ORIGINAL ARTICLE

# The use of dead-end and cross-flow nanofiltration to purify prebiotic oligosaccharides from reaction mixtures

.

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## Abstract

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**Nanofiltration (NF) of model sugar solutions and commercial oligosaccharide mixtures were studied in both dead-end and cross-flow modes. Preliminary trials, with a dead-end filtration cell, demonstrated the feasibility of fractionating monosaccharides from disaccharides and oligosaccharides in mixtures, using loose nanofiltration (NF-CA-50, NF-TFC-50) membranes. During the nanofiltration purification of a commercial oligosaccharide mixture, yields of 19% (w $w^{-1}$) for the monosaccharides and 88% (w $w^{-1}$) for di, and oligosaccharides were obtained for the NF-TFC-50 membrane after four filtration steps, indicating that removal of the monosaccharides is possible, with only minor losses of the oligosaccharide content of the mixture.**

**The effects of pressure, feed concentration, and filtration temperature were studied in similar experiments carried out in a cross-flow system, in full recycle mode of operation. The rejection rates of the sugar components increased with increasing pressure, and decreased with both increasing total sugar concentration in the feed and increasing temperature. Continuous diafiltration (CD) purification of model sugar solutions and commercial oligosaccharide mixtures using NF-CA-50 (at 25°C) and DS-5-DL (at 60°C) membranes, gave yield values of 14 to 18% for the monosaccharide, 59 to 89% for the disaccharide and 81**

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**to 98% for the trisaccharide present in the feed. The study clearly demonstrates the potential of cross flow nanofiltration in the purification of oligosaccharide mixtures from the contaminant monosaccharides.**

**Key words :** nanofiltration, oligosaccharide, prebiotic, fractionation

Certain oligosaccharides are considered to be functional food ingredients, having prebiotic properties, beneficial to the human health (Gibson and Roberfroid. 1995). Prebiotic oligosaccharides, generally consisting of 2-10 linked sugar monomers, are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and /or activity of one or a limited number of bacteria in the colon (i.e. *Bifidobacterium* sp. and *Lactobacillus* sp.). Some oligosaccharides have also been reported to act as soluble dietary fibre and as anticarcinogenic agents (Playne and Crittenden, 1996). They are produced by enzymic trans-glycosylation or controlled degradation reactions in complex synthesis mixtures (Playne and Crittenden. 1996).

Microfiltration and ultrafiltration, are well established separation processes in the biotechnology and fermentation industry, which can be used as a means of purifying oligosaccharides from high molecular weight enzymes and polysaccharides (Mountzouris *et al*., 1998). However, these commercial products often contain low molecular weight sugars that do not contribute to the beneficial properties of the higher molecular weight oligosaccharides. Nanofiltration (NF) may have potential as an economic industrial scale method for purification and concentration of oligosaccharide mixtures, although chromatography is currently the principal method (Lopez Leiva and Guzman, 1995). Matsubara *et al*. (1996) reported partial concentration of oligosaccharides from steamed soybean wastewater using NF membranes, while Sarney *et al*. (2000) used NF for the fractionation of human milk oligosaccharides and produced biologically active oligosaccharide mixtures with very little contaminating lactose.

The molecular weight cut-off values (MWCO) of NF membranes lie in the approximate range 200 to 1000 Daltons, between the ultrafiltration and reverse osmosis separation ranges. Mass transport in NF is based on two mechanisms: sieving and charge effects. Some sugars, such as pectate oligosaccharides (which are acidic in aqueous solutions), carry an electric charge, which affects their separation properties by NF membranes. However, most sugars are neutral molecules in aqueous solution, and their mass transport through NF membranes is controlled by convection and diffusion. Diffusive transport of sugars depends on the concentration gradient and remains pressure independent, whereas convective transport increases with pressure.

Although many literature reports have stated that NF separations of sugars, with differences in their molecular size in the range of a few glucosyl units, are not feasible due to poor selectivity, the present study is an attempt to evaluate NF as a possible fractionation and purification process for oligosaccharide mixtures.

The aim of the present study was to investigate the feasibility of nanofiltration for purification of oligosaccharides from mixtures containing monosaccharides. Preliminary studies using a “dead end” stirred cell system compared three types of membrane in terms of their rejection characteristics with simple sugar solutions. Commercial oligosaccharide mixtures were then fractionated using the same membranes and the extent of their purification was monitored. The study went on to evaluate cross-flow NF as a possible fractionation and purification process for oligosaccharide mixtures. Pressure dependence experiments were first carried out with a model solution containing a mono, di, and trisaccharide. The significance of the feed concentration and temperature were then studied to optimise the separation. Finally, taking into account the previous experiments, continuous

diafiltration (CD) purification of a model solution, and of a commercial oligosaccharide, was performed.

## Materials and Methods

### Chemicals

All solutions were prepared in demineralised water.

Analytical grade purity D(-)-fructose and sucrose were supplied by BDH Laboratory Chemicals (Poole, Dorset, UK), and D(+)-raffinose pentahydrate was supplied by Sigma Chemicals (Poole, Dorset, UK). These were used for the preparation of the model sugar solutions and as standards for HPLC analysis.

