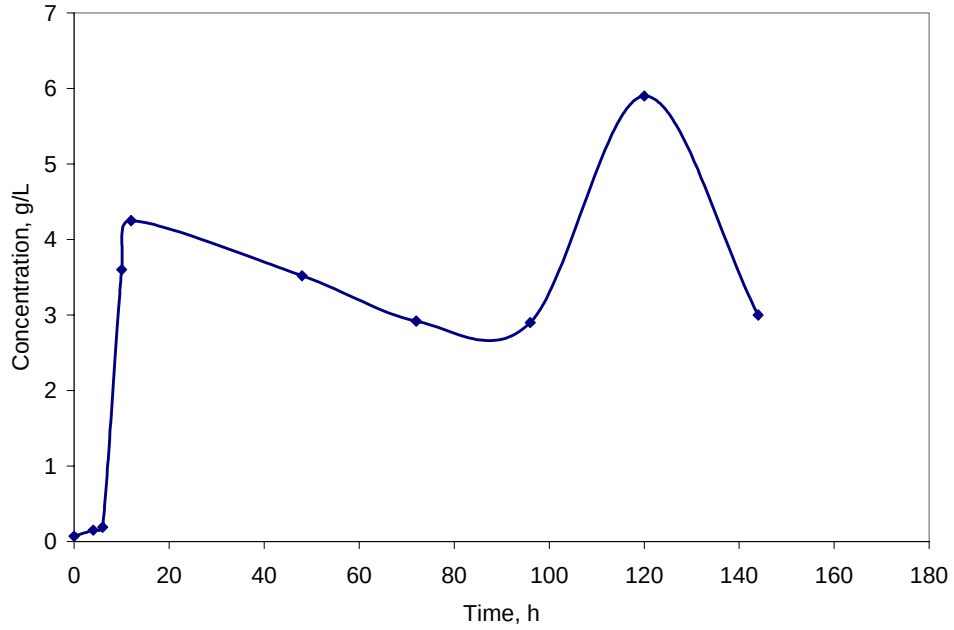

Figure 4.2 Cell mass concentration at 1.5 vvm aeration rate.

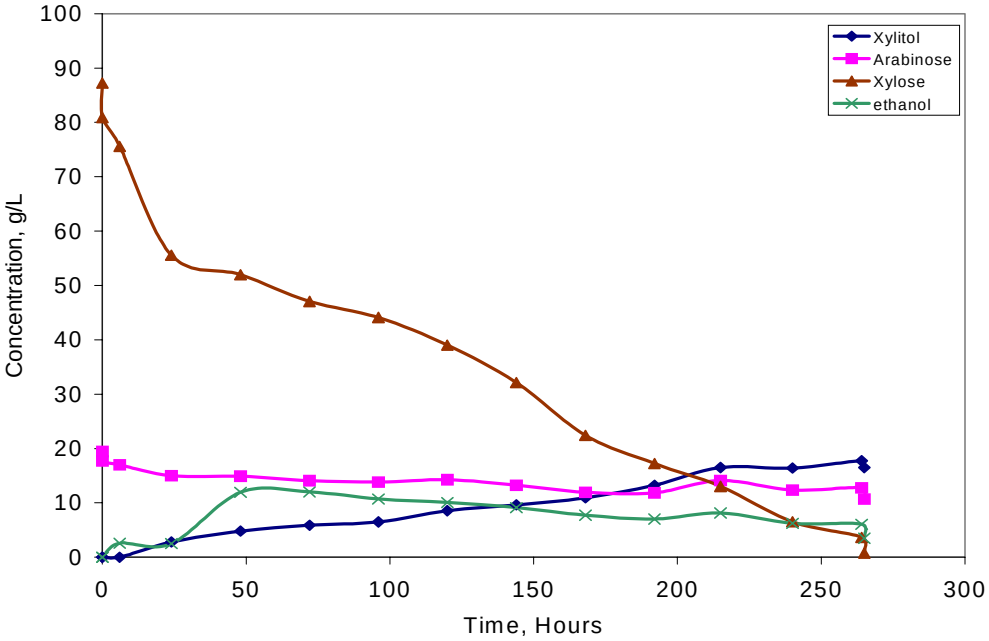

Figure 4.3 Xylitol, xylose, arabinose and ethanol contents at aeration rate of 0.5 vvm.

4.2 Membrane Selection

Eleven membranes were investigated to select the most suitable one for xylitol recovery. The criteria used for selecting the membrane were: (1) high xylitol permeation of the membrane and (2) high retention of impurities such as Lowry positive material and carbohydrates. With the aid of the data in the following sections, the proper membrane for xylitol recovery was chosen and the permeate crystallized. Two other membrane purification methods were also investigated for comparison (polyethersulfone membrane 150,000 MWCO and Maillard reaction treatment).

4.2.1 Polyethersulfone Membrane Separation

The initial membrane tested was a polyethersulfone membrane that had a nominal molecular weight cutoff (MWCO) of 150,000. The polyethersulfone membrane was tested in an OPTISEP ® unit (North Carolina SRT, NC). This unit was run with crossflow filtration using the model sugar fermentation broth described previously. The test was the least severe of all the membrane tests, and it removed the proteins (>150,000 Daltons (Da)). There was a decrease in Lowry positive material, which analyzed impurities (such as oligopeptides and peptides) during the 150,000 MWCO membrane test. The decrease in Lowry positive material was due to protein removal because the sugar molecules and peptides that could interfere with the Lowry's method were too small to be retained by the 150,000 MWCO membrane. Therefore, the decrease in Lowry positive material must be due to the removal of proteins. Two examples of the 150,000 MWCO crossflow ultrafiltration separation are shown in Table 4.1.

The data shown in Table 4.1 show complete removal of yeast cells. Optical density analysis of permeate for cell mass showed no absorbance at 640 nm (Tables 4.1). In addition, there was hardly any change in the concentration of sugars or xylitol, and the crossflow prevented any excessive fouling of the membrane. The 150,000 MWCO membrane is therefore suitable for efficient removal of yeast cells and some Lowry positive material with flux ranging from 622.4 to 912.7 L/day·m^2 at 13.8 to 27.6 kPa. In

Table 4.1 (test 1 and test 2) the polyethersulfone membrane not only removed yeast cells and some Lowry positive material, but also allowed xylitol to permeate the membrane.

Table 4.1 OPTISEP ® ultrafiltration of xylitol fermentation broth using the polyethersulfone membrane (150,000 MWCO)

Sample	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	Lowry positive Material (g/L)	Cell Mass (g/L)
---------- Test 1 ----------					
12/21 150000 Feed	3.5	23.7	79.8	15.2	12.3
12/21 150000 ultrafiltered	3.4	22.5	76.5	8.8	0.0
12/21 150000 End Feed	3.6	23.7	79.5	16.6	12.9
---------- Test 2 ----------					
Mix Feed	9.4	17.3	42.4	8.4	7.5
Mix 150000 ultrafiltered	10.2	19.2	46.9	6.8	0.0
Mix End Feed	8.3	15.7	38.0	25.1	17.6

The permeate from the polyethersulfone membrane was collected and tested with SDS-PAGE to determine the effect of protein removal. The distribution of protein subunits in the filtered fermentation broth was analyzed using SDS-PAGE and the image of the gel containing the 150,000 MWCO filtered fermentation broth can be seen in Figure 4.7. The gel shows light protein bands for the polyethersulfone permeate at molecular weights of about 31,000, 50,000 and at the bottom of the gel, less than 14,400. The SDS-PAGE gel showed that proteins having subunit molecular weight between 14,400 and 50,000 were still in the filtered fermentation broth when using the 150,000 MWCO membrane.

The permeate from the polyethersulfone membrane was concentrated by vacuum evaporation and crystallized. The crystallized xylitol was contained in a yellow-orange colored, viscous mother liquor, which made it difficult to recover the crystals after crystallization. The viscous mother liquor was dealt with by allowing the crystals to form, and the flask was rotated 90° to allow gravity to pull the viscous mother liquor to the bottom of the flask, while the crystals stuck to the top of the flask. The crystals were filtered and the protein impurities were washed away with methanol. Unfortunately, the xylitol in the mother liquor that had not yet crystallized was also washed away. The recovered crystals were dissolved in water and analyzed for xylitol, sugars, Lowry positive material, and inorganic salt content. Table 4.2 shows the results of the crystallization for polyethersulfone membrane treatment of model sugar fermentation broth.

Table 4.2 Purity of recovered crystals treated with polyethersulfone membrane (150,000 MWCO)

	Repetition 1 (%)	Repetition 2 (%)	Repetition 3 (%)
Xylitol	4	4.4	8.9
Arabinose	2.8	4.5	2.5
Xylose	0.0	0.0	0.0
Lowry positive material	8.7	8.6	3.8
Phosphate positive material	69.3	68.7	27
Other	15.2	13.8	57.8
Ash content	52.4	20.5	43.9

The crystals recovered using the polyethersulfone membrane separation contained xylitol ranging from 4 to 8.9% xylitol with the major impurities being inorganic salts and other

molecules. Impurities could include phosphates salts, which were measured to be between 27 and 69.3 percent of the crystals and other salts such as $MgSO_4$. The ash content of the crystals was performed to validate the presence of inorganic materials such as, salts.

Yields of xylitol crystals from the polyethersulfone separation ranged from 0.009 g xylitol /g xylitol in permeate to 0.07 g xylitol /g xylitol in permeate. The crystals were obtained from solutions containing 43.4 to 76.5 g/L xylitol and the solutions were concentrated to 340 to 374 g/L xylitol. The low yields were due to the presence of colored, viscous mother liquor, caused by Lowry positive material, which inhibited crystallization and made crystal recovery difficult. The crystallization process was not optimized. Complete crystallization of this solution was possible, but was stopped early to avoid trapping all impurities in the crystals.

The important thing to note from this experiment and the following crystal experiments is the crystalline purity of xylitol. Xylitol yields could be improved through recycle of the mother liquor and fractional crystallization methods, such as, those reported by Jaffe (1978) for crystallization of xylitol. In addition, cleaner xylitol solutions would make for easier crystallization and recovery. While this polyethersulfone membrane was not very effective in removing impurities it could be used as a pretreatment for further purification steps.