The commercial oligosaccharide mixtures were as follows (compositional data supplied by manufacturers):

a) Panorich® (Nihon Shokuhin Kako Co., Ltd, Tokyo, Japan), high panose syrup, produced by corn starch hydrolysis and transglucosylation. The product consists of 23%w.w$^{-1}$ glucose, 26%w.w$^{-1}$ maltose and isomaltose, 30%w.w$^{-1}$ panose and other branched oligosaccharides.

b) Biotose®#50 (Nihon Shokuhin Kako Co., Ltd, Tokyo, Japan) a branched-oligosaccharide corn syrup containing 41%w.w$^{-1}$ glucose, 27%w.w$^{-1}$ maltose and isomaltose, 18%w.w$^{-1}$ panose and isomaltotriose.

c) Vivinal®GOS a gift from Friesland Coberco Diary Foods (Deventer, The Netherlands), with typical specifications: 73%w.w$^{-1}$ dry matter of which, 57%w.w$^{-1}$ were galacto-oligosaccharides, 23%w.w$^{-1}$ lactose, 19% w.w$^{-1}$ glucose and 0.9%w.w$^{-1}$ galactose (values provided by the manufacturer).

### Membrane filtration equipment

*Dead-end* NF was carried out with a Gyrosep™ 300 stirred cell (Techmate Ltd., Milton Keynes, UK), modified to permit the use of pressures up to 50bar using compressed nitrogen (Figure 1). Flat sheet membranes with an effective membrane area of 40cm$^2$ were employed. A PTFE-coated magnetic stirrer bar was employed

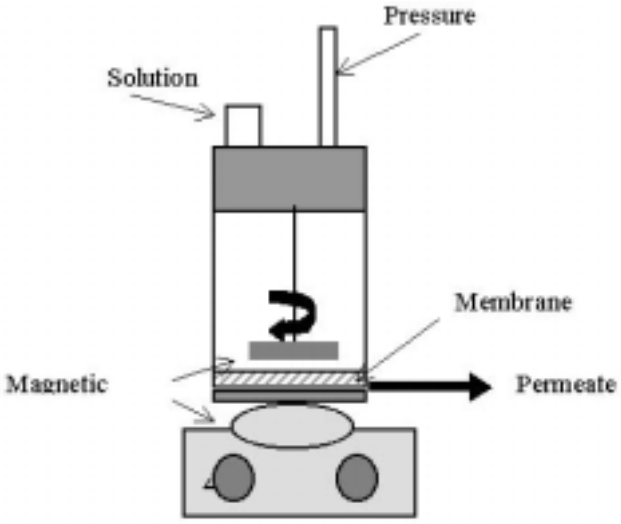


**Figure 1. Dead end filtration cell.**

at a stirring rate of 150rpm, adjusted so that the depth of the vortex was no more than one third of the stirred solution level. Reverse stirring was also applied every 5 seconds to ensure that the feed solution was well mixed.

*Cross-flow* NF used a high pressure test cell unit (Figure 2) supplied by Osmonics Desal (Le Mee sur Seine, France), which consisted of a tri-piston pump, a feed tank and two stainless steel high pressure cross-flow cells connected in parallel to the pump outlet. The unit had a maximum operating pressure of 70bar, a maximum operating temperature of 90°C, a pH operating range of 1-13, and the pump had a feed flow rate of 220.8L h$^{-1}$. The feed tank (2-5L capacity) had a vertically placed baffle to prevent aeration and turbulence, and a cooling/heating coil was connected to a temperature controlled water-bath. Each high pressure filtration cell consisted of a square stainless steel base, in which a stainless steel porous support disk was centrally positioned. The support disk had an effective membrane area of 81 cm$^2$ and 0.16 cm thickness. In order to maximise the turbulence in the feed and thus minimize concentration polarization, the feed solution inlet on the stainless steel cover was positioned to the perimeter of the cylindrical space above the membrane, and the concentrate