4.2.2 Ultrafiltration, Nanofiltration and Reverse Osmosis Selection with Constructed Test Cell

Investigation of smaller MWCO membranes (<10,000 molecular weight) for xylitol purification was done using the constructed test cell to obtain high pressures. The constructed test cell apparatus allowed for recycling of the fermentation broth and each membrane was tested with a model sugar solution and the permeate analyzed. The results of the reverse osmosis, nanofiltration, and ultrafiltration membrane tests at 1.4 MPa are shown in Table 4.3. The individual membrane tests with the pressure

differential and concentrations of sugars, xylitol and Lowry positive material obtained from each membrane tested are shown in Tables 4.4 through Tables 4.13.

Xylitol is currently concentrated by evaporation and can be costly due to the sophisticated equipment, steam costs, cooling water and electric power costs (Rutskaya, 1989). The significant thermolability of xylose and other carbohydrates present can result in carmelization and condensation, resulting in the need for further purification. If reverse osmosis could be used for the concentration of xylitol it might save on production costs, such as steam for heating during evaporation.

From Table 4.3 it can be seen that the reverse osmosis membranes (MS19, ST10, SR10) allowed little carbohydrate, little xylitol, and little Lowry positive material to pass through. The SR10 membranes would be ideal for investigating the concentration of xylitol produced by fermentation broth. The SR10 (Table 4.11) was the most effective membrane for the retention of sugars and xylitol. At 4.8 MPa the flux attained for SR10 (966 L/day·m^2) was higher than that for the ST10 (710 L/day·m^2) and it also retained a higher percentage of the xylitol. About 99% of the xylitol was retained by the SR10 membrane at 4.8 MPa, but this membrane is rated for 6.9 MPa. The increased pressure would increase the flux and reduce xylitol permeation. There was no permeate from the MS19 (Table 4.13) membrane in two hours operating at pressures up to 4.8 MPa. This lack of permeate provided indirect proof that the membrane separation unit was leak-free. The SF10 membrane (Table 4.10) also retained a large portion of the xylitol in solution. However, SF10 would not be suitable for concentration because xylitol permeation was relatively high (2.2% at 4.8 MPa). Reverse osmosis concentration of xylitol was not thoroughly investigated in this experiment. The equipment used for the membrane testing was not designed for collection of large quantities of permeates and therefore could not be used for extensive concentration studies.

Table 4.3 Permeation data for reverse osmosis, nanofiltration, and ultrafiltration membranes at 1.4 MPa

Membrane type		Ms19	St10	Sr10	Sf10	Sx10	Sx01	Sv10	Mx07	Bq01	Hg19
MWCO		200	220	300	400	500	500	600	600	1000	10000
Flux, L/(day·m^2)	1.4 MPa	0	6.6	3.79	14.2	47.3	55.4	87.6	391.0	151.5	882.9
Permeation %											
(at 1.4 MPa)	xylitol	-	-	-	-	31	50	41	76	75	86
	arabinose	-	-	-	-	34	48	42	70	79	85
	xylose	-	-	-	-	37	57	51	83	73	75
	*Lowry positive material	-	3.0	4.2	4.0	10.2	14.7	10.0	15.6	21.4	50
	[x]UV absorption at 260 nm (%)	-	-	-	10.5	5.7	5.1	11.3	3.5	5.5	13.1

* Lowry's method, for protein detection, can have interferences from sources other than proteins, such as sugars, amino acids and peptides.
x Relative UV absorption as calculated from eqt. 3.1.

The selection of the appropriate membrane for xylitol separation was based on the data shown in Table 4.3. The membranes, MX07, BQ01, and HG19, all allowed a large portion of xylitol to pass through the membrane with HG19 allowing the highest percentage of xylitol to permeate (86%), while retaining 50% of the Lowry positive material. The other membranes tested (SX10, SX01, SV10) retained 50% or more of the xylitol that could otherwise be recovered by the MX07, BQ01, or HG19 membranes. For industrial separation of xylitol by membrane, the flux through the membrane would need to be high. HG19 polysulfone membrane was determined to be the best for xylitol separation with respect to xylitol permeation, retention of proteinaceous impurities analyzed with Lowry's method, and high flux rate (883 L/day·m^2 at 1.4 MPa).

The effect of pressure on the flux of the system was investigated to determine the appropriate operating pressure for the HG19 membrane. As the pressure was increased, the membrane allowed smaller molecules such as water to permeate the membrane more rapidly. While larger molecules, like carbohydrates, could not pass through the pores as quickly. Therefore, when pressure was increased the permeation of water increased, but the permeation of xylitol decreased. This trend was seen for all ten membranes tested. Figure 4.4 shows the result of increased pressure on the flux and permeation of xylitol for the HG19 membrane. With this information, the HG19 membrane was tested with fermentation broth.

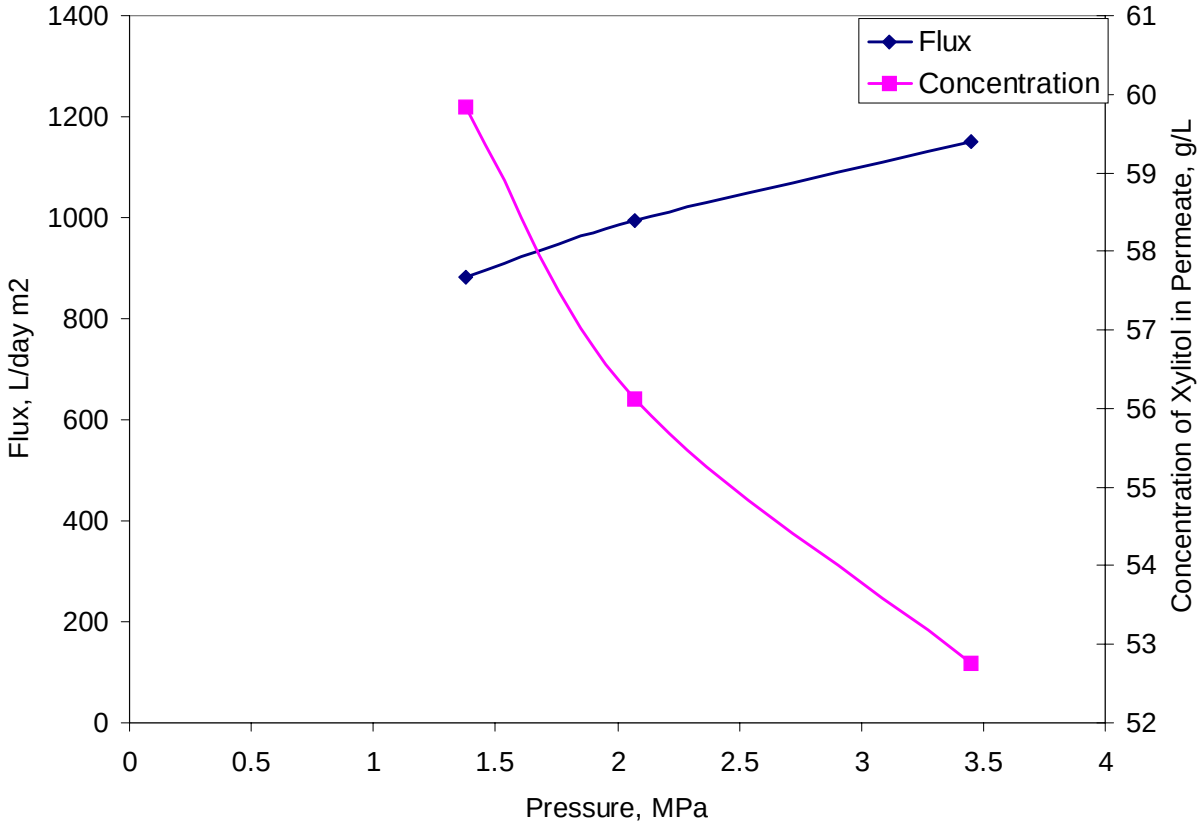

Figure 4.4 Flux and xylitol concentration in the permeate versus pressure for the HG19 membrane.

Table 4.4 HG19 membrane selection test using model sugar mixture

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
HG19 Feed	-	31	-	2.3	17.5	68.1	100	3.9
HG19 80	1.4	32	902	1.7	14.9	59.5	14.7	1.9
HG19 100	1.4	32	881	1.8	15.0	59.4	12.3	1.9
HG19 120	1.4	32	866	1.8	15.7	60.7	12.3	2.0
HG19 140	2.1	33	994	1.2	13.6	56.1	6.3	2.1
HG19 160	3.4	34	1150	1.2	12.8	52.8	10.7	1.4
HG19 End Feed	-	34	-	2.4	18.3	70.9	91.8	4.3

* Relative UV absorption calculated from eqt. 3.1.

Table 4.5 BQ01 membrane selection test

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
BQ01 Feed	-	22	-	2.5	14.0	46.5	100	3.7
BQ01 80	1.4	25	170	1.9	11.2	36.2	4.5	0.9
BQ01 100	1.4	26	142	2.1	12.3	39.6	8.9	0.7
BQ01 120	1.4	25	142	2.0	12.3	39.5	3.2	0.8
BQ01 140	2.1	27	241	1.7	10.9	34.2	3.5	0.9
BQ01 160	3.4	30	426	1.3	8.3	25.2	10.2	0.6
BQ01 End Feed	-	27	-	2.5	15.6	53.6	100	3.2

* Relative UV absorption calculated from eqt. 3.1.

Table 4.6 MX07 membrane selection test

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
MX07 Feed	-	28	-	3.2	18.2	64.0	100	5.4
MX07 80	1.4	29	412	2.7	12.7	48.3	3.4	1.0
MX07 100	1.4	29	416	2.9	13.9	46.9	3.3	0.9
MX07 120	1.4	28	345	3.0	13.4	48.0	3.9	0.7
MX07 140	2.1	29	503	2.7	12.5	42.6	3.1	1.2
MX07 160	3.4	30	724	2.4	11.1	38.2	1.4	0.8
MX07 End Feed	-	31	-	4.0	19.9	68.4	82.6	4.1

*Relative UV absorption calculated from eqt. 3.1.

Table 4.7 SV10 membrane selection test

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
SV10 Feed	-	24	-	3.4	19.4	67.6	100	4.9
SV10 80	1.4	25	92	1.7	8.4	27.8	11.2	0.4
SV10 100	1.4	25	78	1.6	7.6	25.6	11.5	0.3
SV10 120	1.4	25	92	1.7	8.2	27.9	11.2	0.8
SV10 140	2.1	26	199	1.2	5.8	17.5	4.3	0.6
SV10 160	3.4	29	739	-	3.4	8.2	3.4	0.4
SV10 End Feed	-	29	-	2.9	17.8	59.8	81.9	3.8

*Relative UV absorption calculated from eqt. 3.1.