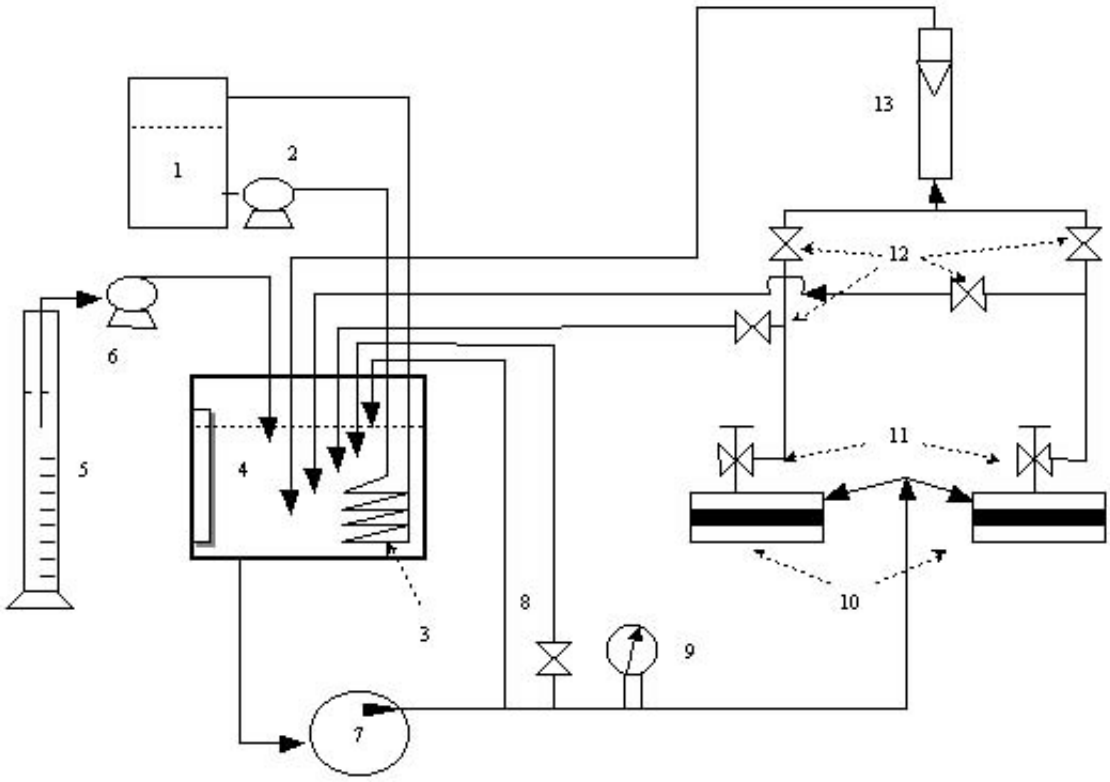


**Figure 2. Cross flow membrane unit: 1,2,3) Tempered water bath, Pump, and Cooling or heating coil for temperature control of the feed, 4) Feed tank, 5,6) Volumetric cylinder and pump for water addition, used only during the CD experiments, 7) Tri-piston pump, 8) By-pass valve kept closed during the experimental runs, 9) Pressure gauge, 10) Nanofiltration cells 11) Back-pressure regulator valves, 12) Valves used for redirecting the retentate flow of each cell towards the flow meter (one at the time) in order to equalise the pressure applied to the two cells, 13) Flow meter. In the figure the permeate streams are recycled in the feed (total recycle mode), but when CD was used the permeates from the two cells were removed.**

outlet was positioned centrally. A back-pressure regulator on the concentrate outlet was used to control the pressure.

Due to the parallel configuration of the two cells, a flow meter was used to ensure that the same pressure was applied to both cells. By adjusting the feed flow rate of the two cells to the same level, the same trans-membrane pressure was applied to both cells. In addition to the temperature control coil in the feed tank, the cells were maintained in a water-bath, to ensure accurate control of the filtration temperature.

**Membranes**

Stirred cell experiments employed two nanofiltration membranes: NF-CA-50 composed of cellulose acetate, and NF-TFC-50, a thin film trilaminate membrane composed of polyether-sulphone, both of which had a nominal 50% sodium chloride rejection (Intersep Ltd., Wokingham, UK). In addition, an ultrafiltration membrane composed of cellulose acetate with nominal molecular weight cut-off 1000 Daltons (UF-CA-1, Intersep Ltd.) was included in the study. New membranes were conditioned to avoid changes in the separation characteristics due to compaction. For that purpose, 300 ml of

demineralised water were filtered through the membrane, at a constant pressure of 30 bar, to wash off any protective materials (glycerine, azide) from the membrane and compress the membrane to a steady state.

The cross- flow experiments used three thin film composite membranes, DS-5-DL (NF), DS-51-HL (NF) and DS-GE (UF), supplied by Osmonics Desal (Le Mee sur Seine, France) as well as the two cellulose acetate membranes used in the dead-end experiments. According to the manufacturer, DS-5-DL and DS-51-HL had a MWCO on sucrose and glucose and a 96% and 95% rejection of $MgSO_4$ respectively. The DS-GE had a MWCO of 1000Da. Membranes were cut to size and soaked overnight in demineralised water. To facilitate fitting of the membranes, a 25%v.v$^{-1}$ ethanol solution in demineralised water was used to wet the effective filtration surface of the cells where the membranes were placed. Membranes were conditioned by compressing them to a steady state of compaction (determined by the flux of permeate), with demineralised water as feed, at an intermediate pressure according to their pressure limits (at 25$^{o}$C).

Membranes were stored in 10%v.v$^{-1}$ ethanol solutions at 4$^{o}$C after each experimental run with sugar solutions.

## Experimental procedure

### Stirred cell experiments

The water flux of the new preconditioned membranes was measured over a range of pressures to establish criteria that would allow comparison with respect to damage or fouling. The volumetric flux of permeate was measured and expressed as litres per square meter per hour (l m$^{-2}$ h$^{-1}$):

$$J_V = \frac{V_p}{A \times t} \qquad \textbf{Eq.1}$$

where $V_p$ is the permeate volume, $A$ the membrane effective area (0.004 m$^2$), $t$ the time (hours) necessary for the production of $V_p$ litres of permeate. The flux values were calculated, taking into account the time necessary for the collection of a certain volume of permeate. This volume determined the volume concentration ratio (VCR, see Eq.2) value at which the flux measurement was taken.

Preliminary experiments using glucose and lactose solutions (10 g kg$^{-1}$) were carried out to establish the rejection characteristics with each membrane. According to the literature, pressure does not have a significant effect on the rejection of sugars (Aydogan, 1998; Sarney, 2000). However, membrane compaction caused by the applied pressure may alter their separation characteristics. The sugar solution filtration experiments were carried at constant pressure 40 bar, and the volume of the initial feed solution was 300ml in all cases. During the course of the filtration, 25 and 50 ml batch samples of permeate were collected in the single sugar and oligosaccharide experiments respectively, and permeate flux was calculated. The final retentate volume was 50ml and stirring was applied throughout all filtrations. The operating temperature of the filtration cell was in the range of 20-25$^{o}$C.

In experiments with commercial oligosaccharide mixtures, four stage discontinuous diafiltration was used to improve the purification of the retained solutes. Discontinuous diafiltration refers to the operation where permeable solutes are cleared from the retentate by volume reduction, followed by redilution with water to the original volume and the operation repeated.

At the end of each experimental run the permeate flux with demineralised water was measured at three different pressures (10, 30 and 50 bar) in order to determine if any irreversible fouling had occurred. In fact, water flux was restored to the initial value in all cases, indicating that no irreversible fouling had occurred.

Volume concentration ratio (VCR) was calculated from the following equation:

$$VCR = \frac{V_f}{V_r} \qquad \textbf{Eq.2}$$

where $V_f$ and $V_r$ the volume of the initial feed

solution and the volume of the retentate respectively.

The observed rejection for a given solute in a batch process is given from either the permeate or the retentate concentrations and the corresponding VCR:

$$R_i = \frac{\ln(C_r - C_f)}{\ln(VCR)} \qquad \textbf{Eq.3}$$

where $R_i$ is the rejection of a certain solute i and $C_r$, $C_f$, are the concentrations of that solute in the retentate and the initial feed respectively (Kulkarni and Funk 1992).

The yield ($Y$) of any component was calculated from:

$$Y = \frac{C_r C_r}{C_f C_f} \qquad \textbf{Eq.4}$$

**Cross flow experiments**

After conditioning of the membranes the water flux was measured at various pressures at 25±1°C. These initial water flux measurements, were compared with measurements carried out at the same conditions after each experiment to indicate any irreversible fouling or membrane damage. However, irreversible fouling was never observed in this study. Total recycle mode was used to study the effect of operational variables on separations, while purification of oligosaccharide mixtures was performed with continuous diafiltration (CD).

Sugar solutions (2L), were added to the filtration system (after drainage of the existing demineralised water) and circulated for 10 min, before the initial feed concentrations were measured. All permeate concentration and flux values presented in this study are the average value of both filtration cells of the unit.

For the total recycle experiments, the pressure and temperature were adjusted to the desired levels, and the system was allowed to stabilize for 45 min. Flux measurements were taken, and the feed and permeates were sampled. In experiments where the sugar concentration or the temperature were varied, the pressure was kept constant, and the system was allowed to stabilize for 45 min before any measurements or samples were taken.

In the CD experiments, the desired pressure and temperature were set, and the system was allowed to stabilize for 1h in total recycle mode. Permeate flux measurements and samples (zero cumulative permeate) were then taken and CD started. At intervals of 30 or 60 min, samples of the feed and permeates were taken, and the permeate flux and total cumulative permeate collected, during this period, were measured.

**Pressure, temperature and concentration dependence**

To investigate the effect of pressure on the separation of sugars, four experiments were carried out using a model solution of approximately 0.07-0.08g $ml^{-1}$ total sugars, at 25±1°C. The model solution, composed of approximately equal concentrations of fructose, sucrose, and raffinose pentahydrate, was tested at four pressures, varying for each membrane according to their pressure range.

The thin film composite DS-5-DL membrane, which in preliminary experiments had given high rejections of monosaccharides, was used to perform a temperature dependence study. This membrane had a maximum operating temperature of 90°C and a high porosity, which was reflected in the high permeate fluxes obtained even at low temperatures. The experiments were carried out using a model solution (total sugar 0.055 g $ml^{-1}$), at 13.8 bar pressure, and temperatures 25, 35, 45, 60 ±1°C. In addition, based on the results from the temperature dependence experiments, the effect of pressure on the separation characteristics of this membrane was tested at 25 and 60°C.

The effect of feed concentration on the separation characteristics of the sugar components in an oligosaccharide mixture, was studied with varying concentrations of the commercial oligosaccharide mixture Vivinal®GOS, at 25±0.5°C.

**Continuous diafiltration**

The NF-CA-50 and DS-5-DL (at 60°C) membranes, gave the best separation characteristics of monosaccharides from oligosaccharides with respect to the retention of the oligosaccharides, which were the target components in this study. For that reason, CD purification of a model solution and a commercial oligosaccharide mixture, was carried out using these membranes at 13.8bar pressure, and temperature regulated at 25±0.5°C for the NF-CA-50 and at 60±0.5°C for the DS-5-DL. The total cumulative permeate necessary to be removed in order to achieve a five fold purification of these mixtures from the monosaccharide was calculated to be in the range of 6.0 to 6.5L using the equation:

$$\ln\left(\frac{C_i}{C_f}\right) = (1-R)\times\left(\frac{V_c}{V_s}\right) \qquad \textbf{Eq. 5}$$

and assuming that the rejection of this sugar (taken from the data of the pressure dependence experiments) remained constant throughout the CD process.

**Analytical methods**

Sugar solutions were analysed using an Aminex HPX-87C $Ca^{+2}$ resin-based column (300 × 7.7mm) supplied by Bio-Rad Laboratories Ltd (Hertfordshire, UK) and an HPLC analyser coupled to a refractive index detector. The column was maintained at 85°C and HPLC grade water was used as mobile phase at a flow rate 0.6 ml $min^{-1}$.

Calibration curves were prepared for each sugar separately, and for the commercial oligosaccharide mixtures. The oligosaccharide mixture appeared as three separate peaks, the third and the second corresponding to the retention times of glucose and lactose respectively. Thus, in the discussion and presentation of the results, the term glucose is used to refer to all the monosaccharides present in the mixture (since it is the predominant one), the term lactose stands for all the disaccharides (since it is the predominant one), and the term "oligos" for all the sugars which had a higher molecular weight than a disaccharide. Each sample was analysed twice and the average was used.

The phenol sulphuric acid assay for carbohydrates (Saha and Brewer 1994), was used as a rapid, non-specific determination of the total carbohydrate content of the samples. Results from the analysis of the oligosaccharide samples were used for mass balance calculations and to determine the concentration of lactose.

Glucose was measured by the glucose oxidase-peroxidase assay using a commercial kit (procedure No.510, Sigma Aldrich, Poole, UK).