Table 4.8 SX01 membrane selection test

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
SX01 Feed	-	24	-	3.6	21.6	75.8	100	5.2
SX01 80	1.4	25	68	1.8	9.6	31.8	8.1	0.9
SX01 100	1.4	25	38	2.0	10.7	39.0	4.0	0.7
SX01 120	1.4	25	60	2.1	10.3	37.5	3.3	0.7
SX01 140	2.1	27	114	2.1	10.8	38.4	5.2	0.4
SX01 160	3.4	30	469	1.2	6.3	19.3	4.2	0.4
SX01 End Feed	-	27	-	3.5	21.5	71.7	62.8	5.0

*Relative UV absorption calculated from eqt. 3.1.

Table 4.9 SX10 membrane selection test

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
SX10 Feed	-	22	-	3.0	17.0	60.0	100	4.2
SX10 80	1.4	24	50	1.2	6.0	18.7	5.3	0.4
SX10 100	1.4	24	50	1.3	6.4	20.7	6.0	0.3
SX10 120	1.4	24	43	1.1	5.8	18.5	5.7	0.5
SX10 140	2.1	26	241	0.9	4.1	11.3	7.2	0.6
SX10 160	3.4	29	781	-	2.7	5.10	2.0	1.0
SX10 End Feed	-	29	-	2.8	15.8	54.9	88.3	4.3

*Relative UV absorption calculated from eqt. 3.1.

Table 4.10 SF10 membrane selection test

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
SF10 Feed	-	24	-	2.4	15.5	57.6	100	4.0
SF10 80	1.4	25	14.0	-	-	-	10.5	0.2
SF10 100	3.4	32	639	-	0.4	2.6	12.8	0.2
SF10 120	3.4	33	511	-	0.2	2.1	0.8	0.3
SF10 140	3.4	34	568	-	0.5	2.9	3.8	0.2
SF10 160	4.8	36	994	-	0.02	1.2	2.3	0.2
SF10 End Feed	-	35	-	2.6	16.8	62.5	100	4.1

*Relative UV absorption calculated from eqt. 3.1.

Table 4.11 SR10 membrane selection test

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
SR10 Feed	-	25	-	1.1	15.9	59.1	100	3.3
SR10 80	1.4	27	3.8	-	-	-	-	0.1
SR10 100	3.4	33	454	-	-	0.4	14.5	0.2
SR10 120	3.4	34	469	-	-	1.2	15.9	0.3
SR10 140	3.4	35	500	-	-	1.2	0.7	0.2
SR10 160	4.8	38	966	-	-	0.7	10.6	0.3
SR10 End Feed	-	37	-	1.5	14.7	51.4	100	3.8

*Relative UV absorption calculated from eqt. 3.1.

Table 4.12 ST10 membrane selection test

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
ST10 Feed	-	28	-	4.8	20.4	70.5	100	5.4
ST10 80	1.4	28	6.6	-	-	-	-	0.2
ST10 100	3.4	34	312	-	2.2	5.7	6.0	0.2
ST10 120	3.4	34	335	1.2	1.6	3.0	3.1	0.2
ST10 140	3.4	35	284	1.2	1.6	3.2	0.1	0.2
ST10 160	4.8	38	710	1.3	1.4	2.2	0.04	0.3
ST10 End Feed	-	37	-	4.6	19.9	69.8	97.6	4.9

*Relative UV absorption calculated from eqt. 3.1.

Table 4.13 MS19 membrane selection test

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	*UV absorption at 260 nm (%)	Lowry positive material (g/L)
MS19 Feed	-	27	-	3.0	15.5	57.3	100	3.5
MS19 60	1.4	29	0	-	-	-	-	-
MS19 120	3.4	32	0	-	-	-	-	-
MS19 140	4.8	35	0	-	-	-	-	-
ST10 End Feed	-	35	-	2.9	16.3	56.4	100	3.3

*Relative UV absorption calculated from eqt. 3.1.

4.3 Membrane Filtration of Fermentation Broth

Once the HG19 polysulfone membrane was determined to be the most appropriate for xylitol separation with the model xylose/xylitol mixture, model sugar fermentation broth was then investigated. Three runs were conducted to determine the performance of the HG19 membrane. The HG19 membrane separation efficiency of the fermentation broth was similar to the results using the xylose/xylitol model mixture. About 82.2 to 90.3% of the xylitol permeated the membrane while 49.2 to 53.6% of the Lowry positive material was retained (Table 4.14).

Table 4.14 Testing HG19 membrane with fermentation broth

	Permeation (%) Repetition 1	Permeation (%) Repetition 2	Permeation (%) Repetition 3
Xylitol	82.2	90.3	87.6
Arabinose	79.3	91.3	88.2
Xylose	45.5	92.3	70.2
Lowry positive material	49.2	51.8	53.6

The permeate from the HG19 membrane was analyzed with SDS-PAGE to determine the efficiency of protein removal (Figure 4.7). The SDS-PAGE gel has a detection limit of 100 ng depending on the molecular weight of the protein molecule and the staining method. The gel showed that no protein subunit bands were present for the HG19 10,000 MWCO membrane. This indicated that the HG19 membrane removed all proteins above 14,400 (The lower molecular weight limit of the particular gel used). However,

the Lowry's method showed that 49.2 to 53.6 % of the proteinaceous impurities in the fermentation broth were present in the permeate. This is due to the permeation of peptide molecules less than 14,400 molecular weight. These peptides produced a colored viscous liquid when the permeate was concentrated by reduced vacuum evaporation. The viscous liquid interfered considerably with the recovery of the xylitol crystals because it was very difficult to filter the crystals. Three replications were performed and the results of the crystal analysis are shown in Table 4.15. The range of xylitol purity for the HG19 treatment was 82.8 to 90.3%.

Table 4.15 Crystal purity for HG19 separation

	Repetition 1 (%)	Repetition 2 (%)
Xylitol	82.8	90.3
Arabinose	19.4	3.4
Xylose	0.0	0.0
Lowry positive material	0.0	4.9
Phosphate positive material	15.9	8.7

The analysis of the third repetition was not included due to insufficient sample size for accurate testing. For the second repetition, the phosphate analysis was done with crystals taken from the same batch, but were not the same crystals analyzed by HPLC for sugar and xylitol content. In addition, the sample sizes of crystals used for analysis averaged 14 ± 4.7 mg.

The yields for the HG19 crystals ranged from 0.014 g/g to 0.03 g/g from the initial xylitol in the permeate collected. The permeate contained 30.4 to 56.7 g/L of xylitol and was concentrated by evaporation to 426 to 475 g/L. The low yields were due to the slow crystallization process. Crystallization was stopped early to aid in the separation as described previously for the polyethersulfone membrane (150,000 MWCO). If

crystallization was continued to completion, the entire mother liquor crystallized with all the impurities trapped in the crystal structure. If the solution had been further purified to remove more proteins, carbohydrates, and salts, either by more severe membrane separation (e.g. MX07), ion exchange, or chemical reactions, the viscosity of the mother liquor may have been reduced and crystal yields would have increased.

4.4 Separation Aided by Chemical Reaction

In order to recover xylitol from other sugars via membrane separation, there must be a difference in physical characteristics between the molecules. The major components in the fermentation broth at the end of the fermentation cycle were biomass, proteins, xylitol, arabinose, xylose and inorganic salts. The cells and proteins can be separated from xylitol by size difference. However, one of the other main contaminants requiring removal before crystallization was arabinose. Xylitol and arabinose are both neutral molecules of similar molecular weight (152.15 and 150.13, respectively). Some difference in characteristics had to be created between these molecules in order for the molecules to be separated by the membrane method. Urea and $NaHSO_3$ were reacted with arabinose to add bulk to the arabinose molecule and prevent it from permeating the membrane. However, reaction yields were low (less than 50%) and little molecular weight was added, an alternative chemical reaction was investigated.

The Maillard reaction was performed to react residual reducing sugars in the fermentation broth with the residual proteins present in the fermentation broth. Several tests were conducted to react proteins in yeast extract with the reducing sugars (arabinose and xylose) present in the fermentation broth. The initial test was conducted on a model sugar mixture with similar composition as the test solution used for the membrane selection, except the yeast extract content was varied. The rate of reaction at 90 °C for the model mixture containing 10 g/L yeast extract is shown in Figure 4.5. There was only 15% and 17% conversion of the arabinose and xylose respectively for the Maillard reaction complex. Using similar conditions, but with increased yeast extract content (40 g/L), the conversion of arabinose (33%) and xylose (29%) doubled (Figure 4.6).

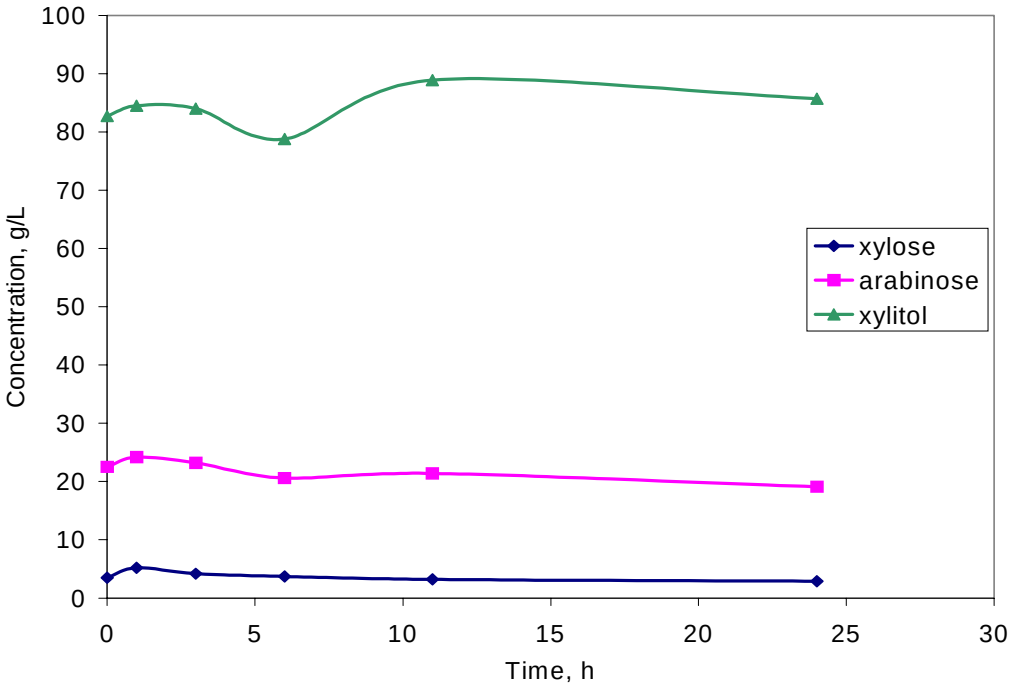

Figure 4.5 Conversion of residual xylose and arabinose to Maillard reaction products. Reaction at 90 °C, 24-hours and 10 g/L yeast extract.

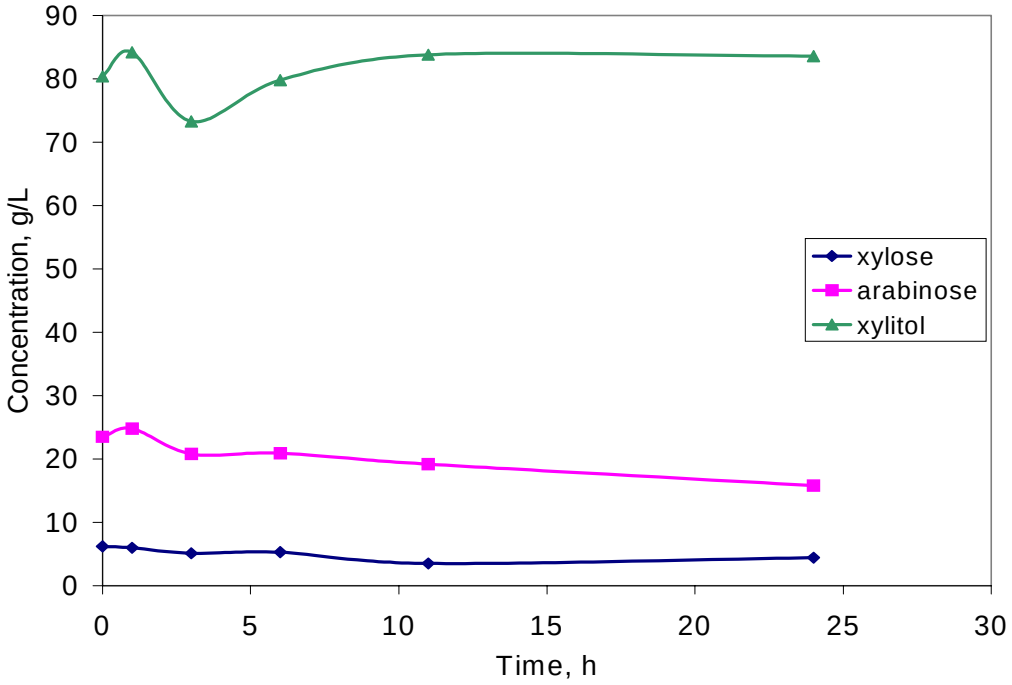

Figure 4.6 Reactivity of xylose and arabinose during Maillard reaction. Reaction at 90 °C, 24-hours and 40 g/L yeast extract.

The Maillard reaction was also conducted at higher temperatures (121°C) to increase the amount of arabinose reacted with proteins. The reaction was conducted using a true fermentation broth consisting of residual sugars (xylose, arabinose), proteins, and yeast. However, the protein concentration in the broth was not enough to react with the reducing sugars in the fermentation broth (see Table 4.16).

Table 4.16 Maillard reaction with actual fermentation broth at 121 °C for 1 to 2 hours with and without yeast cells

Sample	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)
Before Maillard no yeast cells	2.8	22.1	73.5
After Maillard no yeast cells 1h reaction time	4.8	18.9	78.9
Before Maillard, broth with yeast cells	3.6	27.0	88.8
After Maillard, broth with yeast cells 1h reaction time	5.6	19.1	83.5
After Maillard, broth with yeast cells 2h reaction time	6.3	17.3	92.5

The yeast cells did not rupture and release as much protein as expected. More severe conditions were applied. The temperature was increased to 125 °C and the time was increased to 3 hours. In addition, the samples were supplemented with yeast extract to increase free proteins for the arabinose-protein reaction (Table 4.17).

Table 4.17 Maillard reaction with fermentation broth at 125 °C for 3 hours with and without yeast cells and yeast extract added

Sample	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	Lowry positive material (g/L)
Starting Concentration	21.3	16.0	29.1	8.8
20g/L yeast extract added	8.8	6.2	32.3	59.5
40g/L yeast extract added	7.7	3.0	32.0	67.7
60g/L yeast extract added	7.5	4.8	32.6	88.5
Maillard with cells	14.1 ± 0.4	11.8 ± 0.5	35.7 ± 2.5	25.9 ± 1.6
Maillard no cells	12.1 ± 2.3	11.2 ± 0.9	33.6 ± 1	27.7 ± 3.3

As shown in Table 4.17, 81% of the arabinose reacted when 40 g/L yeast extract was added and yielded the best results at 125 °C for 3 hours. In subsequent Maillard studies 40 g/L yeast extract was added and the solution was reacted for 3 hours at 125 °C.

The Maillard reaction was not optimized because it was desired to see if it would have any effect on the membrane separation of xylitol before any further Maillard reaction study was performed. The Maillard reaction samples were then treated with the HG19 membrane to determine how well Lowry positive material and arabinose could be removed. Maillard samples treated with the previous procedure (unfiltered and HG19 filtered Maillard samples) were analyzed using SDS-PAGE (Figure 4.7). The gel showed a bright protein band around molecular weight (MW) of 14,400 for the unfiltered Maillard product. This indicated that the protein subuints in the Maillard reaction product were on the order of 14,400 and less in molecular weight. The gel indicated that the HG19 membrane was successful in removing protein greater than 14,400 MW because no protein bands were present. However, from the Lowry's method it could be seen that 9.6 ± 4 g/L of the proteinaceous impurities were still in the permeate after treatment with the HG19 membrane (Table 4.19). These impurities could be the peptides with molecular weight less than 14,400.

There were large amounts of proteins and peptides in the fermentation broth from the addition of yeast extract and this made it difficult to filter the Maillard solution. The maximum flux of 129 L/day·m^2 was obtained for the Maillard solution with the HG19 membrane. In addition, there were large amounts of Lowry positive material (9.6 ± 4 g/L) left in the Maillard HG19 treated permeate (Table 4.19). This made for a difficult crystallization process because it created a colored, viscous mother liquor.

The Maillard crystals were analyzed and the results are given in Table 4.18. The average weight of xylitol crystals recovered for the Maillard reaction was 13 ± 4.2 mg and insufficient quantities made it difficult to get an accurate impurity content by Lowry's method for repetition 3. The yields of xylitol recovery for Maillard reaction followed by HG19 membrane treatment ranged from 0.01 g/g to 0.016 g xylitol/g xylitol in permeate. Again, the low yield is due to the fact that the solution was not thoroughly crystallized and the recovery was very difficult due to the colored, viscous mother liquor.

Table 4.18 Maillard crystal purity treated with HG19 membrane

	Repetition 1 (%)	Repetition 2 (%)	Repetition 3 (%)
Xylitol	25.3	18.6	42.1
Arabinose	0.0	11.4	0.0
Xylose	0.0	0.0	0.0
Lowry positive material	5.3	4.6	N/A
Phosphate positive material	63.7	38	N/A
Other	5.7	27.4	57.9

Table 4.19 Average Maillard permeate collected from HG19 filtrations

Sample	Pressure (MPa)	Temperature (°C)	Flux (L/day·m^2)	Xylose (g/L)	Arabinose (g/L)	Xylitol (g/L)	Lowry positive material (g/L)
Maillard Feed	-	30.6 ± 0.5	-	3.9 ± 2	2.4 ± 0.7	49 ± 12	23.3 ± 7
Maillard Ultrafiltered	1.4	30.4 ± 2	68 ± 37	2.8 ± 1	2.2 ± 0.7	42 ± 12	9.6 ± 4
Maillard End Feed	-	30.6 ± 2	-	3.1 ± 1	2.0 ± 0.3	46 ± 13	22.7 ± 8

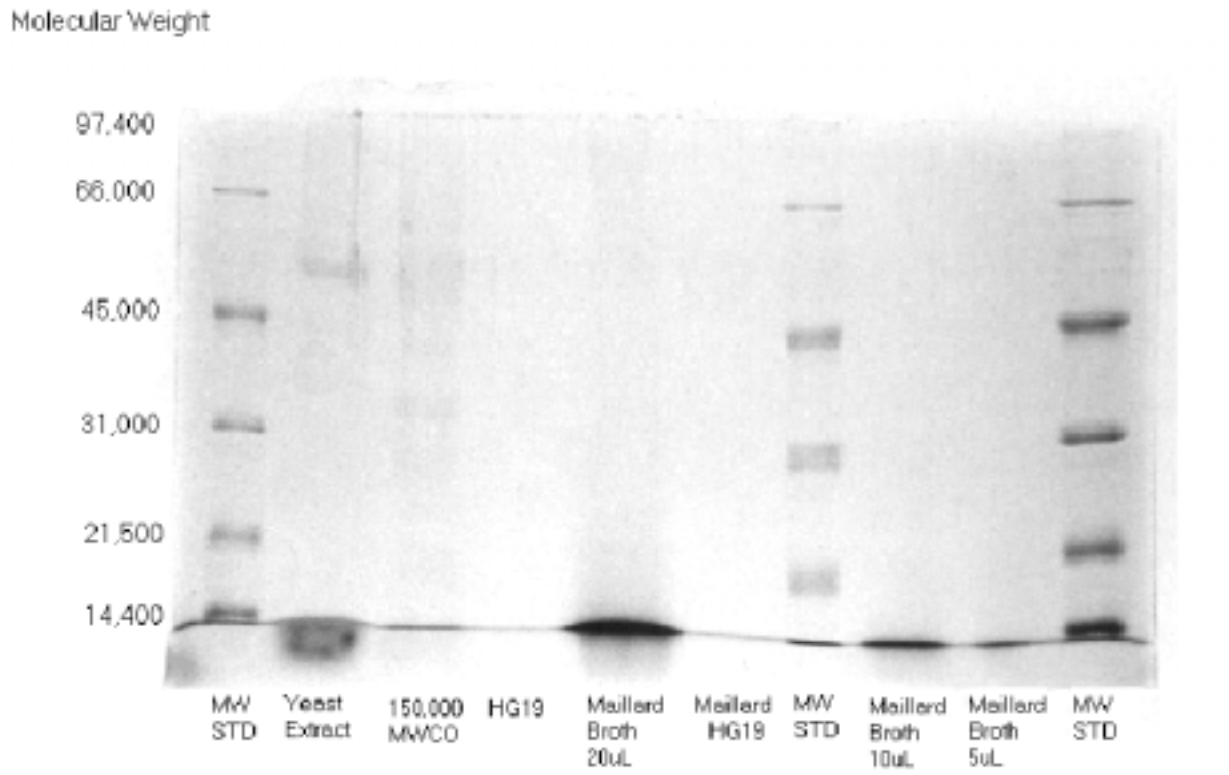

Figure 4.7 SDS-PAGE for fermentation solutions.