## Results and Discusstion

**Stirred cell experiments**

The permeate flux (e.g. for glucose shown in Figure 3), determined for the single sugar and the oligosaccharide fractionations respectively, showed an overall decrease throughout the volume reduction from 300ml to 50ml (VCR: 6), as expected due to the increased concentration polarization. Initial flux was greater, but flux decline was more marked for the NF-TFC-50 than the other membranes. In the diafiltration purification of oligosaccharides, the flux recorded in the first filtration run was lower than in subsequent

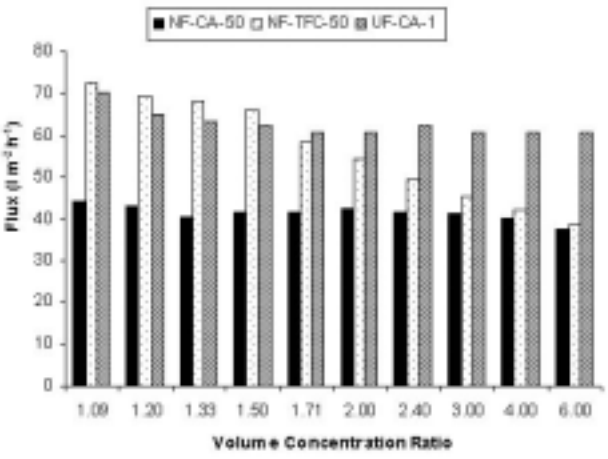


**Figure 3. Permeate Flux vs Volume Concentration Ratio for the fractionation of 10g $L^{-1}$ Glucose at 40bar constant pressure in stirred cell unit.**

runs at the same VCR, due to reduced osmotic pressure following removal of permeating solutes.

NF-CA-50 and NF-TFC-50 membranes had the highest rejection for glucose (R values of 0.86 and 0.69 respectively) and lactose (R value of 0.99 for both membranes). UF-CA-1 membranes had rejections of 0.82 and 0.61 for glucose and lactose respectively. These experiments suggest that separation of mono and disaccharides is more effective with the NF-TFC-50 membrane than the two cellulose acetate membranes. The concentrations of glucose and lactose in the permeate increased with increasing VCR in the single sugar experiments for all three membranes (Data for lactose shown in Figure 4), the increase being most marked with the NF-TFC-50 for glucose and with UF-CA-1 for lactose. Vellenga and Tragardh (1998) found, with membranes too dense for sugars to permeate, that in combined sugar and salt solutions the salt rejection decreased as the sugar concentration increased. This was explained as a direct effect of the increased concentration polarization layer viscosity, due to the sugar concentration, which caused back diffusion of the salt to be hindered, resulting in reduced salt rejection. In the same way the sugar concentration in the feed affects the rejections of individual sugars, causing them to decrease as the total concentration increases.

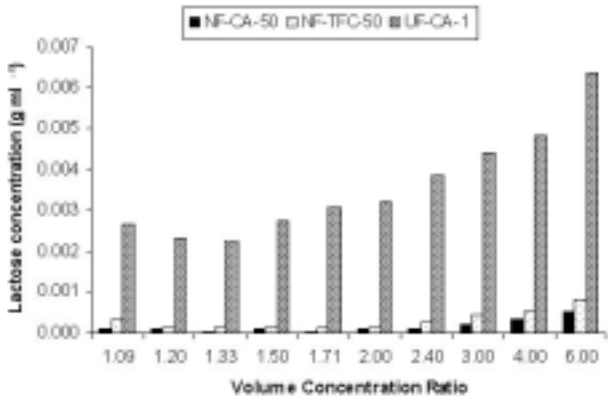


**Figure 4. Concentration of Lactose in the permeates vs Volume Concentration Ratio during the filtration of 10g $L^{-1}$ Lactose at 40bar constant pressure in stirred cell unit.**

Mass balance data obtained during diafiltration of Panorich solution are given in Table 1. All the membranes gave distinct differences in the retention of mono- and di- and oligosaccharides. It is clear that the most appropriate membrane for purifying oligosaccharides from monosaccharides in this system, is the NF-TFC-50, which gave 80% removal of monosaccharides from the initial feed solution with only a relatively small loss of di, and oligosaccharides. The final yields in the retentate after four diafiltration runs were 88% for di- and oligosaccharides, and 19% for monosaccharides.

**Cross flow experiments**

All membranes tested with model sugar solutions showed a linear relationship between the permeate flux and the applied pressure, the magnitude of fluxes were:

UF-CA-1 > DS-51-HL > NF-CA-50 > DS-GE

Table 2 summarizes the rejection values for the sugars of the model solution. The observed differences between the rejection values of the sugars indicate the potential of some NF membranes for purifying oligosaccharides from monosaccharides. The rejection values for the three sugars were directly dependent on the total sugar concentration of the feed solution, and increased with pressure due to membrane compaction and also due to increased solvent flux. Compaction reduces the membrane thickness that normally would lead to an increased permeate flux. However, pore size reduction, caused also by compaction, is the predominant characteristic upon which the rejection of neutral solutes is dependent (sieving effect), hence causing an overall increase to the rejections observed. Increased pressure also led to reduced differences between the rejections of the three sugars, and hence a less effective separation. That is because the effect of pressure on rejection is less marked as the molecular weight of the sugar increases, with respect to the pore size of the membranes used. As pore size decreases, increasing pressure causes the convective flux of solutes to decrease to a much

**Table 1. Total mass of sugars present in the initial feed solution and the retentates of the 20g $L^{-1}$ Panorich purification at 40bar constant pressure.**

| | | Mass of sugars in the total volume of solution [g] | | |
|---|---|---|---|---|
| | | Total sugars | di, oligo-saccharides | monosaccharides |
| | **Feed sol.** | **6.03** | **4.62** | **1.41** |
| | **Filtration** | **Retentates** | | |
| **NF-CA-50** | Step No1 | 5.81 | 4.61 | 1.20 |
| | Step No2 | 5.65 | 4.63 | 1.02 |
| | Step No3 | 4.70 | 3.96 | 0.74 |
| | Step No4 | 4.60 | 3.86 | 0.75 |
| **NF-TFC-50** | Step No1 | 5.87 | 4.69 | 1.18 |
| | Step No2 | 5.12 | 4.34 | 0.78 |
| | Step No3 | 4.82 | 4.32 | 0.50 |
| | Step No4 | 4.34 | 4.08 | 0.27 |
| **UF-CA-1** | Step No1 | 4.13 | 3.41 | 0.72 |
| | Step No2 | 3.24 | 2.90 | 0.34 |
| | Step No3 | 2.60 | 2.43 | 0.17 |
| | Step No4 | 2.22 | 2.14 | 0.08 |

*** Mass of the sugars was calculated from the concentrations found for each sugar in the feed and the retentate solutions taking in account the actual volume of each solution.**

greater extent than the diffusive flux (Pontalier *et al.* 1997). Thus, with NF membranes, and neutral solutes, the significance of convective solute flux becomes greater as the molecular size of the sugar decreases leading to greater changes in rejection. The increase in the rejection rate at higher pressures caused by the higher solvent flux was observed because the diffusive transport of solutes through the membranes remained the same at higher pressures, thus reducing the solute concentration in the permeate stream.

The effect of temperature on flux and solute rejection are shown for DS-5-DL membranes in Figure 5. As expected, flux increased with increasing temperature in both water and sugar solutions. Increased temperature also caused the sugar rejections to decrease, but the effect was quite different for the three sugars in the model solution. The monosaccharide (fructose) showed the greatest change in rejection values, followed by the disaccharide (sucrose). The rejection of raffinose remained constant at elevated temperatures. These results are in agreement with Tsuru *et al.* (2000), for the effect of temperature on the transport performance of inorganic NF membranes. Since diffusion of molecules through pores is an activated process, because of the hydrodynamic drag forces inside the pores, higher temperatures supply thermal energy increasing the diffusivity of these molecules. This increase in diffusivity, in relation to the higher permeate flux, results in a decrease in solute rejection, with the fructose rejection being affected more, because of the higher convective flux of permeate. The unaffected rejection of raffinose shows that the actual pore size of the membrane was not affected by temperature, although the effective pore diameter may have changed due to the thinner layer of adsorbed water molecules on the pore walls. The effect of pressure on rejection and permeate flux was the same at elevated temperatures (Table 2). The

**Table 2. Rejection values for Raffinose, Sucrose and Fructose at a range of applied pressures during cross-flow NF of a model solution of the three sugars.**

| | NF-CA-50[a] / 0.0761 (g.ml$^{-1}$)[b] | | | | UF-CA-1 / 0.0754 (g.ml$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| Pres [bar] | 6.9 | 13.8 | 20.7 | 27.6 | 6.9 | 13.8 | 20.7 | 27.6 |
| Raffinose | 0.80 | 0.90 | 0.93 | 0.95 | 0.71 | 0.80 | 0.84 | 0.87 |
| Sucrose | 0.56 | 0.73 | 0.79 | 0.83 | 0.45 | 0.60 | 0.66 | 0.72 |
| Fructose | 0.10 | 0.26 | 0.35 | 0.42 | 0.11 | 0.22 | 0.29 | 0.35 |
| | DS-GE / 0.0775 (g.ml$^{-1}$) | | | | DS-51-HL / 0.0702 (g.ml$^{-1}$) | | | |
| Pres.[bar] | 6.9 | 13.8 | 20.7 | 24.1 | 6.9 | 10.3 | 13.8 | 17.2 |
| Raffinose | 0.70 | 0.79 | 0.80 | 0.80 | 0.84 | 0.92 | 0.95 | 0.96 |
| Sucrose | 0.41 | 0.56 | 0.61 | 0.62 | 0.77 | 0.87 | 0.92 | 0.93 |
| Fructose | 0.08 | 0.18 | 0.24 | 0.25 | 0.51 | 0.67 | 0.76 | 0.78 |
| | DS-5-DL (25 °C) / 0.0496 (g.ml$^{-1}$) | | | | DS-5-DL (60 °C) / 0.0496 (g.ml$^{-1}$) | | | |
| Pres. [bar] | 6.9 | 13.8 | 20.7 | | 6.9 | 13.8 | 20.7 | |
| Raffinose | 1.00 | 1.00 | 1.00 | | 0.99 | 1.00 | 1.00 | |
| Sucrose | 0.99 | 0.99 | 0.99 | | 0.94 | 0.97 | 0.97 | |
| Fructose | 0.54 | 0.72 | 0.77 | | 0.28 | 0.48 | 0.53 | |

[a] **Membrane type**
[b] **Feed concentration (Feed concentrations varied due to dilution of the hold-up volume of the system. In the DS-5-DL experiment lower concentration was used due to lack of raffinose)**

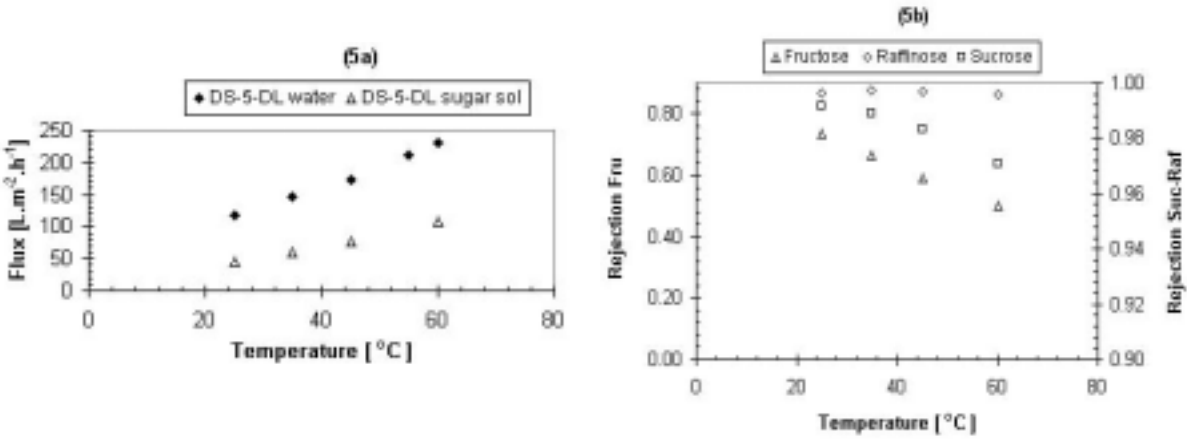


**Figure 5. a) Permeate Flux vs. Temperature and, b) Rejection vs. Temperature in the cross-flow NF of the model solution with the DS-5-DL membrane at 13.8bar pressure and 0.055g.ml$^{-1}$ total feed concentration, in cross flow unit.**