4.5 Recovery System Comparisons

Xylitol recovery from fermentation broth by membrane separation was investigated with three different membrane treatments; 150,000 MWCO polyethersulfone membrane, 10,000 MWCO HG19 polysulfone membrane, and Maillard reaction with HG19 membrane treatment. In each case, the treated product was crystallized and analyzed for purity. Each treatment had its benefits for xylitol purification. In addition, activated carbon treatment of fermentation broth was also investigated.

The 150,000 MWCO polyethersulfone membrane worked well for rapid cell removal. As much as 913 L/day·m^2 of fermentation broth was treated with this membrane at 13.8 kPa. The membrane is rated for pressures up to 0.7 MPa and flux rates could be increased. This membrane was not sufficient for xylitol purification, but did remove yeast cells with no absorbance at 640 nm. If rapid removal of yeast cells is required prior

to further membrane treatment the 150,000 MWCO polyethersulfone membrane was found to be ideal for this task.

Rapid filtration with high flux is important for industrial membrane processes. Decreasing the time for filtration saves on operating costs, including energy. The flux for the membrane treatment depended on the pressure applied (Figure 4.4). The flux for the 150,000 MWCO membrane was the highest of all the membranes tested at 913 L/day·m^2 at 13.8 kPa and can be increased with pressure. The HG19 membrane had a flux of 883 L/day·m^2 at 1.4 MPa that was slightly less than the 150,000 MWCO membrane. The flux of the HG19 membrane decreased when Maillard solution was passed through the membrane. The maximum flux was 129 L/day·m^2 for the HG19 membrane when Maillard solution was treated at 1.4 MPa. This was only 14.3% of the maximum flux attained (902 L/day·m^2) when regular fermentation broth was used. The decrease was caused by the addition of yeast extract to achieve the Maillard reaction. Clearly the addition of yeast extract affected the fouling of the membrane and decreased the flux of the HG19 membrane. However, flux was inconsequential when the desired purification was not being achieved.

Lowry positive material removal for the three membrane treatments varied. The 150,000 MWCO membrane removed 19 to 42% of the Lowry positive material, which could be large proteins (>150,000) from the fermentation broth. Notable improvement in impurity removal was seen for the HG19 membrane with Lowry positive material removed ranging from 46.4 to 50.8% and allowing 82.2 to 90.3% of the xylitol to permeate the membrane with Lowry positive material concentration ranging from 0.9 to 4.9 g/L in the permeate. For the Maillard reaction, Lowry positive material removal in the permeate ranged from 49.9 to 66.9%, but the concentration ranged from 5.5 to 15 g/L. When more Lowry positive material was removed, crystallization of xylitol was more rapid. While the 150,000 MWCO membrane was not tested for Maillard purification, it could be used as a pretreatment for Maillard fermentation broth to remove large proteins and improve further filtration by reducing fouling. Of the three treatments, the HG19 membrane with

regular fermentation broth was the most effective at removing Lowry positive material (such as oligopeptides, amino acids, and peptides).

When activated carbon was used for color removal and protein purification, between 21 and 27% of the relative UV absorbed material at 260 nm was removed. The solution was clear, but 40-60% of the xylitol was adsorbed by the activated carbon. Ultrafiltration removed 46.4 to 50.8% of the relative UV absorbed material with only 13.3% loss of xylitol compared to 60% loss of xylitol by activated carbon.

The maximum xylitol crystal yield from all the treatments in this study was 0.07 g/g for the 150,000 MWCO membrane. The maximum yields for xylitol crystals for HG19 membrane treatment and Maillard with HG19 membrane treatment were 0.03 g/g and 0.016 g/g, respectively. These yields are not representative of the true yields that can be obtained from membrane treatment for xylitol because the crystallization process was not optimized. However, it shows that xylitol can be recovered in a crystalline form with membrane treatment.

Most important, was the crystal purity. Crystals with 90.3% xylitol (by HPLC) were obtained from this study using the HG19 membrane to filter model hemicellulose hydrolysate fermentation broth. The purity of xylitol crystals for the three membrane treatments are shown in Table 4.20. The 150,000 MWCO membrane and Maillard treated with HG19 membrane resulted in crystals containing less than 25% xylitol. A large number of impurities (such as Lowry positive material, arabinose, and xylose) were present. The purest crystals were obtained from the HG19 membrane treatment of model fermentation broth and yielded crystals that had an average purity of 86.6 ± 5% xylitol. This high purity was a result of removing sufficient quantities of Lowry positive material from the permeate. A common problem for all of the xylitol crystals recovered by membrane treatment was lack of inorganic salt removal. In future studies, if salts and proteins could be removed by membranes and the proper chemical reactions could be found for carbohydrate removal, then yields of xylitol could be much higher and crystal purity could be higher than the 90.3% obtained in this study.

Table 4.20 Purity of xylitol crystals after membrane treatment

	150000 MWCO (%)	HG19 (10000 MWCO) (%)	Maillard reaction & HG19 (%)
Xylitol	5.8 ± 2.8	86.6 ± 5	28.7 ± 12.1
Arabinose	3.2 ± 1	11.4 ± 11.3	3.8 ± 6.6
Xylose	0.0	0.0	0.0
Lowry positive material	7 ± 2.8	2.5 ± 3.5	5 ± 0.5
Other	74	0.0	62.5

*note: other could include salts, such as phosphate and sulfate

4.6 Membrane Separation as Pretreatment for Chromatography

Membrane separation has not been refined sufficiently to obtain xylitol crystals that are pure enough for commercialization at this point. Alternatively, membrane separation could be used in conjunction with chromatography to extend the service life of chromatography resins. The proteins in the fermentation broth would slowly foul the ion-exchange resins used in the chromatography column. The resins would need to be regenerated more often for the fermentation process than for the current chemical process because of increased protein content. A flowsheet of membrane separation for fermentation production of xylitol is shown in Figure 4.8.

The proposed process for xylitol production includes pretreatment even though it was not researched in this study. However, acid extraction and enzyme hydrolysis could be used for pretreatment of corn fiber prior to fermentation (Leathers, 1996). Following acid treatment and hydrolysis, the hydrolysate can be neutralized with $Ca(OH)_2$ or other calcium chemicals to prepare the solution for fermentation. The hydrolysate would be fermented and the xylitol produced must then be separated from the impurities contained in the fermentation broth. Yeast cells and large proteins can be removed with a high MWCO ultrafiltration membrane, such as, the 150,000 MWCO polyethersulfone membrane used in this study. This separation prepares the fermentation broth for further membrane separation by removing impurities, which would otherwise foul the membrane. The membrane used for further separation would remove proteins and macromolecules. Such a membrane would be the HG19 membrane tested in this experiment.

The permeate collected from the HG19 membrane was crystallized with purity up to 90.3%. Xylitol crystal purity could be improved with further membrane research or by following membrane separation with chromatographic separation. The use of chromatography can further remove most impurities following membrane separation. Chromatography, using a sulfonated polystyrene resin cross coupled with divinylbenzene in a Ca^{++} form, would remove any remaining peptides, arabinose or residual

carbohydrates. Anion and Cation exchange membranes could be used to remove any residual salts. This would result in a purified xylitol solution ready for concentration and crystallization.

Reverse osmosis was investigated in this experiment for concentration of xylitol prior to crystallization. The experimental results from this study showed that reverse osmosis can concentrate xylitol. Rutskaya (1989) reported that xylitol can be concentrated from 5-6 wt% to 15-16 wt% xylitol by reverse osmosis, and this procedure reduced cost over evaporation. Xylitol was crystallized at as little as 35 wt% xylitol in this study. If reverse osmosis is not sufficient to obtain a sufficient weight percent of xylitol for crystallization, then evaporation could be used to obtain the desired concentration of xylitol. For this reason, evaporation is included with reverse osmosis for concentration of xylitol.

Following concentration of xylitol, the solution is crystallized. The crystals would be filtered or centrifuged, and the mother liquor containing uncrystallized xylitol would be recycled for further membrane separation. The result would be highly pure xylitol crystals ready for utilization in xylitol products such as chewing gum and tooth paste.