difference in rejections between fructose and sucrose was much larger, particularly with the lower MWCO membranes, than the difference between sucrose and raffinose, emphasising that the spatial configuration of the molecules and their conformation in solutions is very important

for their separation characteristics.

The rejections of the three sugar components during processing of Vivinal®GOS decreased as the total sugar concentration of the solution increased (Table 3) in agreement with the stirred cell experiments. The effect was more pronounced as the molecular weight of the sugars decreased and the MWCO of the membranes increased, as a direct effect of the convective solute flux. According to this, for each individual sugar component of the mixture, total sugar concentration determines the rejection values observed, which is also confirmed in the CD experimental results.

The NF-CA-50 and the DS-5-DL membranes were chosen to perform CD purification using a model solution and an oligosaccharide mixture, because these membranes showed greater differences in rejection values between the monosaccharides and the higher molecular weight sugars, with particular respect to retention of oligosaccharides. The pressures chosen for the CD purification represented a compromise between permeate flux and rejections observed at

**Table 3. Rejection values for oligosaccharides, lactose, and glucose at varying concentrations of Vivinal®GOS during cross flow NF.**

| | NF-CA-50 / 13.8 (bar) [a] | | | | | |
|---|---|---|---|---|---|---|
| Feed con. ($g.ml^{-1}$)[b] | 0.0160 | 0.0300 | 0.0408 | 0.0557 | 0.0682 | 0.0793 |
| Oligos | 0.96 | 0.95 | 0.95 | 0.95 | 0.94 | 0.93 |
| Lactose | 0.89 | 0.87 | 0.86 | 0.85 | 0.83 | 0.83 |
| Glucose | 0.58 | 0.53 | 0.48 | 0.48 | 0.42 | 0.43 |
| | UF-CA-1 / 13.8 (bar) | | | | | |
| Feed con. ($g.ml^{-1}$) | 0.0155 | 0.0296 | 0.0426 | 0.0557 | 0.0666 | 0.0775 |
| Oligos | 0.93 | 0.93 | 0.92 | 0.91 | 0.91 | 0.89 |
| Lactose | 0.82 | 0.81 | 0.79 | 0.77 | 0.75 | 0.72 |
| Glucose | 0.50 | 0.47 | 0.43 | 0.40 | 0.37 | 0.32 |
| | DS-51-HL / 6.9 (bar) | | | | | |
| Feed con. ($g.ml^{-1}$) | 0.0159 | 0.0307 | 0.0435 | 0.0558 | 0.0679 | 0.0780 |
| Oligos | 0.97 | 0.96 | 0.94 | 0.93 | 0.89 | 0.83 |
| Lactose | 0.95 | 0.94 | 0.91 | 0.87 | 0.80 | 0.72 |
| Glucose | 0.83 | 0.81 | 0.72 | 0.61 | 0.46 | 0.36 |
| | DS-GE / 13.8 (bar) | | | | | |
| Feed con. ($g.ml^{-1}$) | 0.0160 | 0.0294 | 0.0422 | 0.0535 | 0.0643 | 0.0736 |
| Oligos | 0.86 | 0.87 | 0.85 | 0.85 | 0.83 | 0.83 |
| Lactose | 0.64 | 0.67 | 0.62 | 0.61 | 0.58 | 0.58 |
| Glucose | 0.30 | 0.33 | 0.28 | 0.26 | 0.23 | 0.23 |

[a] **The pressures were chosen by taking in account the separation achieved at the pressure dependence experiments and also the permeate flux at each pressure.**

[b] **From the total sugars in each solution on average 40.4% was galacto-oligosaccharides as (defined in the introduction), 40.0% was lactose, and 19.6% was glucose.**