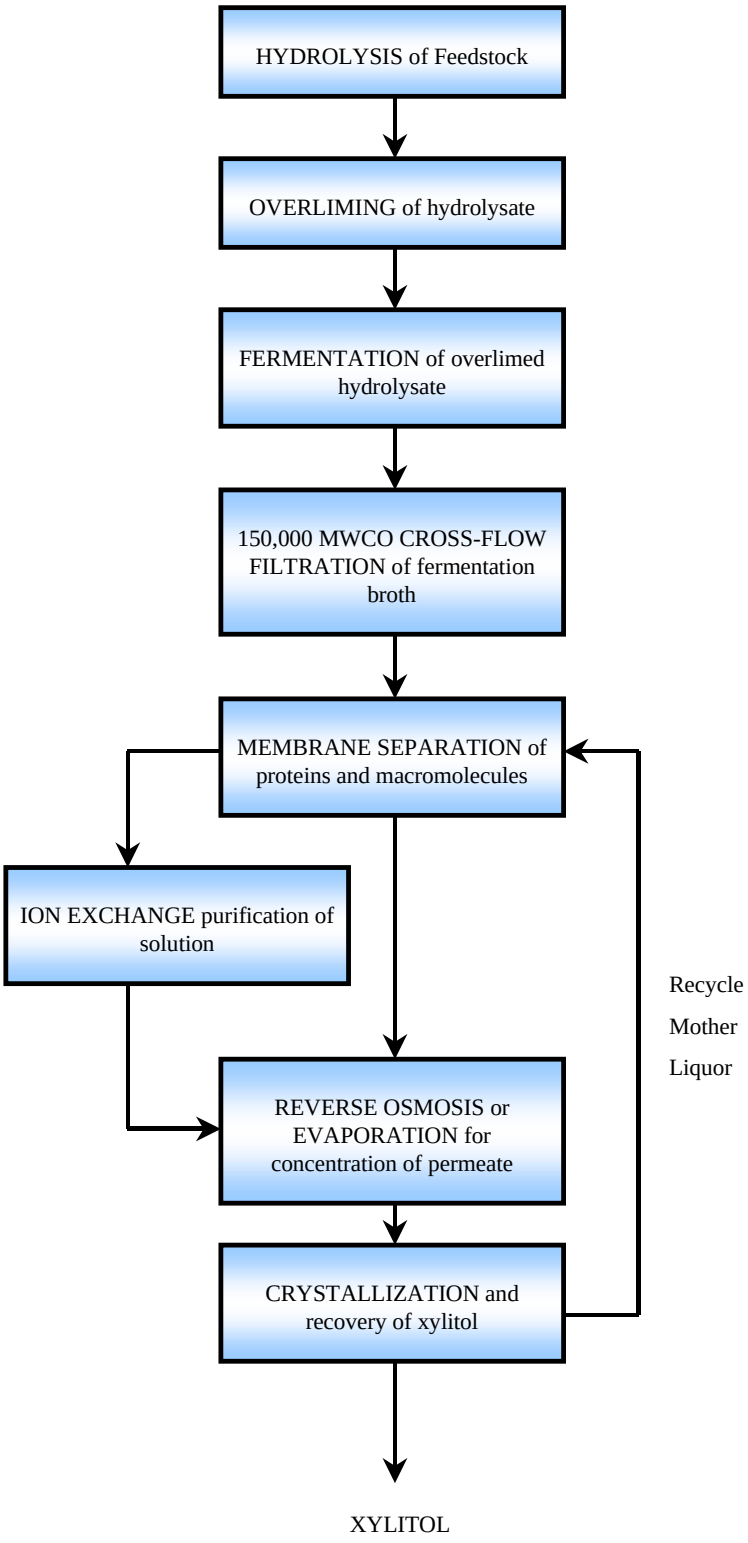

Figure 4.8 Microbial xylitol production and recovery using membrane method.

CONCLUSIONS

- Xylitol can be produced from D-xylose by *Candida tropicalis* under high air flow rate conditions. The high air flow rate (1.5 vvm) reduced the ethanol content of the fermentation broth and therefore improved the yield of xylitol. A yield of 0.6 g xylitol/g xylose was obtained by fermenting a model corn fiber hemicellulose hydrolysate.

- D-xylose, glucose, mannose, and galactose can be completely consumed by *Candida tropicalis*, but the arabinose utilization by this yeast species is very low. From a starting concentration of 25 g/L arabinose, only about 40% of the arabinose was consumed after 170 hours of fermentation.

- The activated carbon treatment can remove color from xylitol fermentation broth by adsorbing the UV absorbing material at 260 nm. As much as 79.5% of the UV absorbing material was removed by activated carbon. However, the activated carbon adsorbs about 25-50% of the xylitol in the solution.

- The polyethersulfone 150,000 MWCO membrane was used successfully in removing yeast cells. The 150,000 MWCO membrane achieved complete removal of yeast cells and removed some Lowry positive material.

- The HG19 10,000 MWCO polysulfone membrane can be used to separate xylitol and Lowry positive material, allowing over 87% of the xylitol to permeate while retaining over 50% of the Lowry positive material (including proteins).

- Adding yeast extract to the fermentation broth increases the Maillard reaction. There was an 81.3% conversion of arabinose when 40 g/L of yeast extract were added and reacted at 125 °C for three hours.

- The SR10 reverse osmosis membrane can be used for the concentration of xylitol for crystallization. At a pressure of 3.4 MPa, the SR10 membrane consistently concentrated xylitol, while allowing an average of $1.7 \pm 0.8\%$ of the xylitol to permeate the membrane.

- Over 87% pure xylitol crystals were obtained using membrane separation and crystallization techniques. Xylitol crystals with purity as high as 90.3% xylitol (by HPLC) were obtained through membrane separation with the HG19 polysulfone membrane.

RECOMMENDATIONS

The final results of the HG19 membrane separation of xylitol appear promising, therefore the effect of MX07 and BQ01 membranes on the increased removal of Lowry positive material and the subsequent increase in xylitol crystal purity, should be investigated. The HG19 membrane was chosen for further study based on the removal of relative UV absorption of proteinaceous materials. Later the impurities were analyzed with Lowry's method and showed that the MX07 membrane may give better impurity removal than the HG19 membrane and result in higher xylitol crystal purity. There would be a higher loss of xylitol, but further research is needed to determine if use of the MX07 membrane is worthwhile.

Recrystallization of xylitol crystals could improve xylitol crystal purity. Quantities of crystals obtained in this study were insufficient for a recrystallization procedure. When the xylitol crystals are redissolved in water and recrystallized, further purification results and greater than 90.3% purity can be obtained.

Scale-up of the membrane separation for recovery of xylitol should be performed with larger membrane surface area. The membranes MWCO range for xylitol and some membrane examples have been shown in this experiment and have narrowed the range of membranes needed to be tested.

The Maillard reaction was successful with yeast extract added, but the impurities added (such as peptides) were too small to be removed by the HG19 membrane. Perhaps a larger, inexpensive protein source could be identified and added to the fermentation broth to aid in the Maillard reaction and be separated out by the membrane method. For use with the HG19 membrane the protein would need to have a molecular weight on the order of 10,000.

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APPENDIX A

Design of Reverse Osmosis Pressure Vessel

The determination of the vessel wall thickness is important because thick-walled and thin walled vessels distribute stress in different ways. If the ratio of the mean radius of the vessel to its wall thickness is 10 or greater, the stress is very nearly uniform and it can be assumed that all the material of the wall shares equally to resist the applied forces. Such pressure vessels are called thin-walled vessels (Mott 1990). For the design of the test cell used in the separation of xylitol, it was determined that the vessel was thick-walled using Equations A.1 and A.2.

Determination of thick walled vessel

$$D_m = \frac{D_o + D_i}{2} \qquad (A.1)$$

Dm - Mean diameter (m),
Do - Outside diameter (m), and
Di - Inside diameter (m)

Thick walled vessel

$$\frac{D_m}{t} \leq 20 \qquad (A.2)$$

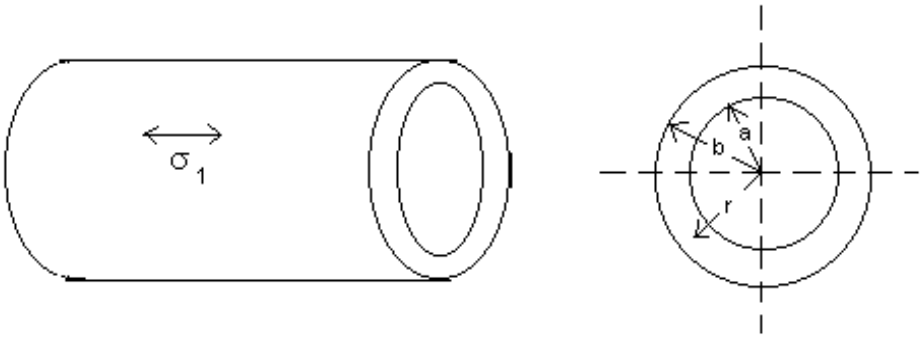
t - Wall thickness (m)

The minimum thickness of a thick-walled cylinder was calculated to withstand a pressure of 1500 psi or 10.3 MPa. There are three types of stress exerted on a pressure vessel, the longitudinal stress, the hoop stress and the radial stress. The longitudinal stress was found to be the critical stress on the pressure vessel with the smallest minimum wall thickness. The calculation is shown in Example 1.

Example 1.

Longitudinal stress

$$\sigma_1 = \frac{pa^2}{b^2 - a^2} \qquad Mott(1990)$$



Tensile stress for stainless steel = 90000 psi

$$90000 = \frac{1500\,psi(0.5625)^2}{b^2 - (0.5625)^2}$$

b = 0.5672 in

t = b – a = 0.0047 in

Multiply by safety factor of 6 and the minimum wall thickness for the pressure vessel is equal to 0.028 inches. The vessels walls were designed to be 1 inch thick, well within the safety limit. The reverse osmosis pressure vessel is held together by two plates connected by 6 (1/4inch) steel bolts. The pressure on the bolts at a fluid pressure of 1500psi or 10.3 MPa is calculated in Example 2.

Example 2.

Pressure P is the pressure exerted over the area of the top of the cylinder, A is the area, which the pressure is exerted on, s is the tensile or compressive stress.

P = 1500psi x π $(r)^2$ = 1491 pounds

$$s = \frac{P}{A}$$

$$A = \frac{\pi D^2}{4} \bullet 6$$

The area is multiplied by six because the design called for 6 bolts to get uniform compression around the reverse osmosis pressure vessel.

s = 5062 psi

At 1500psi pressure the stress in each bolt is equal to 5062 psi, which falls well within the tensile strength of steel (80000 psi).

APPENDIX B

Fermentation Results

Eight fermentations were performed during this experiment to improve growth conditions and collect product for membrane separation of xylitol. The fermentations are given in the order that they were performed to show the progression. The concentrations vs. time are shown along with the yield and productivity when calculated.