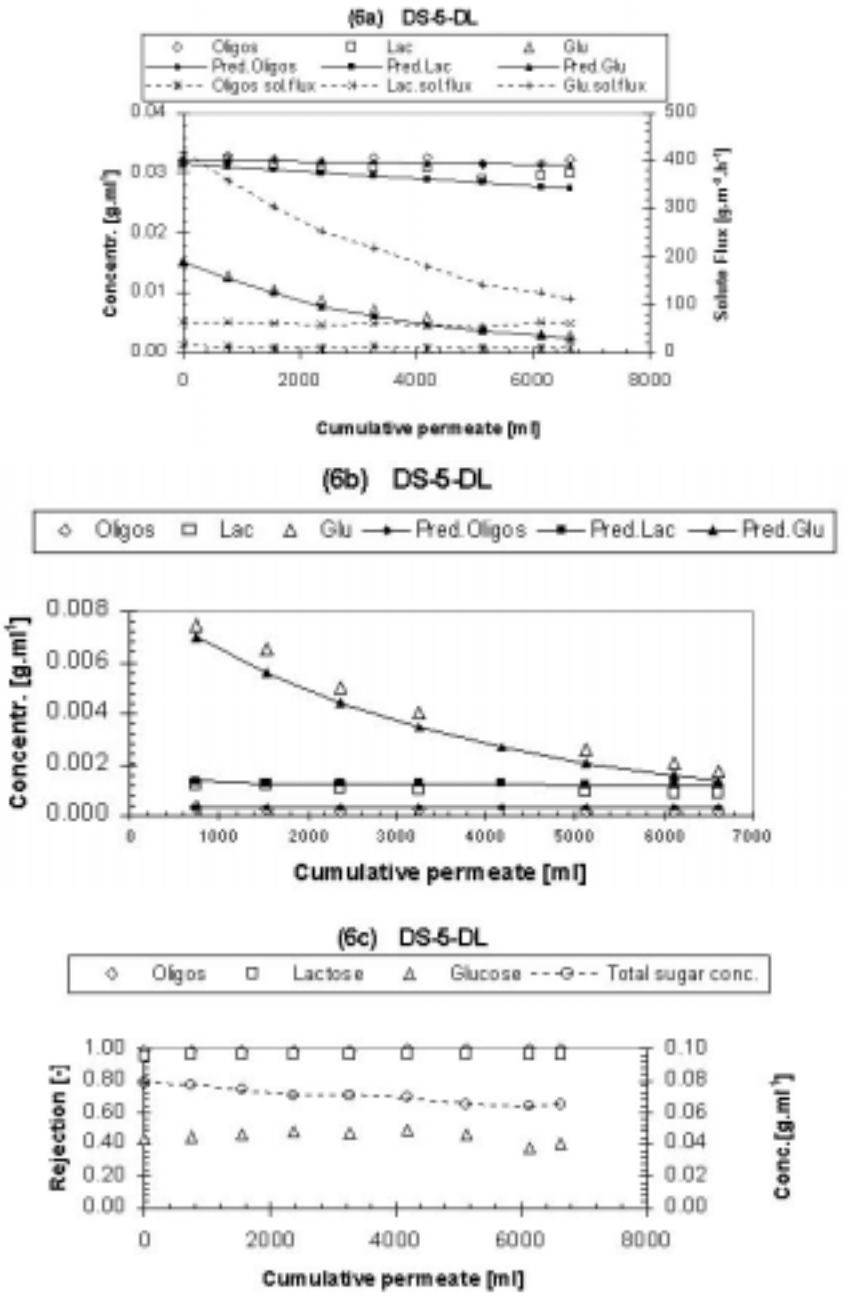


**Figure 6. Vivinal®GOS CD with the DS-5-DL membrane at 60°C at 13.8 bar pressure, a) Feed concentration vs Cumulative permeate, b) Permeate concentration vs. Cumulative permeate, c) Sugar Rejections and Total sugar feed concentration vs. Cumulative permeate.**

each pressure.

CD experiments with the NF-CA-50 membrane were carried out at 25°C and at 60°C with the DS-5-DL. The zero cumulative permeate rejection values were used with equations 4 and 5 to predict the course of the CD purification. During the course of CD, the permeate flux increased slightly (results not shown), due to de-

**Table 4. Yield values from the continuous diafiltration experiments with the NF-CA-50 and DS-5-DL membrane with the model solution and the commercial oligosaccharide mixture.**

| **NF-CA-50[a] / 13.8 (bar) Yield [%]** | | | **DS-5-DL[b]/ 13.8 (bar) Yield [%]** | | |
|---|---|---|---|---|---|
| Feed 0.077 ($g.ml^{-1}$)/ TCP[d] 6562 (ml) | | | Feed 0.0551 ($g.ml^{-1}$)/ TCP 9068 (ml) | | |
| Raffinose | Sucrose | Fructose | Raffinose | Sucrose | Fructose |
| 81 | 59 | 15 | 98 | 89 | 14 |
| Feed 0.0821 ($g.ml^{-1}$)/ TCP 6388 (ml) | | | Feed 0.0791 ($g.ml^{-1}$)/ TCP 6623 (ml) | | |
| Oligos[c] | Lactose | Glucose | Oligos | Lactose | Glucose |
| 84 | 62 | 18 | 98 | 89 | 18 |

**[a] Experiments carried out at 25°C ; [b] Experiments carried out at 60°C**
**[c] The term oligos stands for all the galacto-oligosaccharide content of the mixture used**
**[d] Total Cumulative Permeate removed during the CD**

creased concentration polarization resulting from reduction of the feed concentration. Figure 6 shows actual and predicted data, and solute rejections for CD of Vivinal®GOS using DS-5-DL membranes. Rejection of the sugars remained fairly constant throughout the purification (Figure 6c). As sugar rejection values remained virtually constant, the mathematical analysis applied to predict the concentration in the feed and permeate, using the zero cumulative permeate rejection values observed, were very close to the experimental results (Figure 6a, b). This mathematical analysis provides a useful tool in predicting the course of similar CD purifications only in cases where the total feed concentration does not change greatly. However such a prediction would be restricted in cases where high protein content is present in the oligosaccharide synthesis mixture, since proteins have different separation characteristics from sugars.

Examination of the solute flux values (Figure 6a), which are directly dependent on the concentration of the sugar in the feed solution, allows determination of the extent of purification achievable without significant loss of the oligosaccharide content. This level is where the solute flux of the monosaccharide coincides with the solute flux of the oligosaccharides.

Excellent yields of oligosaccharide were obtained from both model sugar mixtures and a commercial oligosaccharide preparation, with greater than 80% removal of monosaccharides (Table 4). The DS-5-DL membrane (at 60°C) generally performed better than the NF-CA-50, in terms of monosaccharide removal, retention of oligosaccharide and permeate flux.

In conclusion, excellent purification of oligosaccharides with respect to contaminating monosaccharides could be obtained by NF in stirred cell and cross flow membrane units.

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