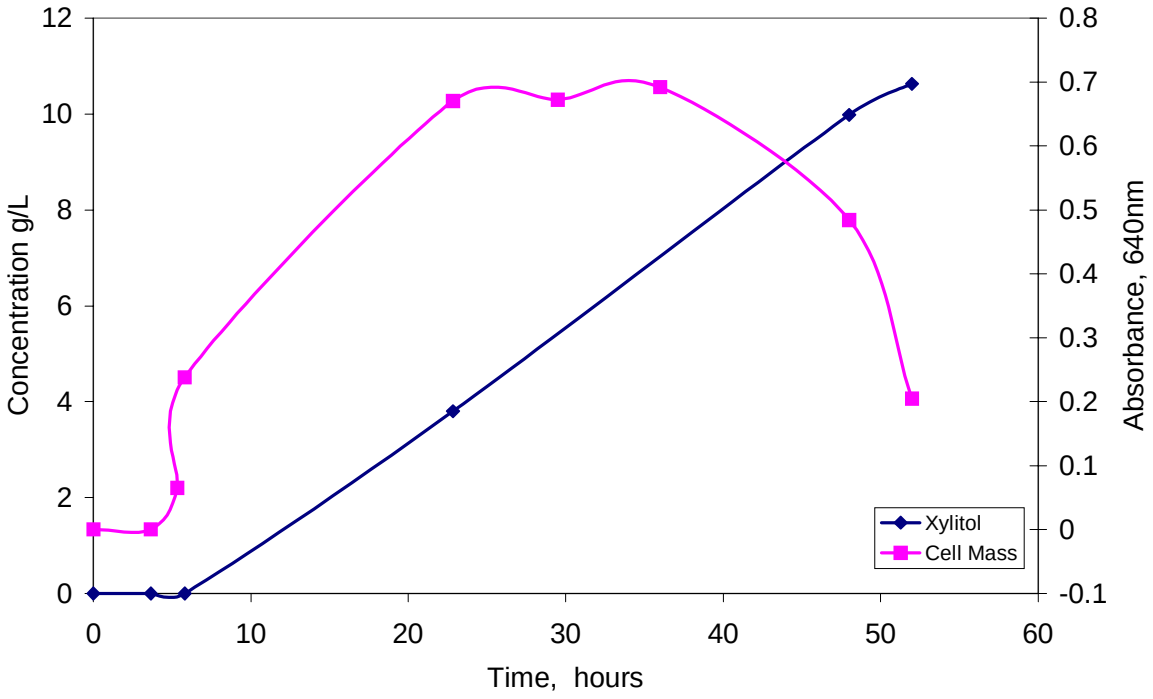


Figure B.1 7/7 Fermentation conditions: pH uncontrolled, aeration 1.5 vvm, 30 °C and agitation 130 rpm.

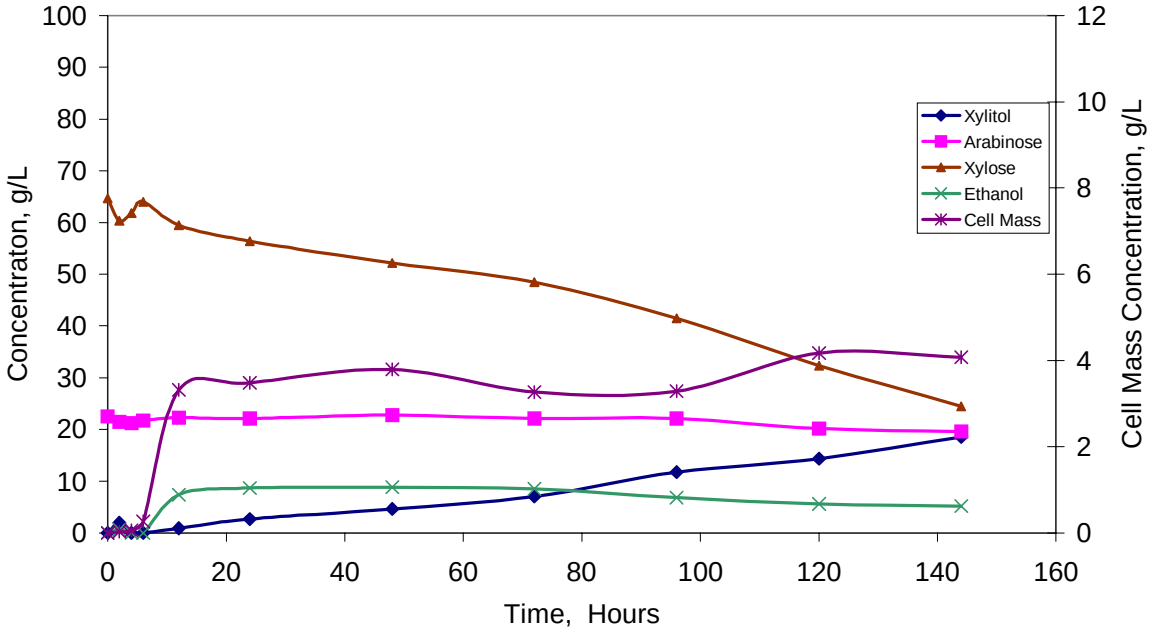


Figure B.2 10/19 Fermentation conditions: pH uncontrolled, aeration 0.2 vvm, 30 °C, and agitation 130 rpm.

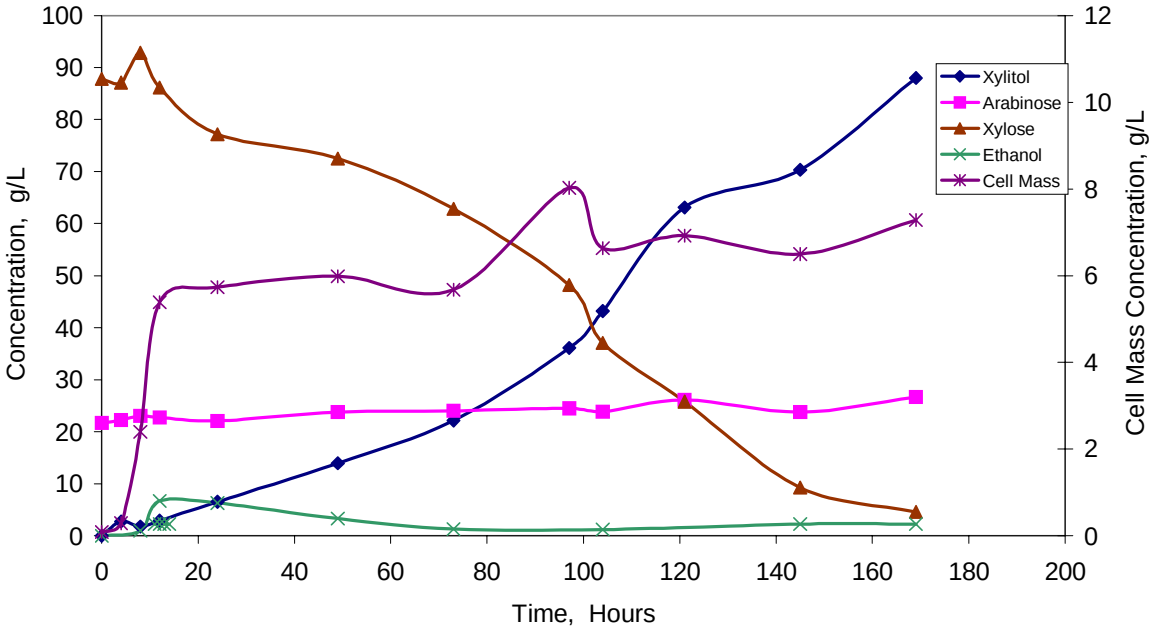


Figure B.3 10/28 Fermentation conditions: pH uncontrolled, aeration 1.5 vvm, 30 °C, and agitation 130 rpm.

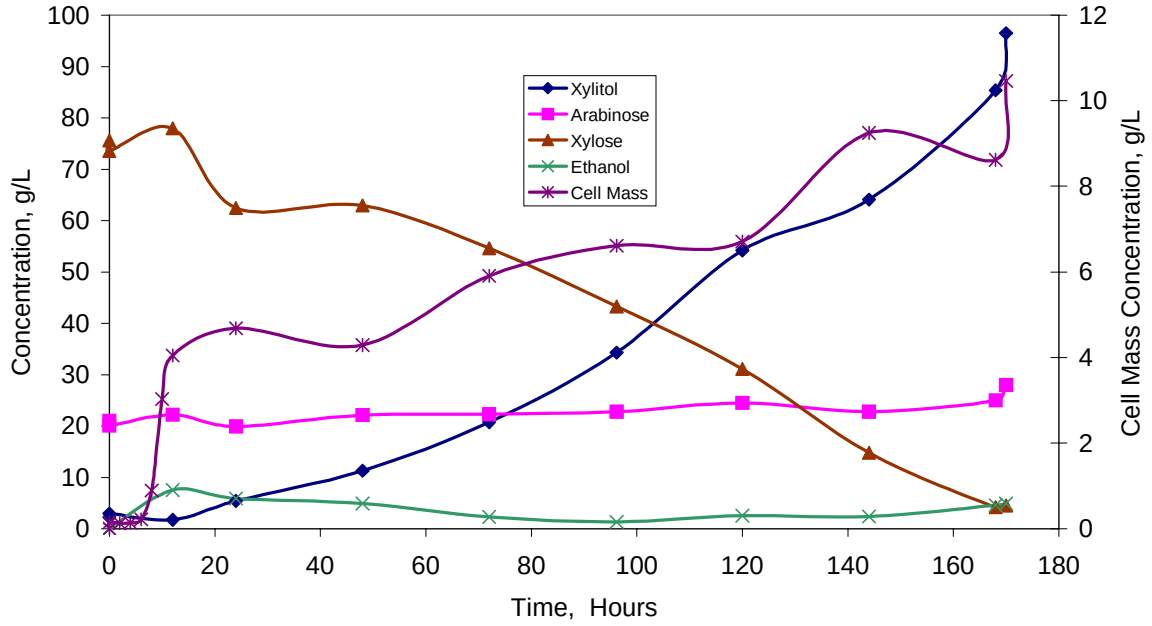


Figure B.4 12/8 Fermentation conditions: pH uncontrolled, aeration 1.5 vvm, 30 °C, agitation 130 rpm.

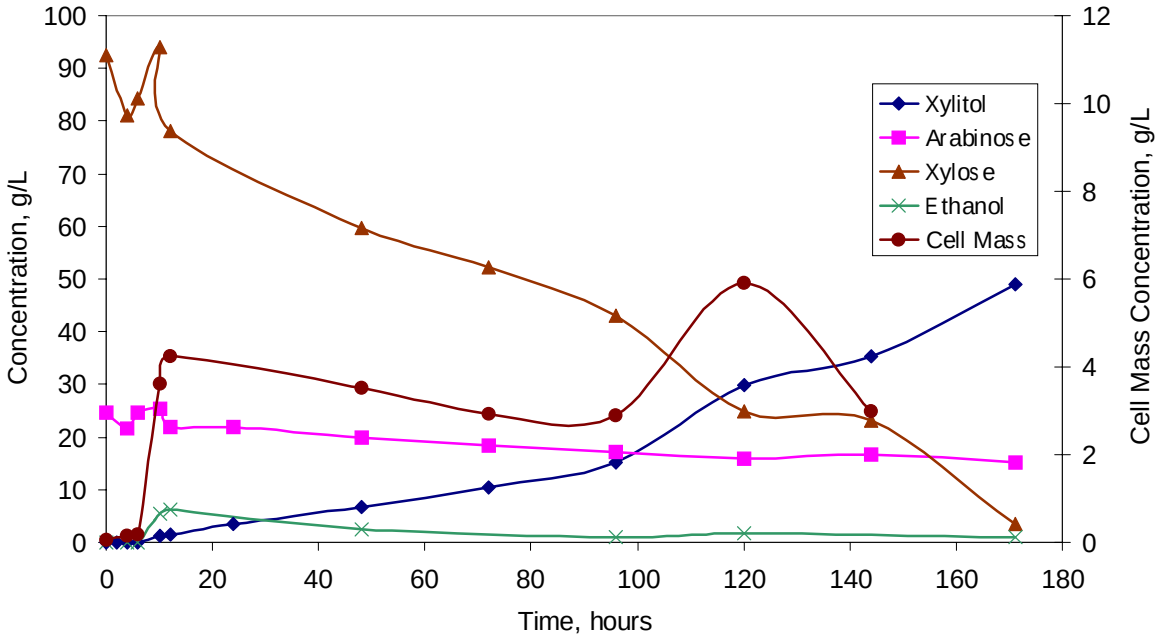


Figure B.5 12/21 Fermentation conditions: pH uncontrolled, aeration 1.5 vvm, 30 °C, agitation 130 rpm, yield 0.52 g/g, and productivity 0.262 g/L·h (adjusted for evaporation).

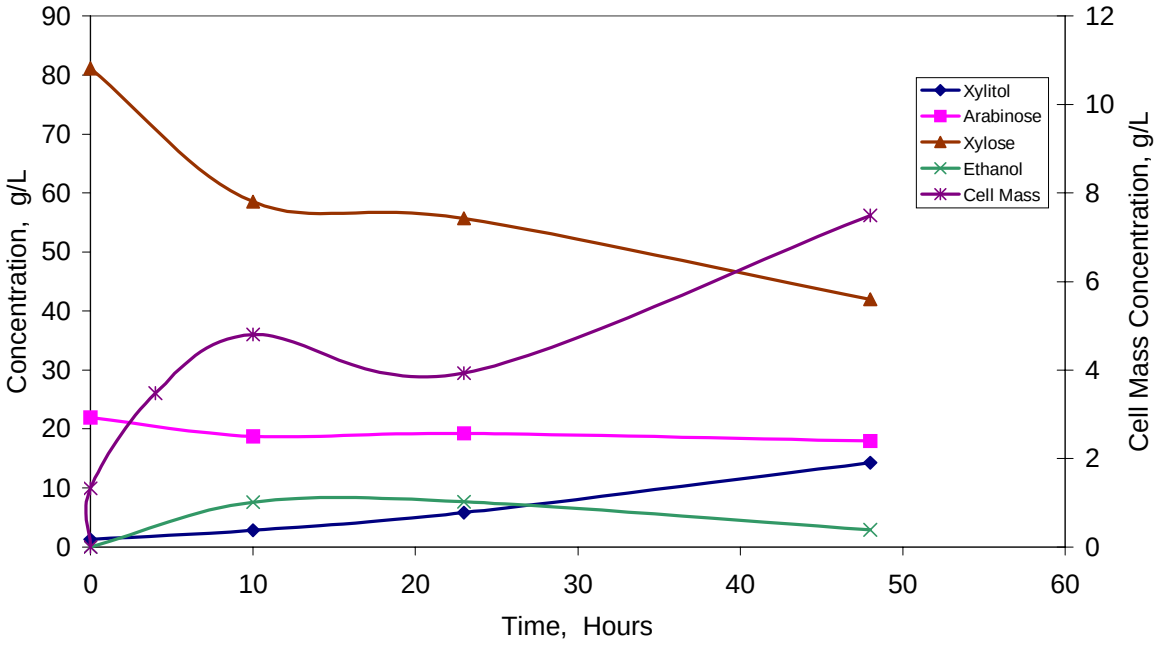


Figure B.6 1/15 Fermentation conditions: pH 5, aeration 1.5 vvm, 30 °C, agitation 130 rpm, yield 0.508 g/g, and productivity 0.276 g/L·h (adjusted for evaporation).

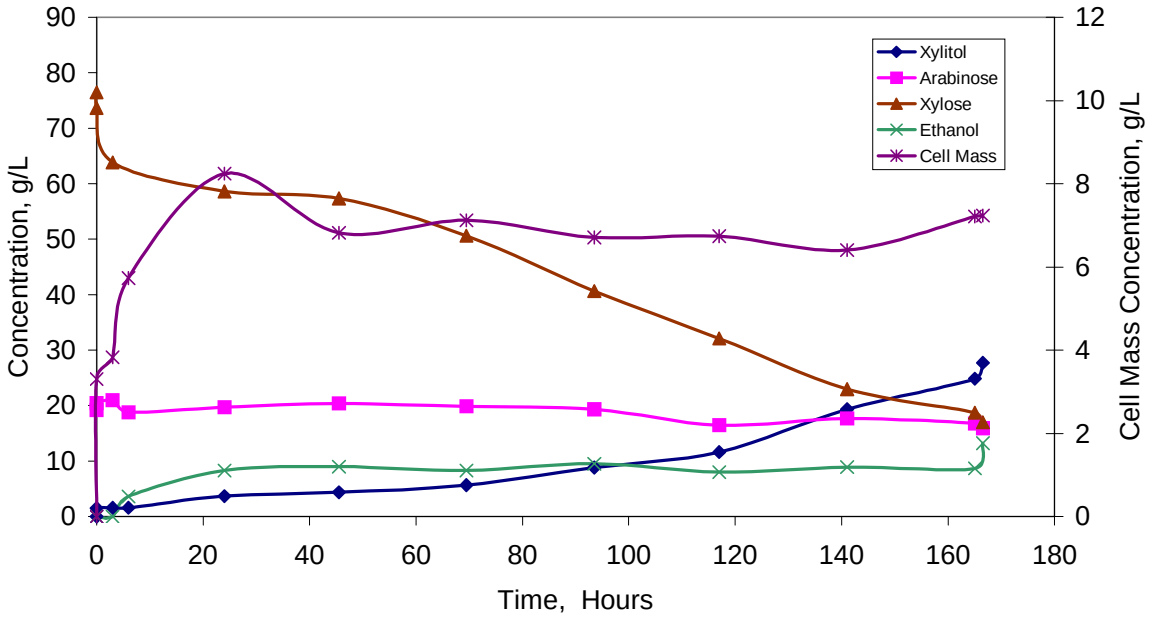


Figure B.7 1/21 Fermentation conditions: pH 5, aeration 0.5 vvm, 30 °C, agitation 130 rpm, yield 0.512 g/g, and productivity 0.141 g/L·h (adjusted for evaporation).

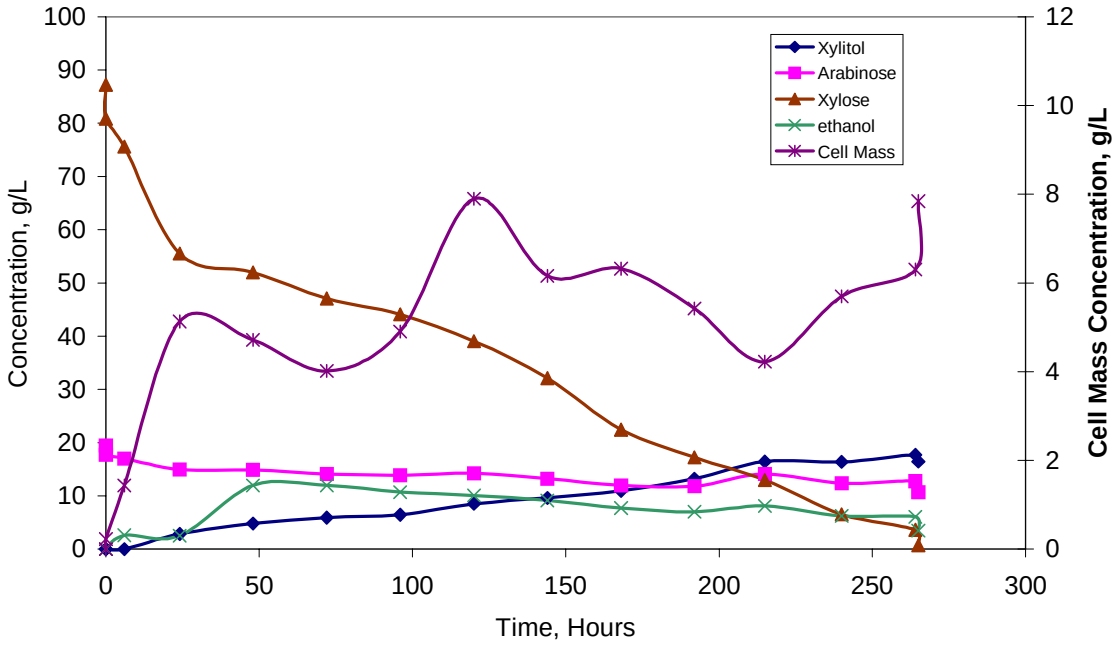


Figure B.8 5/10 Fermentation conditions: pH uncontrolled, aeration 0.5 vvm, 30 °C, agitation 200 rpm, yield 0.213 g/g, and productivity 0.066 g/L·h (adjusted for evaporation).

APPENDIX C

Flux calculation for membrane testing

$$Flux = \frac{V}{T \bullet A} \qquad (C.1)$$

Where

V – Volume of the permeate sample (L),
T – Time of permeate collection (days), and
A – Surface area of the membrane (m^2).

Calculation for crystal yield of xylitol

$$Crystal\ Yield = \frac{(Wcry - Wseed) * Purity}{Conc \bullet V} \qquad (C.2)$$

Where

Wcry – weight of crystals recovered (g),
Wseed – Weight of seed crystals added prior to crystallization (g),
Purity – Percent purity of xylitol crystals recovered (%),
Conc – Xylitol concentration in permeate collected (g/L), and
V – Volume of permeate for concentration (L).

VITA

Richard P. Affleck

Richard Affleck was born on June 21, 1973 in Tucson, Arizona. He grew up in Fairfax, Virginia and graduated from W.T. Woodson High School in 1991. Richard graduated with a Bachelor of Science degree in Chemical Engineering from VA TECH in 1997. In August of 1997, he began the study for a Master of Science degree in Biological Systems Engineering at VA TECH. After completion of his Master's degree, Richard plans to pursue a career in process development in biotechnology or pharmaceuticals.